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Research

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Biological Control - Parasitoids and Predators

Spatial and temporal dispersion of *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) in orchards infested with *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae)

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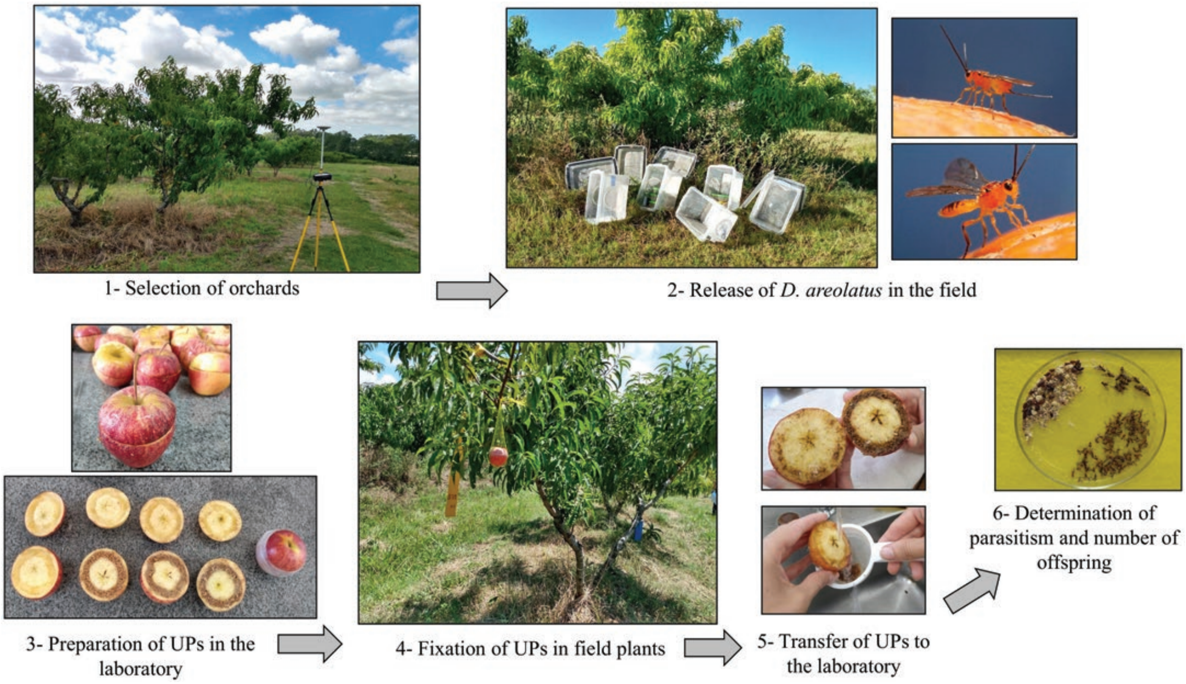
Doryctobracon areolatus (Szépligeti) is an endoparasitoid and promising fruit fly control agent. The objective of the study was to determine the spatial (horizontal and vertical) and temporal dispersion of *D. areolatus* in the field. To evaluate the horizontal and temporal dispersion, two peach orchards were selected. In each orchard, 50 points were marked at different distances from the central point, from where 4,100 couples of *D. areolatus* were released. Four hours after release, parasitism units (PU) (3 per point) were fixed to the trees at a height of 1.5 m from the ground. The PUs were composed of ripe apples artificially infested with second instar larvae of *Anastrepha fraterculus* (30 larvae/fruit). For the evaluation of vertical dispersion, in an olive orchard six points were selected (trees of ≈4 m in height). Each tree was divided into three heights in relation to the ground (1.17, 2.34, and 3.51 m). *Doryctobracon areolatus* was able to disperse horizontally at a distance >60 m from the release point. However, the highest parasitism rates [15–45% (area 1); 15–27% (area 2)] were observed up to 25 m. Higher percentages of parasitism and of recovered offspring occur in the first days after the release of the parasitoid (2 DAR). As for vertical dispersion, *D. areolatus* parasitized *A. fraterculus* larvae up to the highest attachment height of the evaluated PUs (3.51). The results showed the potential use of *D. areolatus* in the management of fruit flies in the field.

Key words: fruit fly, biological control, parasitoid release

Graphical Abstract



Introduction

The search for control methods that cause the least impact on the environment has been growing in recent decades. In this context, biological control has assumed relevant importance as a management strategy associated with other pest control techniques (Paranhos et al. 2021). The global biological agents market reached \$3.4 billion in 2016, with an estimated \$7.6 billion by the end of 2022. Brazil is the world leader in the use of biological control in crops, with application in more than 23 million hectares (Embrapa 2019). In 2020, 95 products were registered in the country for this purpose (Mapa 2021). Although the country is one of the leaders in the use of biological control, there are still difficulties regarding its application in the management of pest tephritids (Diptera: Tephritidae), especially for the main species that occur in Brazil *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) (Zucchi 2000). The damage to the fruit is caused by the females by oviposition and later by the feeding of the larvae, which destroy the fruit pulp, accelerate ripening, and lead to premature fall (Zucchi 2015, Botton et al. 2016). Annually, the estimated losses from these pests in Brazil represent about R\$ 180 million, including losses in production, commercialization, and control costs.

Among the great diversity of potential parasitoid species that can be used in tephritid management is *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) (Zucchi and Moraes 2008, Paranhos et al. 2019), an endoparasitoid native to the Neotropics that parasitizes the early stages of the development of fruit flies (Murillo et al. 2015, Paranhos et al. 2021). Only in Brazil, there are reports of parasitism of 23 species of fruit flies, where the genus *Anastrepha*, especially the *A. fraterculus* species stands out. This species forages for larvae in ripe fruit on the tree and, unlike some related species, seldom investigates fallen fruit (Sthul and Sivinski 2015). After locating the host inside the fruit, mediated by antennation (touching the antennae), the adult female inserts a single egg inside the body of the fly larvae that will later give rise to a new wasp (Sthul and Sivinski 2015). Several works have already elucidated aspects related to taxonomy, rearing techniques, and biology of this species (Sthul and Sivinski 2015, Gonçalves et al. 2018, Paranhos et al. 2019, 2021, López-Arriaga et al. 2021).

Because it is a native species, *D. areolatus* has advantages regarding its evolutionary interaction with hosts and greater adaptation to local environmental conditions, compared to exotic species (Eitam et al. 2004). As an example, one can cite the introduction and release of the exotic parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) for fruit fly management. Despite the control efficiency, the parasitoid has not become established in releases made in Southern Brazil, probably due to the colder winters than in other regions of the country (Paranhos et al. 2019). This fact may trigger an inefficient management of the pest in regions with predominantly low temperatures, as occurs in Southern Brazil, a region where the largest producing areas of temperate climate fruit trees in the country, such as apple (*Malus domestica* Borkh.) and peach (*Prunus persica* (Linnaeus)) (Rosales: Rosaceae) are found (IBGE 2020).

Thus, the wide geographic distribution and abundance of *D. areolatus*, in practically all Brazilian states, associated with biological characteristics, make this species one of the most promising for use in biological control programs for fruit flies (Paranhos et al. 2019). Although laboratory-rearing techniques have already been established (Gonçalves et al. 2018) and have verified the high potential of parasitism on larvae of *A. fraterculus* and *C. capitata* (Nunes et al. 2011), there is still a lack of information about the dispersion behavior in the field. According to Hougardy and Mills (2006), the scarcity of information in this regard for most biological control agents is mainly due to the difficulty of assessing the dispersal ability of small insects. Knowledge of the species' ability to disperse is essential to establish successful inundative biological control strategies (Montoya et al. 2000, Paranhos et al. 2007, Zappala et al. 2012). In addition, information related to the species' ability to disperse, both spatially (distance and height) and temporally (survival of the parasitoid in the orchard), is essential to define the number of

Table 1. Meteorological information recorded on the release dates of *D. areolatus* in peach (horizontal and temporal dispersion) and olive (vertical dispersion) orchards

Peach orchard					
Release date	Average temperature (°C)[a]	Relative humidity (%)	Rain (mm)	Wind direction	Wind speed (m/s)
19 January 2021	20.6	81.1	0.0	ESE	5.1
23 February 2021	23.0	84.8	3.8	NNW	5.4
6 April 2021	22.5	89.0	0.2	NNE	5.1
Olive orchard					
Release date	Average temperature (°C)*	Relative humidity (%)	Rain (mm)	Wind direction	Wind speed (m/s)
18 November 2021	15.8	76.0	15.4	ESE	5.0
23 Novemer 2021	20.3	72.4	0.0	SE	10.7
1 December 2021	19.7	79.2	0.0	E	8.8

[a]Data referring to the average of the day.
Source: Embrapa Clima Temperado—Pelotas—RS/Brazil.

insect release points in order to obtain the best results (Oliveira et al. 2020). Thus, the aim of this study was to know the spatial and temporal dispersion of the parasitoid *D. areolatus* in fruit orchards in temperate climates.

Material and methods

Characterization of study sites

The experiments were carried out in peach orchards (0.87 and 0.64 hectares) and olive trees [*Olea europaea* Linnaeus (Lamiales: Oleaceae)] (0.96 hectares), located in the experimental area of Embrapa Clima Temperado, Pelotas, RS, Brazil (latitude 31° 41′S, longitude 52° 26′W, elevation 57 m). For both study areas (orchards), the adjacent landscape is made up of forest remnants, in addition to the sporadic occurrence of fruit species, both native [(Surinam cherry—*Eugenia uniflora* Linnaeus), (Cherry guava—*Psidium cattleianum* Sabine) (Myrtales: Myrtaceae)], and exotics [(Peach and plum—*Prunus* spp. Linnaeus) (Rosales: Rosaceae), (citrus—*Citrus* spp. Linnaeus) (Sapindales: Rutaceae)]. Two experiments were carried out during the period of absence of fruits on the trees. This criterion was established to avoid the overlapping of peach fruits with parasitism units (PUs), consisting of apples artificially infested with second instar larvae. Therefore, the first experiment was carried out to determine the horizontal and temporal dispersion capacity of *D. areolatus*, while the second experiment aimed to establish the capacity of vertical dispersion, through the evaluation of different heights looking for parasitism in larvae of *A. fraterculus*.

For both experiments, prior to the release of the parasitoids, 25 PU were randomly distributed in each orchard, composed of ripe apples artificially infested in the laboratory with second instar *A. fraterculus* larvae (30 larvae per fruit). This procedure aimed to verify the existence or not of natural parasitism. According to Harbi et al. (2015), parasitism observed in fruit traps represents the movement of the female and, therefore, can be used as an indirect method to detect adult dispersal. The PUs were prepared by making a horizontal cut in the fruit, then opening two cavities in the flesh (top and bottom). Using a spatula (stainless steel with 3 mm micro scoop), larvae were added inside the fruit together with wheat fiber and corn meal artificial larval diet.

In the orchards, the PUs were fixed to the trees at 1.5 m from the ground and placed individually inside a plastic net (polyethylene) for fruits (1 kg of apples infested with larvae), remaining in this location for a period of 24 h. For each experiment installed in the field, three PUs were maintained as controls under controlled laboratory conditions (temperature: 25 ± 2°C, relative humidity: 70 ± 10%, and photophase: 12 h) in order to verify the natural mortality of the population of insects and carry out the correction by Abbott (1925).

During the experiments, local climatic conditions such as temperature, relative air humidity, precipitation, and wind speed and direction were monitored using data obtained from the meteorological station at Embrapa Clima Temperado, Pelotas, RS at a distance of about 1 km from the experimental areas (Table 1).

Rearing and multiplication of *D. areolatus*

The parasitoids used in the experiments were reared in the Entomology Laboratory of Embrapa Clima Temperado, where the adults were raised inside plastic cages (40 cm × 27 cm × 23 cm), fed with honey, and kept in an acclimatized room (temperature of 25 ± 2°C, relative humidity of 70 ± 10%, and photophase of 12 h). The multiplication of *D. areolatus* was carried out in the second instar larvae of *A. fraterculus* reared on an artificial diet according to the methodology proposed by Gonçalves et al. (2018). After the period of exposure to parasitism (12 h), the larvae mixed with hydrated wheat germ were transferred to an artificial diet of corn flour, where they remained until the formation of the first pupae. For pupation, corn flour was used as substrate. Then, the pupae were separated and placed in emergence cages.

Capacity of horizontal and temporal dispersion

Two peach orchards were selected, area 1 (A1—0.87 hectares) and area 2 (A2—0.64 hectares), and georeferencing was carried out using topographic GPS (Leica, model GS20 PDM) and drone (DJI, model Phantom 4 PRO) to capture aerial images and generate georeferenced maps (Supplementary Materials S1 and S2). Subsequently, in each orchard, 50 points were identified (with yellow nappa fabric ribbons), in addition to a central point (CP) (represented by peach trees). The distance between points and in relation to the CP was defined through the georeferenced maps of the areas, generated in the software ArcGIS. After that, at the CP of each area, a density of approximately 4,100 couples of *D. areolatus*, reared to 6 to 8 days old, were released (≈5,800 female/ha). The density of released parasitoids was established based on previous studies (Unpublished data).

Three releases of *D. areolatus* were carried out in the months of January, February, and April 2021, and on each release were offered PUs on three occasions (on the day of release (1 DAR);

two days after release (2 DAR); and 6 days after release (6 DAR). Releases were carried out in the early hours of the day, between 7 am and 8 am (UTC-3, Brazilian time). Four hours after release, the PUs, composed of apples, were fixed, as previously described, to the 50 points previously marked. Three PUs were fixed at each point, at a height of 1.5 m from the ground, covering the entire surroundings of the peach tree. After 24 h of field exposure, the PUs were removed and, in the laboratory, the second instar larvae were removed from the fruits by washing in running water and with the aid of a fine mesh sieve. Subsequently, the larvae were placed in plastic containers (80 ml) (one PU per container) containing an artificial diet (20 ml) composed of corn flour to complete the larval development and were kept in an acclimatized room. At the end of the third instar (approximately 4 days), the larvae were transferred to plastic pots (80 ml) containing medium corn flour (1 cm) as a substrate for pupation. Subsequently, the pupae were separated from the flour with the aid of a sieve (coarse mesh) for later emergence (flies and/or parasitoids). Upon reaching emergence, the parasitoids of each PU were removed, counted, and transferred to containers (10 ml) containing 70% alcohol, for identification using a specific key for braconids to determine the species (Canal and Zucchi 2000, Marinho et al. 2018). The pupae that remained intact were dissected for the presence of flies or unemerged parasitoids, in order to determine the actual rate of parasitism.

The parameters evaluated were: number of descendants (ND), calculated by ND = number of emerged parasitoids + number of non-emerged parasitoids (present inside the pupae) and percentage of parasitism (%P), calculated by P (%) = [(number of parasitoid adults emerged)/(total number of adults emerged (parasitoids and flies)] × 100.

Vertical dispersion capacity

Six points were randomly marked (on trees approximately 4 m high) in an olive orchard (0.96 ha). Each tree was considered a repetition and divided into three strata of height in relation to the ground: lower (1.17 m), medium (2.34 m), and higher (3.51 m). At each height, three apples infested with second instar *A. fraterculus* larvae (30 larvae/fruit) were fixed to the outside of the tree, as previously described, totaling nine PUs per tree. After fixing the PUs to the trees, 250 females and 50 males (≈1,562 females/ha), 6–8 days of age, were released between the rows of olive trees (about 3 m away from the plant containing the PUs). After 24 h of field exposure, the PUs were removed and transported to the laboratory for further evaluation of the number of offspring (ND) and percentage of parasitism (%P), following the procedures described above. In this study, 3 parasitoid releases were performed during 3 consecutive weeks (November 2021), maintaining the same density of parasitoids per point. PUs were put out on every day of release.

Statistical analysis

All data obtained were submitted to the Shapiro–Wilk test in order to verify normality. In order to determine the effect of distance on the percentage of parasitism and number of offspring, an analysis of variance was performed using the Generalized Linear Models (GLM) method, using distance as the independent variable and as dependent variables: percentage of parasitism (% P) with a binomial distribution and the ND with a Poisson's distribution. This analysis was carried out for each area independently and for each day after the release of parasitoids (1 DAR, 2 DAR, 6 DAR), using the "glm2" package from the R Studio software (RStudio Team, 2021). Regarding the effect of height (independent variable) on the percentage of parasitism and the ND (dependent variables), the data were initially submitted to the Kruskal–Wallis test. When the statistic obtained a significant P value ($P \leq 0.05$), Dunn's test was applied at 95% probability, using the statistical software Bioestat (version 5.0).

Results

The first evaluation of the orchards, through the distribution of PUs infested with second instar larvae of *A. fraterculus*, indicated the absence of natural parasitism in the areas where the study was carried out. In all PUs evaluated, no parasitoid specimens were recovered. No difference was observed between PUs kept in the laboratory (control) and those taken to the field, regarding the number of surviving insects.

Horizontal and temporal dispersion

It was observed that *D. areolatus* adults parasitized *A. fraterculus* larvae located 68.5 m (area 1) and 64.8 m (area 2) from the central release point (Figs. 1 and 2, respectively). However, the highest parasitism rates [15–45% (area 1); 15–27% (area 2)] and recovered descendants [20–35 (area 1); 15–26 (area 2)] in *A. fraterculus* larvae located in the PUs were recorded at points close to the release site within a radius of up to 25 m (Figs. 3 and 4). In addition, there was a trend toward the dispersion of parasitoids toward the west of the release point (Table 1 and Figs. 3 and 4). Through the mathematical models, an effect of distance ($P \leq 0.05$) on the evaluated variables (percentage of parasitism and the number of parasitoids recovered) was evidenced, demonstrating that the greater the distance from the central release point, the lower the parasitism rates and the number of parasitoids recovered in *A. fraterculus* larvae (Table 2).

Regarding temporal dispersion, there was a significant difference ($P \leq 0.05$) when the parasitism rate and the ND were compared in relation to the PUs set out after the release of the parasitoids in the orchards (Tables 3 and 4). For both study sites (Area 1 and Area 2), the highest numbers of descendants of *D. areolatus* were observed up to 2 DAR [1 DAR (total: A1 = 262; A2 = 443) and 2 DAR (total: A1 = 229; A2 = 587)]. While at 6 DAR, the ND of the parasitoid was significantly ($P \leq 0.05$) lower than the other offers (total: A1 = 6; A2 = 29) (Tables 3 and 4 and Figs. 1 and 2).

Vertical dispersion

Significant differences were observed in relation to the percentage of parasitism ($H = 10.89$; df = 2; $P = 0.0043$) and mean ND ($H = 11.62$; df = 2; $P = 0.0030$) to evaluate the three heights of attachment of the PUs to the trees (Figs. 5 and 6). The parasitoid *D. areolatus* managed to disperse vertically up to the highest attachment height of the PUs (3.51 m). At this point, the average number of offspring (2.5) and the average rate of parasitism (1.25%) were always higher compared to the heights of 1.17 and 2.34 m, whose averages were less than 0.5 insects. and less than 0.2% parasitism (Figs. 5 and 6).

Discussion

The results showed that the parasitoid *D. areolatus* reached distances above 60 m in relation to the CP of release, although the highest numbers of descendants recovered were observed at points closest to the release site, within a radius of up to 25 m. In addition, it has also been shown that the greatest parasitism occurs at a height of 3.51 m. This displacement, both horizontally and vertically, demonstrates that the parasitoid has a good ability to explore and search for the host.

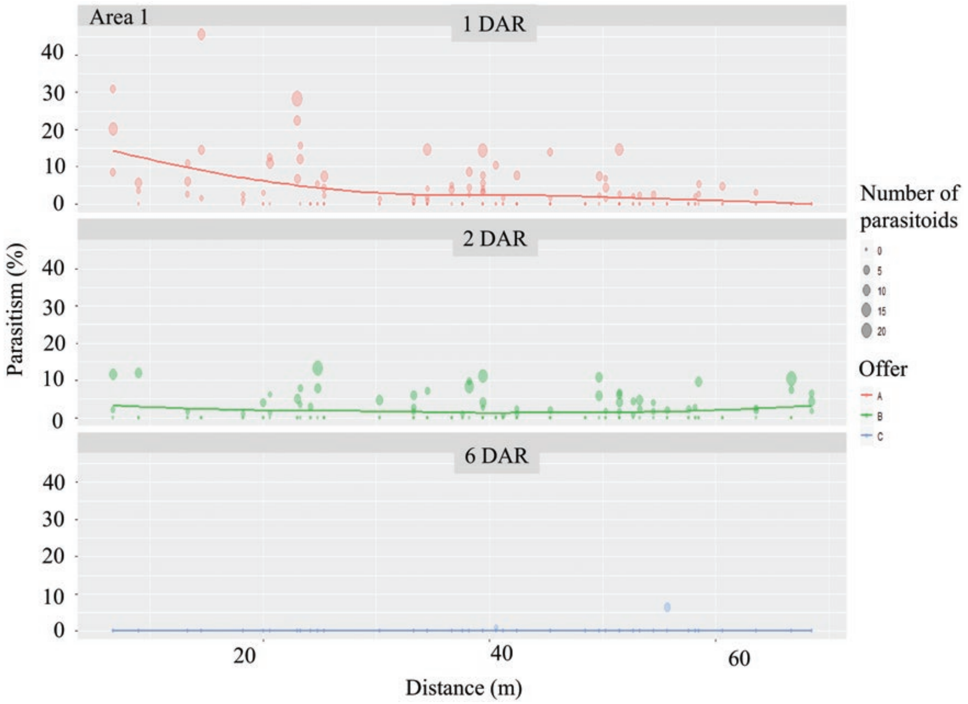


Fig. 1. Number of descendants and percentage of parasitism of *D. areolatus* at different distances and after different times of release in a peach orchard (area 1). (1 DAR) = day of release; (2 DAR) = day after release; and (6 DAR) = 6 days after release.

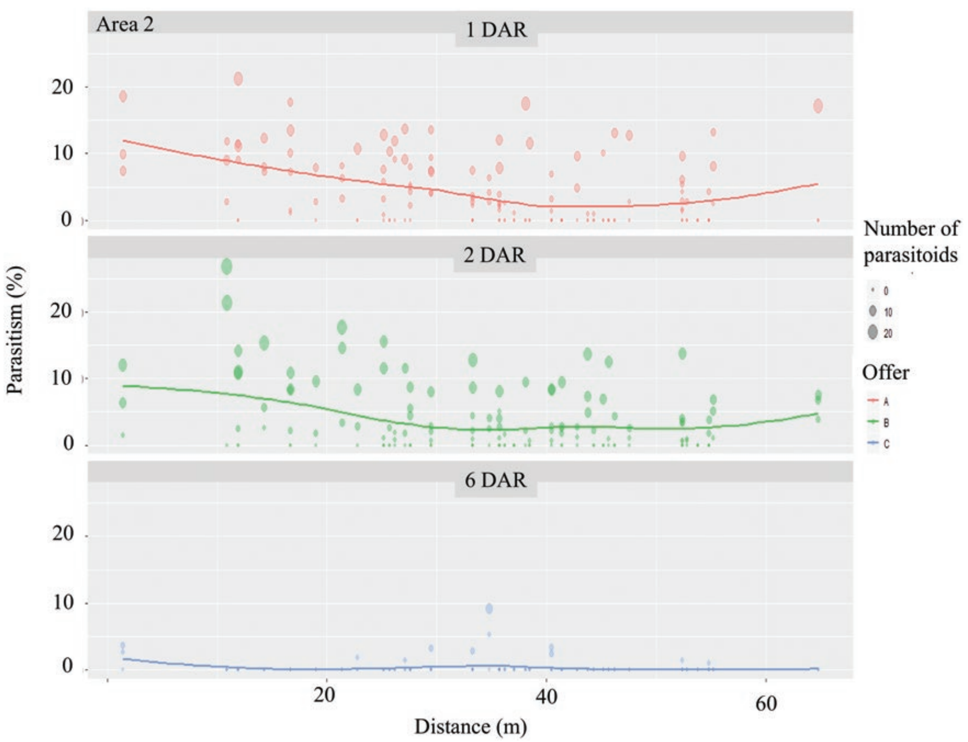


Fig. 2. Number of descendants and percentage of parasitism of *D. areolatus* at different distances and after different times of release in a peach orchard (area 2). (1 DAR) = day of release; (2 DAR)= day after release; and (6 DAR) = 6 days after release.

The decrease in the rate of parasitism with increasing distance from the release point may occur due to the greater energy demand required by the natural enemy in the search for the host (Bueno et al. 2012), since the females direct energy expenditure to ensure their progeny (Jervis et al. 2008). In addition, traveling longer distances increases the chance of natural mortality, both due to biotic and abiotic factors (Geremias and Parra 2014). According to Camargos et al. (2018), due to the small possibility of the parasitoid finding the host and the short lifespan of the parasitoid (average of 16 days) (Nunes et al. 2011), the predator tends

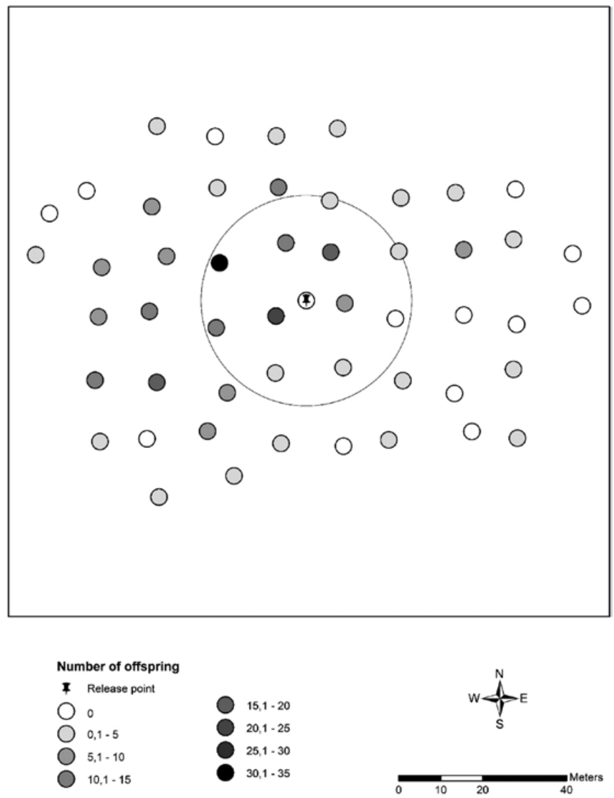


Fig. 3. Number of descendants of *D. areolatus* at different distances from the release point in study area 1 (A1), during the first offer of parasitism units (1 DAR). The circle in the center of the image represents a radius of 25 m.

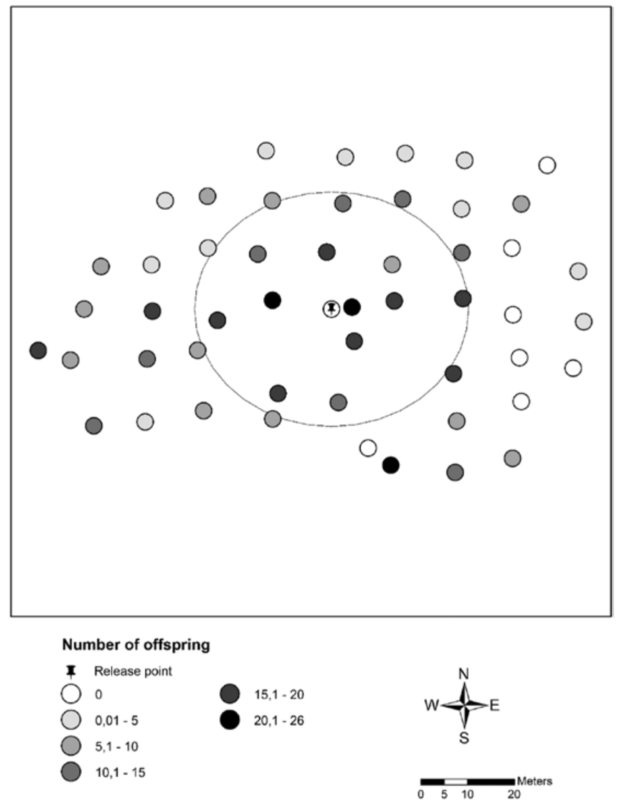


Fig. 4. Number of descendants of *D. areolatus* at different distances from the release point in study area 2 (A2), during the first offer of parasitism units (1 DAR). The circle in the center of the image represents a radius of 25 m.

to lay eggs in a place close to where it was released. The probability of capturing an adult of *D. longicaudata* in a citrus orchard at a distance greater than 40 m was very low (practically null) (Paranhos et al. 2007). However, Harbi et al. (2015) observed that *D. longicaudata* released in citrus orchards was able to parasitize *C. capitata* larvae up to 75 m from the release point. In a guava orchard, *D. longicaudata* caused parasitism in *A. fraterculus* and *C. capitata* larvae located at a distance of 220 m from the release point (Camargos et al. 2018). However, in the same study, the highest rate of parasitism (90% of larvae) of *A. fraterculus* was observed within a radius of up to 22.2 m from the release point, reducing dramatically as the distance increased. Similar behavior was observed in the present study, which was validated through distance estimation models. In studies developed by Zappala et al. (2012), it was evidenced that *D. longicaudata* reached larvae up to 20 m from the release point. However, this fact may have occurred due to the greater distance evaluated in the study. Dispersion data evaluating other Hymenoptera species, such as *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) and mealybug parasitoid *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) demonstrated the ability to move distances of less than 40 m in citrus orchards (Zappala et al. 2012).

The variation in the results of studies on the dispersion of parasitoids in different cultures may be associated with several biotic and abiotic factors that can affect the behavior of insects. Among the biotic factors are the host, density of parasitoids released in the area, adaptability of the species in the location, and the flight capacity of the evaluated parasitoid (Pratissoli et al. 2002). In the case of abiotic factors, local climatic conditions at the time of release, characteristics of the cultivation environments, such as differences in plant architecture and height, planting spacing, and orchard size can directly or indirectly influence horizontal dispersion (displacement) and the search for the host (Pratissoli et al. 2002, Camargos et al. 2018).

In the two horizontal dispersion study areas (Areas 1 and 2), the adults of *D. areolatus* dispersed in all monitored directions, with a trend towards the west of the orchard. This fact can be explained by the greater predominance of the east–west wind observed on the release days. Wind is a regulatory agent of parasitoid dispersion, since passive dispersion is controlled by it (Silva 2007, Harbi et al. 2015). Furthermore, the west direction is in the same orientation as the crop cultivation lines from the central release point. Fewer obstacles (vegetation) in the release line may have favored the displacement of insects in this direction. A fact also observed by Camargos et al. (2018) for *D. longicaudata* after release in guava.

Regarding the temporal dispersion of *D. areolatus*, it was verified that the highest rates of parasitism and of recovered offspring occur in the first days after the release of the parasitoid (2 DAR), and, approximately, after 1 week (6 DAR) these values are significantly reduced. This fact demonstrates that the frequency of release of *D. areolatus* in the field should be performed at weekly intervals, when additional releases are needed, especially in high infestations. A possible explanation for the reduced presence of the insect in the areas after 6 DAR may be associated with the migration of the parasitoids to the native forest around the orchard. The parasitoids are guided to the sites of parasitism through volatile substances released by the fruits, as well as the search for food alternatives (Leyva et al. 1991, Messing and Jang 1992). Therefore, the possibility of searching for food, shelter, and hosts in adjacent areas during the period of absence of PUs (between 3 and 5 DAR) may have triggered the migration of parasitoids out of the study area, thus reducing the permanence rate of insects in the release area.

Table 2. Models for estimating distances on the number of parasitoids and percentage of parasitism of *D. areolatus* released in peach areas

	Area			
	Area 1		Area 2	
Variables	Model[1]	*P* value	Model[1]	*P* value
No. of parasitoids	exp(1.983103 + (−0.040624) × distance)	2.00×10^{-16}	exp(2.026920 + (−0.029291) × distance)	2.00×10^{-16}
% Parasitism	logit-1 (−1.569427 + (−0.054876) × distance)	2.12×10^{-8}	logit-1(−2.064378 + (−0.033218) × distance)	1.31×10^{-15}

[a]Significant model regarding the release date of *D. areolatus* ($P \le 0.05$).

Table 3. Number of descendants and percentage of parasitism of *D. areolatus* in relation to the different offer days of parasitism units observed in Area 1

	No. of parasitoids		
	(1 DAR)	(2 DAR)	(6 DAR)
Intercept	1.7456[a]	1.5267	0.04
P value	2.00×10^{-16}	1.53×10^{-10}	3.15×10^{-15}
Standard error	0.0618	0.0661	0.4082
Diff letter	a	a	b
% Parasitism			
	(1 DAR)	(2 DAR)	(6 DAR)
Intercept	0.0314[a]	0.0167	0.0004
P value	2.00×10^{-16}	2.00×10^{-16}	2.62×10^{-12}
Standard error	0.134	0.1826	1.0598
Diff letter	a	b	c

[a]Intercept values followed by the same letter do not differ significantly from each other ($P \le 0.05$).

Table 4. Number of descendants and percentage of parasitism of *D. areolatus* in relation to the different offer days of parasitism units observed in Area 2

	No. of parasitoids		
	(1 DAR)	(2 DAR)	(6 DAR)
Intercept	2.9533[a]	3.91	0.1933
P value	2.00×10^{-16}	7.75×10^{-6}	2.00×10^{-16}
Standard error	0.0475	0.0413	0.1857
Diff letter	b	a	C
% Parasitism			
	(1 DAR)	(2 DAR)	(6 DAR)
Intercept	0.0418[*]	0.0353	0.0025
P value	2.00×10^{-16}	2.26×10^{-1}	1.02×10^{-11}
Standard error	0.0995	0.1076	0.3947
Diff letter	a	a	B

[a]Intercept values followed by the same letter do not differ significantly from each other ($P \le 0.05$).

Several studies have shown that parasitoids, for example the species *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), a parasite of the cruciferous moth [*Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae)] commonly feed on sugary substances in the field, which suggests that floral resources present in adjacent plants are likely used as food by the parasitoids (Lee et al. 2006). Another hypothesis is non-survival, due to adverse conditions in the environment, including the presence of predators. *D. longicaudata* also showed the highest rates of parasitism in *A. fraterculus* larvae in the first days after release

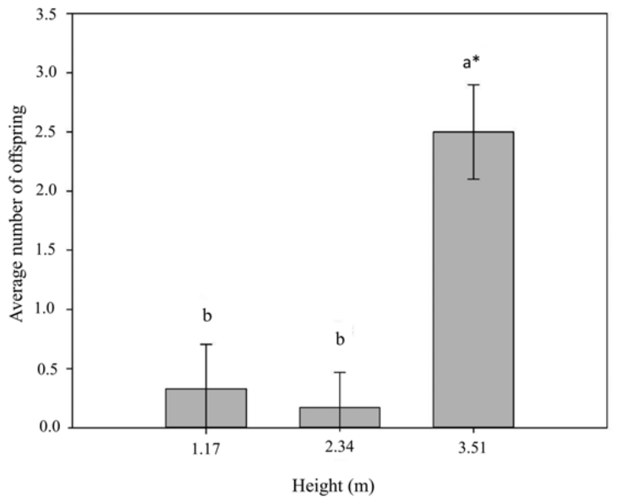


Fig. 5. Mean number of descendants (ND) of *D. areolatus* in different height strata. Lower = 1.17 m; medium = 2.34 m; and upper = 3.51 m. [a]Means followed by the same letter do not differ statistically from each other by Dunn's test at 5% probability.

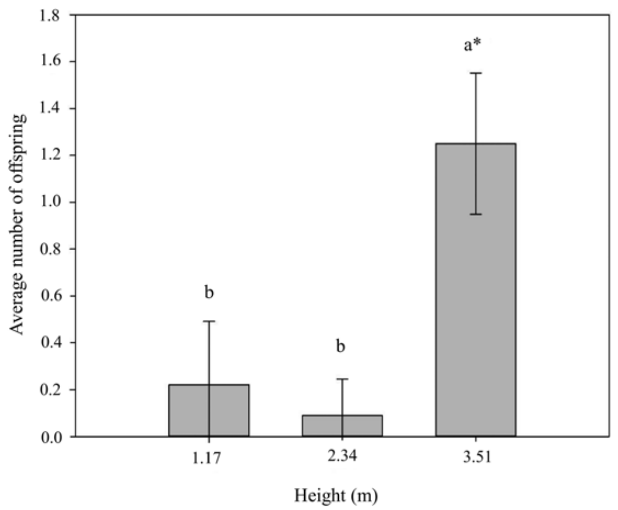


Fig. 6. Percentage of *D. areolatus* parasitism in different height strata. Lower = 1.17 m; medium=2.34 m; and upper = 3.51 m. [a]Means followed by the same letter do not differ statistically from each other by Dunn's test at 5% probability.

in a guava orchard, more specifically in the first 48 h, remaining active until seven days (168 h) after release (Camargos et al. 2018). According to these authors, 44 specimens were recovered after 15 days of release. However, they had a low rate of parasitism (<2.0%). This fact shows that despite the efficiency of parasitism reducing over the days, the species was able to survive during this period (15 days). In the present study, however, no evaluation was performed at 15 days, since at 6 DAR only a low number of *D. areolatus* was recovered.

The parasitoid *D. areolatus* managed to disperse up to the highest height tested (3.51 m). Parasitism was recorded at all heights, with no difference between the lower and middle levels. However, in the upper stratum (3.51 m) the highest average numbers of descendants and percentages of parasitism were found, which demonstrates a good capacity for vertical dispersion. This characteristic is important when considering the choice of parasitoid species because in addition to being efficient as a biological control agent, the insect needs to have adequate dispersion capacity, both horizontally and vertically.

Unlike some species, such as *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae), *D. areolatus* rarely forages for fruits that have fallen on the ground, as they prefer first-instar larvae, which are located in fruits present on trees (Sthul and Sivinski 2015). According to Salles (1995), *A. fraterculus* uniformly infests fruits located between 2 and 10 m in height, which requires the parasitoid to explore the trees to successfully search for the host. When evaluating the parasitoid dispersal behavior of the families Tachinidae, Ichneumonidae, Braconidae, and Chalcidoidea, it was observed that the rate of parasitism in the different hosts (belonging to the families Geometridae, Noctuidae, Tortricidae, Bucculatricidae, Tenthredinidae, and Lymantriidae) decreased from the first height (close to the ground) to the third height and differed significantly between tree species [*Acer campestre* Linnaeus (Sapindales: Sapindaceae), *Carpinus betulus* Linnaeus (Fagales: Betulaceae), *Fraxinus* spp. Linnaeus (Lamiales: Oleaceae), *Quercus cerris* (Fagaceae), *Quercus robur* Linnaeus (Fagales: Fagaceae), *Tilia cordata* Linnaeus (Malvales: Malvaceae), and *Ulmus laevis* Linnaeus (Rosales: Ulmaceae)] (Sigut et al. 2018). In addition, these authors found that the vertical stratification of insect density, diversity, and parasitism rate was more pronounced in taller tree species.

In addition to the flight ability of the parasitoid itself, the vegetation structure of each tree species can influence the vertical displacement of insects (Smith 1988). The mean dispersion height of females of *D. longicaudata* in guava trees was approximately 2.0 m during the period when the tree canopy was at the apex of ripe fruit production (Messing et al. 1994).

The low rates of parasitism of *D. areolatus* observed in the present work may be associated with the methodology used. Due to the need for frequent handling of the PUs and possible loss (mortality) of the experimental units, the number of larvae was overestimated (30 larvae per PU). In the laboratory, the ratio between *A. fraterculus* host larvae and *D. areolatus* females is generally 10 to 1 (10 larvae:1 female).

The results of the present work show that the dispersion of *D. areolatus* under the influence of host stimuli (fruits infested with larvae) is suitable for an augmentative biological control program, since in the presence of hosts, the parasitoids will remain in the area (comparing releases 1 DAR and 2 DAR with 6 DAR) and will allow a release of distinct points within the orchard (perhaps every 50 m—considering the highest action at 25 m (5 points/ha)). This observation is in line with what Hougardy and Mills (2006) comment in their work, that a parasitoid must travel long enough distances to explore the hosts in the target area, but must be limited enough to not easily leave this area. The dispersal behavior of *D. areolatus* up to the highest point demonstrates that it can explore all heights of the plant in the search for the host. These results provide guidelines for future field validation studies of the efficiency of *D. areolatus* in the management of fruit flies.

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Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Supplementary Material S1. Peach orchard with the location of the 50 points of area 1 (0.87 hectares), where the parasitism units were distributed.

Points are represented by the color blue. The central point (CP), in red, corresponds to the site of parasitoid release and the other points correspond to the sites of parasitism evaluation, at different distances (m) from the PC, as follows: A19 = 39.4; A25 = 34.5; B3 = 60.6; B9 = 51.4; B35 = 33.3; B43 = 51.5; C32 = 58.2; D15 = 38.2; E14 = 42.4; E21 = 30.3; E29 = 20.0; E38 = 18.2; E45= 23.3; E58 = 48.5; F25 = 39.4; F37 = 58.5; H 8= 54.5; H40 = 6.7; H48 = 20.6; I27 = 51.5; J24 = 24.2; J31 = 9.0; J58 = 34.5; K2 = 51.5; K9 = 37.6; K42 = 23.0; K60 = 49.7; L1 = 66.7; L35 = 14.5; L41 = 13.3; L58 = 36.7; M7 = 24.8; M49 = 50.3; N17 = 39.4; N36 = 25.4; N43 = 25.4; N51 = 33.3; N60 = 42.4; O5 = 66.7; O13 = 52.7; O29 = 34.5; O82 = 63.6; P22 = 45.4; P47 = 40.6; P55 = 45.4; P74 = 55.7; P80 = 68.5; Q5 = 57.6; Q30 = 41.2; Q54 = 53.3.

Supplementary Material S2. Peach orchard with the location of the 50 points of area 2 (0.64 ha), where the parasitism units were distributed.

Points are represented by the color blue. The CP, in red, corresponds to the site of parasitoid release and the other points correspond to the sites of parasitism evaluation, at different distances (m) from the PC, as follows: A14 = 35.7; A48 = 53.8; B1 = 45.2; B7 = 33.3; B24 = 36.2; B31 = 37.1; B38 = 44.3; C21 = 25.7; C51 = 46.2; D1 = 52.4; D8 = 40.5; D15 = 27. 6; D32 = 22.8; D39 = 26.2; D46

= 35.7; E24 = 16.7; E55 = 41.4; F2 = 52.4; F10 = 38.1; F32 = 11.9; F48 = 29.5; G24 = 25.2; G31 = 11.9; G46 = 19.0; H4 = 64.8; H8 = 52.4; H17 = 39.5; H23 = 27.6; H42 = 1.4; H47 = 14.3; H55 = 27.1; H69 = 54.8; I39 = 10.9; I58 = 40.5; J1 = 55.2; J7 = 43.8; J14 = 33.3; J23 = 16.7; J60 = 52.8; K6 = 25.2; K14 = 21.4; K28 = 29.5; K36 = 41.4; L33= 54.8; M1= 33.3; M13 = 34.8; M21 = 45.7; N2 = 35.7; O5 = 42.8; O12 = 47.6.

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