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BIOLOGICAL CONTROL

Tetrastischus howardi density and dispersal toward augmentation biological control of sugarcane borer

RH Barbosa[1], FF Pereira[1], AVA Motomiya[2], SO Kassab[1], C Rossoni[1], JB Torres[3], RM Mussury[1], PL Pastori[4]

[1]Faculdade de Ciências Biológicas e Ambientais, Univ Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brasil
[2]Faculdade de Ciências Agrárias, Univ Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brasil
[3]Depto de Agronomia, Univ Federal Rural de Pernambuco, Recife, Pernambuco, Brasil
[4]Depto de Fitotecnia, Univ Federal do Ceará, Fortaleza, Ceará, Brasil

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Correspondence
PL Pastori, Depto de Fitotecnia, Univ Federal do Ceará, Av. Mister Hull, 2.977, Campus do Pici, Fortaleza, Ceará60.356-001, Brasil; plpastori@ufc.br

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Abstract
The number of *Tetrastischus howardi* (Olliff) females to be released and their dispersion should be known, that way, used *D. saccharalis* pupae as sentinel host to measure parasitism as function of the release density of the parasitoid and its location in the field. Two sets of trials were run aiming first to define the number of parasitoids to be released and the dispersal of the parasitoid using plots in sugarcane commercial fields, respectively. Pieces of sugarcane stalk holding sentinel pupae were taken to the field and exposed to parasitism in both trials. The parasitoid was released at the rate of 20, 40, 80, and 160 females per sentinel pupa, except for the control plot without releasing. The parasitism rate was calculated based on the recovered pupae after 96 h of exposure time from releasing the parasitoids. The models estimated the best parasitism rate by releasing 102 parasitoids per pupa. In the second trial, sentinel pupae were arranged in five subsequent circles corresponding 4, 8, 12, 16, and 20 m around the central parasitoid releasing point at rate of 4, 8, 12, 16, and 18 pupae per circle. The mean estimated dispersal distance was 7.64 m, with a covering area of 80.07 m^2. Based on these findings, release of *T. howardi* is recommended in 125 points per hectare of sugarcane at rate of 102 females per pupa of the pest aiming to achieve homogeneous distribution and parasitism.

Introduction

Among biofuel crops, sugarcane is one of the most important, and in Brazil alone, more than nine million hectares were cultivated in the 2016/2017 growing season (CONAB 2017). The stem borers (Lepidoptera: Crambidae) include species causing losses in various crops of economic importance including sugarcane with species belonging to the genera *Chilo*, *Ostrinia*, *Diatraea*, *Maruca*, *Dupunchelia*, and *Scirpophaga* (Moolman *et al* 2014, Vargas *et al* 2015). Most of the species within these genera develops through larvae and pupae partially protected inside host plant stalks, precluding control with contact insecticide sprayings (Pannuti *et al* 2013, Rossato Junior *et al* 2013). In contrast, biological control using larval-pupal parasitoids has been successful for this group of insects (Chichera *et al* 2012, Dinardo-Miranda *et al* 2014, Parra 2014, Vargas *et al* 2015). Particularly in Brazil, the larval endoparasitoid *Cotesia flavipes* (Cameron) has been released in more than 35% of cultivated area with sugarcane to control *Diatraea saccharalis* (Fabr.) and *Diatraea flavipennella* (Box.) (Silva *et al* 2012, Vacari *et al* 2012, Dinardo-Miranda *et al* 2014, Parra 2014).

The sugarcane borer complex, *D. saccharalis* and *D. flavipennella*, are the main sugarcane pests of the Americas, infesting all the phenological stages of the crop (White *et al* 2011, Vargas *et al* 2015, Dinardo-Miranda *et al* 2014, Valente *et al* 2018). Sugarcane borer infestations result in direct injuries due to the galleries opened along the sugarcane stalks, which can reduce yield and, indirectly opens entrance to fungi colonization resulting in sugarcane red rot (Pannuti *et al* 2013). In addition, the losses are increased by reduction of sugar content and contamination of the sugarcane juice, which hinders fermentation in the ethanol production process (Rossato Junior *et al* 2013).

Biological control with parasitoids has been the predominant method used against both sugarcane borers in Brazil (Parra 2014). Upon hatching, first instars feed by scraping the leaf or tunneling through the midrib and can be reached with synthetic or biological insecticides. However, larvae after the first or second molt (3–8 days) burrow into the stalk, where they remain during the whole development (40–45 days) gaining partial protection from sprays (Antigo *et al* 2013). In Brazil, the egg parasitoid *Trichogramma galloi* Zucchi and the larval endoparasitoid *C. flavipes* have been widely used against sugarcane borers (Broglio-Micheletti *et al* 2007, Vacari *et al* 2012). Nevertheless, populations of *Diatraea* spp. have exhibited high levels of infestation resulting in losses, even with constant releases of these parasitoids (Silva *et al* 2012). Brought to Brazil in 1970, *C. flavipes* has since been reared and released on a large scale without further introduction or investigation of other efficacious larval parasitoids to offer an alternative or complementary biological control. Another potentially valuable biological control agent is *Tetrastichus howardi* (Olliff), a gregarious endoparasitoid being a primary parasitoid or facultative hyperparasitoid that develops in *D. saccharalis* larvae or pupae (Cruz *et al* 2011, Vargas *et al* 2011, Vargas *et al* 2015, Costa *et al* 2014a, b, Pereira *et al* 2015). This parasitoid is Asian origin (La Salle & Polaszek 2007), widely distributed throughout the world and can be reared in large scale using the sugarcane borer or the alternative host (Oliveira 2013, Costa *et al* 2014a, b). In a single *D. saccharalis* pupae, *T. howardi* produces an average of 190 wasps. The adult lifespan ranges from 15 to 25 days, at 25 ± 2°C (Costa *et al.* 2014a, b). When the parasitoid is multiplied over *Tenebrio molitor* L. pupae, a single host pupae yield 60 to 180 adult wasps with life expectancy over 25 days, at 25 ± 1°C (Oliveira 2013). Additionally, it was observed that *T. howardi* can develop in others Lepidoptera hosts and also in species from different orders, such as Coleoptera, Diptera, and Hymenoptera (Bennett 1965, Moore & Kfir 1995, La Salle & Polaszek 2007, Oliveira 2013).

In America, *T. howardi* was introduced in Trinidad and Tobago in 1963 as a potential biological control agent of *Diatraea* spp. The parasitoid was multiplied over different hosts; however, no mass releases were conducted (Bennett 1965). In 1997, it was reported in Cuba associated to *D. saccharalis* pupae in rice and sugarcane, with a mean parasitism of 16% (Álvarez *et al* 2008). In addition, with the expansion of mass rearing technologies, it has also been used to control other Lepidoptera species (Moreno *et al* 2010). In Brazil, it was found parasitizing *Plutella xylostella* (Linnaeus) (Silva-Torres *et al* 2010) and *D. saccharalis* pupae in sugarcane (Vargas *et al* 2011) and maize (Cruz *et al* 2011). The parasitoid occurrence in Brazil opens a new perspective for the sugarcane borer control, considering the promising results obtained in other countries (Cruz *et al* 2011).

Knowledge concerning release rate and dispersal is central to the development of effective biological control programs (Pereira *et al* 2010, Bueno *et al* 2011, Zappala *et al* 2012). Therefore, in this study, we had three major goals to optimize release of *T. howardi* against *D. saccharalis*: (i) to determine the optimal number of parasitoid females to release per host pupa, (ii) to determine the dispersal ability of the parasitoid in commercial sugarcane fields using sentinel pupae, and (iii) to estimate the number of points per area to release the parasitoid in sugarcane.

Material and Methods

Insect rearings

Viable eggs of the sugarcane borer, *D. saccharalis*, were obtained from colonies maintained at the laboratory. Egg masses close to hatching were transferred to glass vials (8.5 cm diameter and 13 cm height) containing artificial diet based on wheat germ (150 g), soybean meal (540 g), sugarcane yeast (450 g) (*Saccharomyces cerevisiae* Meyen ex Hansen), vitamins, minerals, phagostimulant, and antibiotics prepared after Hensley & Hammond Jr (1968). This diet provides nutrition for the newly hatched larvae that remained in situ at 25 ± 2°C, relative humidity of 70 ± 10%, and 12:12 (L:D) photoperiod in a climate-controlled room until molting to pupae. The pupae were collected and separated by gender (20 males and 30 females) and were kept in cylindrical PVC® cages (10 cm diameter and 22 cm high). The inner surface of the cages was covered with white paper to serve as oviposition substrate. The upper opening was closed with organdy fabric secured by an elastic.

The colony of the factitious host, yellow mealworm *T. molitor*, was initiated with the larvae from the laboratory. Briefly, adults and larvae were reared in plastic container (29.0 × 40.8 × 12.6 cm) containing similar diet for all life stages. This diet was composed of wheat bran (97%), brewer's yeast (3%), and pieces of any available vegetable (carrot, sweet potato, sugarcane stalk, or chayote) serving as moisture source and food supplement. The insects were

maintained at 25 ± 2°C, 70 ± 20% relative humidity (RH), and a photoperiod of 14:10 (L:D) h.

The colony of the parasitoid *T. howardi* was established from naturally parasitized *D. saccharalis* pupae collected from sugarcane crops in the municipality of Dourados, Mato Grosso do Sul State, Brazil. Adult specimens of the parasitoid were sent to Marcelo Teixeira Tavares of the "Universidade Federal do Espírito Santo - UFES" for species confirmation. Voucher specimens were deposited in the insect collection of the UFES, Espírito Santo State, Brazil. In the laboratory, 5–10 *T. howardi* females were individualized in a glass tube (2.5 cm diameter × 8.5 cm height) with a *T. molitor* pupa (24 to 48 h old). The parasitoid females were fed with a small honey bee drop applied on the glass tubes' inner wall that were sealed with cotton plug. The *T. molitor* pupa was exposed to parasitism during 24 h. After this period, the females were removed and glass tubes containing the parasitized pupae were maintained at 25 ± 2°C, 70 ± 10% RH, and a 12:12-h L:D photoperiod until parasitoids emergence (Vargas *et al* 2011). With the parasitoid emergence, the reared procedure was reinitiated. The sex differentiation was made based on the antennae (La Salle & Polaszek 2007).

Parasitism units

The sentinel pupae were prepared using pieces of sugarcane stalks variety SP81-3250; the stalks in which sentinel pupae were placed are referred from hereafter as parasitism units. The pieces were 18–22 cm long and 4–5 cm diameter and included an internode with nodes at each end. Thus, one orifice ~1 cm diameter by 2 cm deep was made between the nodes with the aid of a power drill. A single sugarcane borer pupa 12–24 h old was gently placed inside the single hole in each stalk piece and taken to the field in the same day. In the field, the parasitism units were set on pre-defined points within the studied sugarcane plot. The parasitism units were attached, using an elastic band, at the top of 1.2-m wooden stalks. To avoid opportunistic predators such as ants, the base of the stalk was treated with vegetable oil.

Experimental areas

Field studies were run using two areas belonging to sugar and ethanol production companies (described ahead), both cultivated with the variety SP81-3250 under 1.5 m between plants rows. The selected areas were 7 months old within the second cutting season (ratoon cane), and free of insecticide application during the experimental period.

Parasitoid density to be released

The sugarcane borer field infestation is rated by counting stalk holding with live larvae, and when the infestation index exceeds 10/larvae/hour/man, parasitoids are released (Dinardo-Miranda *et al* 2014, Parra 2014). Thus, the first step was to determine the *T. howardi* female number to be released based on the infestation level. The trial was conducted in a randomized block design with five treatments corresponding to the parasitoid densities tested of 20, 40, 80, and 160 females of *T. howardi* released per *D. saccharalis* pupa and the control without parasitoid release. Each treatment had four replicates. Each replicate consisted of sugarcane plot of 4.5 m wide × 9.0 m length with a minimum distance of 80.0 m between plots. Each plot/replicate received eight parasitism units with each one containing one sentinel pupa. These parasitism units were distributed circularly and 2.2 m apart from the release point. After setting the parasitism units in the plots, females of *T. howardi* were released at central point of the area using the respective densities tested with one release point per plot. The release was carried out at 4:00 p.m. as recommended by Jerbi-Elayed *et al* (2015) to minimize the climatic stress of laboratory-field transference. During the pre-releasing, the females were kept together with males and fed with honey bee. Only parasitoid females 24–48 h old were released. The females were transferred to glass tubes at the respective density using one vial per replicate. In the plots representing the control density (i.e., without parasitoid release), the natural parasitism was measured using 20 parasitism units set in plots at least 100 m apart. The parasitism units were recovered 96 h after releasing the parasitoid and brought to the laboratory. Afterwards, the pupae were transferred to glass vials identified regarding treatment and replication, and set under the same rearing condition waiting for parasitoid or adult moth emergence. The unviable pupae were dissected to observe the presence of dead parasitoid pupae. Parasitized pupae were considered those with parasitoids emergence and dead pupae with parasitoids mummified inside. The percentage of parasitism was calculated by [(number of parasitized pupae × 100) total number of pupae per plot and treatment].

This step was developed in the experimental area of the "Santa Helena" Mill (22°5′28″S and 53°23′26″W and altitude of 430 m a.s.l.) in the municipality of Nova Andradina, Mato Grosso do Sul State, Brazil, in December 2012. During the study period, the temperature ranged from 23.7 to 29.8°C, relative humidity ranged from 54 to 88%, and accumulated rainfall of 14.7 mm was measured in the site.

The data were subjected to regression analysis considering percentage of parasitism as dependent variable (y) as function of the pre-established releasing density from 0 to 160 females, as independent variable (x). The regression model selected was that with all regression parameters significant at 5% probability level, with the highest coefficient of determination (R^2) and representing properly the observed data.

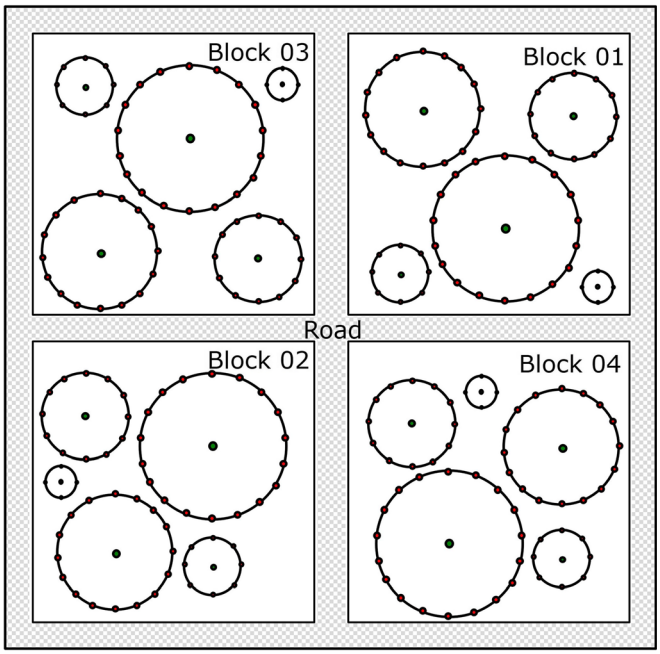

Fig 1 Dispersal of the parasitoid *Tetrastichus howardi* in sugarcane field—experimental field (general) (A) and description of each block (B).

Dispersal of the parasitoid in sugarcane field

This trial was set in the field using a randomized block design consisting of five distances with four replicates each (Fig 1A). Each replicate or block contained five individual concentric circles of 4, 8, 12, 16, and 20 m from the central point (i.e., radius distance) (Fig 1B). The trial was carried out in a sugarcane field of the "Monte Verde" Mill (22°15′43.31″S and 55°11′19.06″W and altitude of 383 m a.s.l.) in the municipality of Ponta Porã, Mato Grosso do Sul State, Brazil, during April 2013.

The field set consisted of pre-established plots at a minimum distance of 100 m apart. Each plot consisted of parasitism units displayed in circles of 4, 8, 12, 16, and 20 m from the central point (i.e., radius distance). Each circle was set up individually. The parasitism units containing the sentinel pupae at the rate of 4, 8, 12, 16, and 18 units per distance were placed in the perimeter of each circle, respectively. Females of *T. howardi* were prepared to be released similarly to the previous study using the best determined density. Again, the release was made at 4:00 p.m. as recommended by Jerbi-Elayed *et al* (2015). In addition, each block, including the control (no parasitoids released), was established more than 80 m apart. The evaluation consisted in recovering the parasitism units 96 h after releasing the parasitoids. The parasitism rate was determined for each distance from the central releasing point considering each parasitism unit within the circle perimeter as a position to allow spatial analysis. The

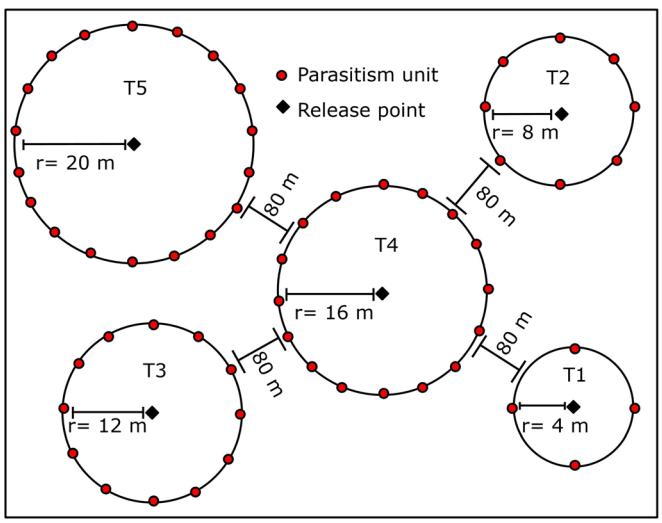

Fig 1 continued.

parasitized pupa identification and the parasitism rate formula followed the same procedure adopted in the previous experiment. Over the study period, the temperature ranged from 21.7 to 24.8°C and relative humidity from 76 to 84%, while the wind showed predominance of a southeast (SE) direction and mean speed of 1.19 m/s.

The mean distance (MD) and the dispersal area (s^2) were calculated 96 h after releasing the parasitoids using the mathematical models proposed by Dobzhansky & Wright (1943). A contour map of the area based on the parameters of the experimental semivariogram was fitted to the spherical theoretical model and to the data interpolated by the ordinary Kriging method (Vieira *et al* 1983) to spatially characterize the parasitism.

Results

Density of the parasitoid to be released

Released females of *T. howardi* found and parasitized the sentinel pupae of *D. saccharalis* set up in commercial plots of sugarcane in the field during 96 h of exposure. The adjusted regression model to the observed data estimates maximum parasitism of 46.4% when 102 parasitoid females are released per one host sentinel pupa (Fig 2). Meanwhile, the observed percentage of parasitism ranged from 9.3 to 39% as a function of the parasitoid densities tested.

Dispersal of the parasitoid in sugarcane field

The best adjusted model explaining the parasitism of *D. saccharalis* pupae by *T. howardi* as function of the distance indicates a linear fashion decrease in parasitism in relation to the distance from the releasing point (Fig 3). The rate of parasitism varied from 62.5% at nearest distance (ca. 4 m) to 1.9% when the sentinel host pupa was set at 20 m from the releasing point (Fig 3).

The flight distance required by females of the *T. howardi* to find and to parasitize *D. saccharalis* pupae was on average 7.6 m from the releasing point 96 h after releasing in the sugarcane plots under the studied conditions. The estimated covered area was 80.1 m^2, with expected parasitism of 39.4% (Table 1). In addition, fitted semivariogram was according to the spherical model indicating strong spatial dependence from the releasing point, with shorter distance resulting in greater parasitism (Table 1; Fig 4).

Finally, in both studies, no natural parasitism was observed in *D. saccharalis* sentinel pupae in the control plots. Therefore, the parasitism obtained in the pupae set up in parasitism units was a result from released parasitoids.

Discussion

The successful searching and parasitism of *D. saccharalis* pupae by *T. howardi* in the field indicate the potential of this parasitoid for biological control against this key pest of sugarcane crop. It provides a new option to complement the already established biological control program using the larval endoparasitoid *C. flavipes* used in Brazil since the 1970, and lately with the egg parasitoid *T. galloi* (Parra 2014). *Cotesia flavipes* is a larval parasitoid (Dinardo-Miranda *et al* 2014), while *T. howardi* specially prefers pre-pupa and pupa

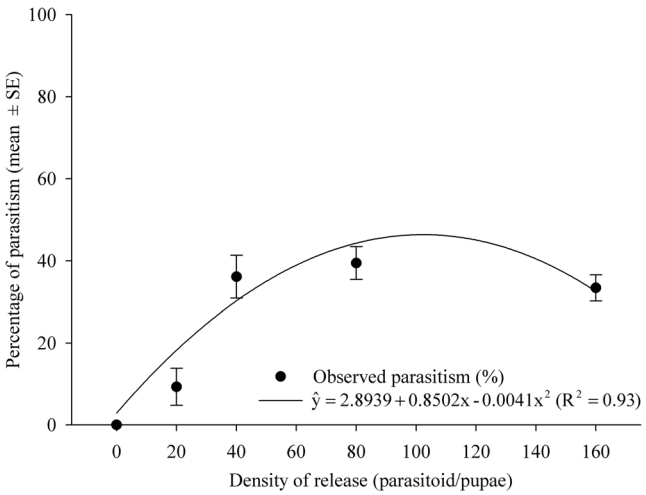

Fig 2 Parasitism of the sugarcane borer *Diatraea saccharalis* pupae by the parasitoid *Tetrastichus howardi* using different releasing densities rated as function of the sentinel pupa set in the field of sugarcane ($F_{3,4,12} = 21.62$, $p < 0.001$). "Santa Helena" Mill, Nova Andradina, Mato Grosso do Sul State, Brazil, 2012.

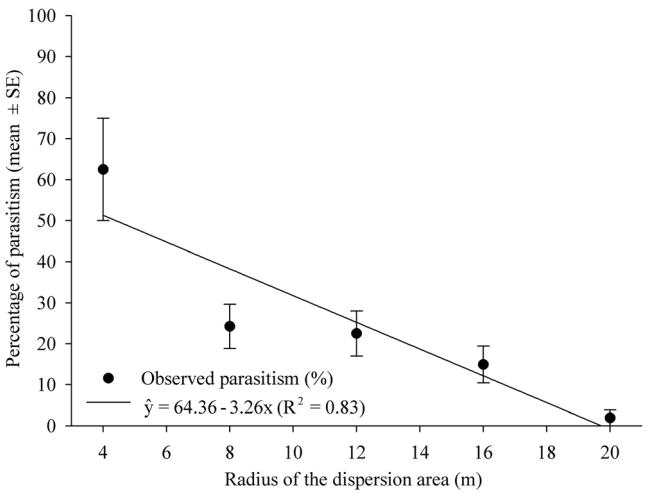

Fig 3 Parasitism (± SE) of *Diatraea saccharalis* pupae by *Tetrastichus howardi* as function of the distance from the releasing point in sugarcane field plots ($F_{3,4,12} = 34.1792$, $p < 0.001$). "Monte Verde" Mill, Ponta Porã, Mato Grosso do Sul State, Brazil, 2013.

Table 1 Mean distance, dispersal area, expected parasitism, parameters of the fitted ', and cross validation of the interpolated data by the ordinary Kriging method for parasitism of *Diatraea saccharalis* pupae by *Tetrastichus howardi* 96 h after released in sugarcane field plots. Mato Grosso do Sul State, Brazil, 2012–2013.

Estimated parameters	Estimates		
Mean distance (MD)	7.6 m		
Dispersal area (s^2)	80.1 m^2		
Expected parasitism*	39.5%		
Estimated parameters	Cross validation		
Pure nugget (C_o)	1.00	*A*	7.13
Structural variance (*C*)	707.50	*B*	0.49
Level ($C_o + C$)	708.50	R^2	0.40
Range (a)	19.85	–	–

*Expected parasitism calculated through mean distance in the model $\hat{y} = 64.36 - 3.26x$.

stages, besides also parasitizing *D. saccharalis* larvae (Pereira *et al* 2015). Therefore, there is good opportunity for additive parasitism during host development, if negative interspecific interactions do not occur.

Searching and parasitism under field conditions critically determine the efficacy of parasitoids in regulating populations of their hosts in the field (Silva-Torres *et al* 2009). A similar (Dinardo-Miranda *et al* 2014) or slightly lower (Chichera *et al* 2012) percentage of parasitism on sugarcane stem borer is reported for eulophids (*T. howardi*, *Palmistichus elaeisis* (Delvare and LaSalle), and *Trichospilus diatraeae* Cherian & Margabandhu) compared to *C. flavipes*. However, we have to consider the specialist behavior and relatively larger body size of *C. flavipes* compared to the eulophids. It is not expected that *T. howardi* release will result in parasitoid substitution. Instead, such release will provide an additional parasitoid attacking stages later of the pest that escaped from parasitism offered by the egg parasitoid *Trichogramma* spp. and the larval parasitoid *C. flavipes*. Further, *T. howardi* acts during immature stages later, such as the pupa stage, will have more impact on the pest population increase. Under natural conditions, *D. saccharlis* may lay 2 to 37 eggs per egg mass (average 11.8 ± 6.3 eggs) on a plant (Lima Filho & Lima 2001); however, only few larvae survive to the pupal stage (Oliveira *et al* 2012). Therefore, further mortality caused by *T. howardi* on surviving old larvae and pupae of sugarcane stem borer likely will have significant impact on the pest population growth.

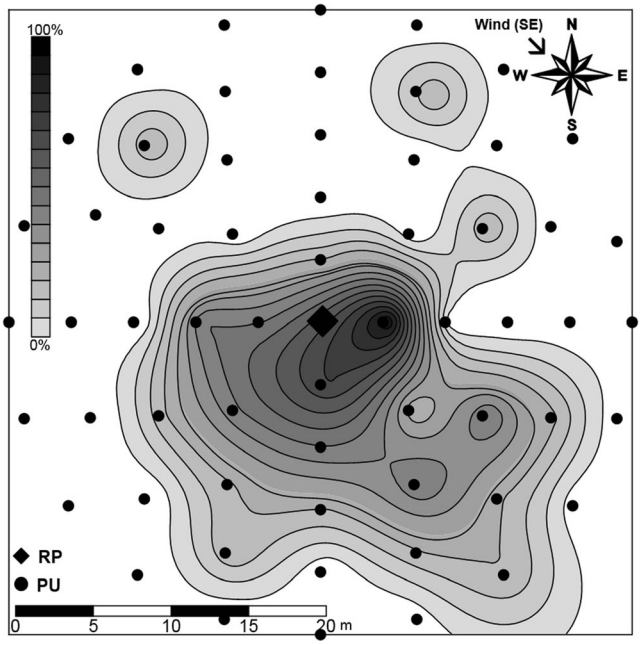

Fig 4 Spatial display of the parasitism units (PU, black dots), the central releasing point of the parasitoids (RP, black square), wind direction and speed (SE and 1.19 m/s), and interpolation of parasitism (%) of *Diatraea saccharalis* pupae by *Tetrastichus howardi* in sugarcane field plots. "Monte Verde" Mill, Ponta Porá, Mato Grosso do Sul State, Brazil, 2013.

Furthermore, the natural presence of stem borer in the experimental areas may have attracted the released *T. howardi* to areas outside study plots, and reduced the calculated parasitism rate. In addition, it is known that host density and chemical and physical signals associated with the hosts and its host plant can influence adult parasitoid's foraging behavior (Heimpel & Casas 2008). These aspects have not yet been clarified for *T. howardi* parasitizing sugarcane stem borer or other natural host. It should be noted that due to the logistics of setting up the experiments, these important components in the process of parasitoids searching in relation to the pupae were not evaluated. Despite that, we can hypothesize that *T. howardi* females expressed their flight potential under our field study considering that the released parasitoids were the same age and rearing conditions.

The study showed the importance for adequate released proportion of *T. howardi* to control *D. saccharalis*. This could be observed in the different pupa parasitism obtained from the five density treatments. The ratio of parasitoid female per host is important to overcome the immune response of the host (Andrade *et al* 2010, Harvey *et al* 2013). In other cases, greater density of parasitoids can increase intraspecific competition and reduce parasitism, as reported for other eulophid species, e.g., *P. elaeisis* and *T. diatraeae* (Pereira *et al* 2010, Favero *et al* 2013). However, even in the field, the probability for a parasitoid to find a non-parasitized host decreased with the parasitoid density increases, as observed for *T. galloi* and *D. saccharalis* eggs (Broglio-Micheletti *et al* 2007).

The maximum estimated parasitism of 46.4%, with 102 females of *T. howardi* per *D. saccharalis* pupa is important to determine the *T. howardi* female ideal number for field released associated with the stem borer infestation index. With knowledge of parasitoid dispersal capacity, the

parasitism rate can be increased by adjusting the spatial distribution of parasitoids in the field (Dinardo-Miranda *et al* 2014, Machtinger *et al* 2015). Our data show a linear decrease in rate of parasitism by *T. howardi* with distance from release point, thus reinforcing the importance of the release point number per area for sugarcane fields. Despite that, *T. howardi* parasitized sentinel pupae up to 20 m from the releasing point under the conditions of this study. This corroborates other data under different conditions and suggests that the dispersal average distance of Eulophidae parasitoids can range from 7 to 24 m, with linear reduction in parasitism as function of the distance (Bueno *et al* 2011, Silva-Torres *et al* 2011). A major factor interfering with dispersal of parasitoids is the plant architecture and the foliar area to be searched for host, parameters strongly influenced by the plant species, and its phenology at the time of parasitoid release (Pomari *et al* 2013). In this case, further studies should be carried out to validate the number of releasing points per area according to the sugarcane phenology.

The semivariogram fitted to the spherical model indicated strong spatial dependency among the samples, showing that parasitism varies in accordance with the distance of the location of release of the *T. howardi* females in relation to the sentinel pupae. This range (radius) is used to characterize the dispersal of parasitoids (Zappala *et al* 2012). In addition, the parameters of the model allow estimating unsampled areas by interpolation techniques and construction of specific maps (Vieira *et al* 1983). Wind speed and direction are also important variables in the parasitoid dispersal process (Zappala *et al* 2012, Machtinger *et al* 2015), with a tendency to concentrate parasitism in the predominant direction of the winds since the wind contributes to passive dispersal when the insect begins its flight (Ávila *et al* 2013, Mahecha & Manzano 2016). This was corroborated in this experiment as greater parasitism occurred in the area located to the southeast (SE) of the releasing point, which resulted in greater aggregation of parasitism in that area. Thus, *T. howardi* should be released at times in which wind speed is less than 1.19 m/s or, otherwise, this factor should be considered by arranging the releasing points considering the direction of the predominant wind.

As expected, viable adults emerged from parasitized sentinel pupae recovered from the experimental areas, showing that the parasitoid will be able to multiply in the sugarcane field where they are released. This species exhibits characteristics that make it an excellent candidate for augmentation biological control. Among those are a large-scale rearing method, female biased offsprings and long adult lifespan such as ease of being raised on a large scale, high proportion of females in the offspring, and adults living up to 15 days (Costa *et al.* 2014a, b).

The parasitism rates and development of *T. howardi* over *D. saccharalis* sentinel pupae based on the parasitoid density and dispersal studies show that this biological agent can contribute for the sugarcane borer control. We estimated that the ideal *T. howardi* is 125 points per hectare with approximately 100 female parasitoids per sugarcane borer pupa. Therefore, further studies are required aiming to refine the recommendation of *T. howardi* releasing against sugarcane borer. The crop phenology, frequency of release of *T. howardi* females in accordance with infestation of sugarcane borer, and methodology to improve release and reduce the walking through considering the high number of releasing points required per hectare are recommended. For example, use of unmanned aerial vehicles (UAV) has been considered a convenient approach to be pursued aiming release of natural enemies in large areas (van Lenteren *et al* 2018). The use of UAV would be very handy to release *T. howardi* considering that it can be produced in large number, released at adult stage, and packed in small volume and weight to be carried by UAV.

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Author's Contribution RHB, FFP, AVAM, SOK, and PLP planned research; RHB, FFP, SOK, and CR conducted experiments; CR, JBT, and RMMFS contributed material and conducted data analyses; RHB, FFP, and AVAM analyzed data and conducted statistical analyses; RHB, FFP, SOK, JBT, and PLP wrote the manuscript; FFP, JBT, RMMFS, and PLP secured funding and additional support; and RHB, FFP, AVAM, SOK, CR, JBT, RMMFS, and PLP read, suggest, and approved the manuscript.

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