

PARASITISM OF FRUIT FLIES (TEPHRITIDAE) IN FIELD, AFTER THE RELEASES OF *Diachasmimorpha longicaudata* (ASHMEAD) (HYMENOPTERA: BRACONIDAE) IN RIO GRANDE DO SUL¹

RAFAEL NARCISO MEIRELLES², LUIZA RODRIGUES REDAELLI³,
SIMONE MUNDSTOCK JAHNKE³, CLÁUDIA BERNARDES OURIQUE⁴,
DÂNIA VIEIRA BRANCO OZORIO⁵

ABSTRACT- *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) was introduced in 1994, in Northeastern Brazil, to evaluate its use in biological control programs of fruit flies. However, the effects of this specie on parasitism rates on the population of native parasitoids and fruit flies in Southern Brazil conditions are unknown. The objective of this study was to evaluate the impact of releases of *D. longicaudata* on the parasitism rates on loquats, peach trees, strawberry guava trees, and persimmons in an experimental area in the city of Eldorado do Sul, Rio Grande do Sul, in southern Brazil. In the first year of the study, fruits were collected and stored in the laboratory to obtain pupae. The flies or parasitoids that emerged were counted and identified. In the second year, adults of *D. longicaudata* were reared in the laboratory on *Anastrepha fraterculus* (Wied.) and larvae were released in the field (1.700 insects/ha) at each harvest time and the parasitism was evaluated in the same manner as in the first year. In the third year, the procedure was the same as the first year, without any releases. A significant increase in the rates of parasitism was recorded in the second year except in the persimmon, in which no parasitoid was recovered in any year. In the second year the number of emerged fruit flies was also lower. In the third year, no *D. longicaudata* were recorded and parasitism rates of parasitism were statistically similar to those found in the first year. The native parasitoids collected were *Aganaspis pelleranoi* (Brèthes), *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti) and *Utetes anastrephae* (Viereck). These species were found in at least one kind of fruit even in the years after the introduction of the exotic parasitoid, indicating that it was not harmful to these species. We conclude that *D. longicaudata* can help to reduce the population of fruit flies in Eldorado do Sul region, in Rio Grande do Sul. **Index terms** – biological control, *Anastrepha fraterculus*, *Aganaspis pelleranoi*, *Doryctobracon*, *Utetes anastrephae*.

PARASITISMO DE MOSCASA-DAS-FRUTAS (TEPHRITIDAE) EM CAMPO, APÓS LIBERAÇÕES DE *Diachasmimorpha longicaudata* (ASHMEAD) (HYMENOPTERA: BRACONIDAE) NO RIO GRANDE DO SUL

RESUMO- *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) foi introduzido Nordeste do Brasil em 1994, para avaliar a possibilidade de uso em um programa de controle biológico de moscas-das-frutas. No entanto, os efeitos desta espécie nos índices de parasitismo e nas populações de parasitoides nativos nas condições do Sul do Brasil, são desconhecidos. O objetivo deste trabalho foi avaliar o impacto das liberações de *D. longicaudata* no parasitismo de moscas-das-frutas em nêspersas, pêssegos, araçás e caquis, no município de Eldorado do Sul, RS, Brasil. No primeiro ano de trabalho, frutos foram coletados e armazenados em laboratório para obtenção de pupários. As moscas ou parasitoides que emergiram foram contabilizados e identificados. No segundo ano, foram realizadas liberações de adultos de *D. longicaudata* provenientes de laboratório, criados em *Anastrepha fraterculus* (Wied.). Os parasitoides foram liberados nos períodos de safras, na densidade de aproximadamente 1.700 parasitoides/ha, e foram realizadas amostragens de frutos da mesma forma que no primeiro ano. No terceiro ano, o procedimento realizado foi o mesmo do primeiro, sem liberações. Um aumento significativo foi registrado nos índices de parasitismo no segundo ano, nas frutíferas amostradas, com exceção do caqui, no qual nenhum parasitoide foi recuperado, em nenhum ano. No segundo ano o número de moscas emergidas também foi menor. No terceiro ano de coletas, nenhuma *D. longicaudata* foi registrada e os índices de parasitismo foram estatisticamente iguais aos verificados no primeiro ano. Os parasitoides nativos coletados foram: *Aganaspis pelleranoi* (Brèthes), *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti) e *Utetes anastrephae* (Viereck), os quais foram constatados em pelo menos uma das espécies de frutífera e mesmo nos anos após a introdução do parasitoide exótico, indicando que este não foi deletério para estas espécies. Conclui-se que *D. longicaudata* pode auxiliar a reduzir populações de moscas-das-frutas na região de Eldorado do Sul, RS.

Termos para indexação – controle biológico, *Anastrepha fraterculus*, *Aganaspis pelleranoi*, *Doryctobracon* spp., *Utetes anastrephae*.

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²Dr. em Fitotecnia -UFRGS, Teresina, PI. CEP: 64003-715. E-mails: rafael.meirelles@ufrgs.br; mundstock.jahnke@ufrgs.br

³Dr^a, Prof^a, Programa de Pós-Graduação em Fitotecnia, Universidade Federal do Rio Grande do Sul. E-mail: luredael@ufrgs.br

⁴Estudante de mestrado, Prog de Pós-Graduação em Fitotecnia, Universidade Federal do Rio Grande do Sul. E-mail: claudiaourique@hotmail.com

⁵Estudante de Agronomia, Universidade Federal do Rio Grande do Sul. E-mail: dania.ozorio@gmail.com

INTRODUCTION

Fruit flies are the most important pests in the fruticulture world, considering the direct damage done to fruit production and the high cost for control and monitoring. In Rio Grande do Sul (RS), Brazil, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) and the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae) are the main species that cause damage to commercial fruit trees, the first specie is the most important (KOVALESKI et al., 2000).

The control of fruit flies is normally done, with chemical products applied as toxic bait or in total area. However, forbidding the use of certain active ingredients and the requirement to reduce chemical residues in fruits allowed by importing countries (OLIVEIRA; LUCCHESI, 2013), give farmers few alternatives for controlling these pests.

In this context, biological control with parasitoids is an important tool to be utilized with other methods, such as the male insect sterile technique. For example, the aim for a decrease in the population of fruit flies. Nowadays, in Rio Grande do Sul, there are eight native species of hymenoptera parasitoids of fruit flies registered, distributed in four families. Including, *Trichopria anastrephae* Lima (Diapriidae), *Pachycrepoideus vindemmia* (Rondani) (Pteromalidae), *Odontosema albinerve* Kieffer and *Aganaspis pelleranoi* (Brèthes) (Figitidae), *Opius bellus* Gahan, *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti) and *Utetes anastrephae* (Viereck) (Braconidae) (GATTELLI, 2006; CRUZ et al., 2011; NUNES et al., 2012; PEREIRA-RÊGO et al., 2013). However, the researchers of these inventories observed an index of parasitism in fruit flies generally below 10%. Gattelli (2006) reported that the lowest parasitism rate was 0.5% (*D. areolatus* and *D. brasiliensis*), verified in the citrus var. "céu" (*Citrus sinensis* L.) and Nunes et al. (2012) observed more than 31.5% of the parasitism (*D. areolatus*, *O. bellus* and *A. pelleranoi*) in wild cherries (*Eugenia involucrata* DC.).

In 1994, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), was introduced in Brazil in order to implement a biological control program for fruit flies (CARVALHO, 2004), due to the success obtained in other countries like United States, Guatemala and Mexico (MONTROYA et al., 2013; VARGAS et al., 2013). After a period in the Quarantine Laboratory Costa Lima in Jaguariúna, São Paulo. In the following years, releases of *D. longicaudata* were made in the region of Lower-

middle São Francisco River (Pernambuco/Bahia) in the north of Minas Gerais and São Paulo, in which the easy adaptation of this organism to different hosts and environments was confirmed, without compromising the native species (CARVALHO, 2004; ALVARENGA et al., 2005).

In this way, the absence of information about survival and the potential of parasitism of *D. longicaudata* under field conditions in southern Brazil, helped to motivate the present study aimed at evaluating the effect of releases of this parasitoid into populations of fruit flies and native parasitoids in loquat, peach, strawberry guava and persimmon trees in the city of Eldorado do Sul, RS, Brazil.

MATERIALS AND METHODS

Rearing of insects

The rearing of the insects used in these studies were obtained in the Laboratório de Biologia Ecologia e Controle Biológico de Insetos (BIOECOLAB) of Universidade Federal do Rio Grande do Sul (UFRGS), in an air-conditioned room (25 ± 2 °C; with a relative humidity of $65 \pm 10\%$ (RU); 14 hours of photoperiod) and in air-conditioned clamber (25 ± 2 °C; $65 \pm 10\%$ RU; without photophase).

Diachasmimorpha longicaudata

The rearing was kept according to the methodology described by Meirelles (2011). The adults were reared in wooden framed cages (60 cm x 25 cm x 50 cm) (400 couples by cage), covered by voile tissue and were given as much water and food (honey, water and agar) as they wanted.

The parasitoid cages received a unit of parasitism daily, with about 400 larvae of third instar of *A. fraterculus* (approximately nine days old) for half an hour. Parasitism units were prepared utilizing a Gerbox with an acrylic lid with a circular opening protected with voile bonded on the outside of the cover. The larvae of *A. fraterculus* were placed inside the opening under the voile fabric, and the lid was pressed against the bottom of a Gerbox® box, preventing the escape of the larvae, but leaving the parasitoid exposed. After exposure, the larvae were placed in a Gerbox® box with sand in the bottom and maintained in air-conditioned clamber until the emergence of parasitoids.

As soon as the parastoids emerged they could be used for farming or kept in cages until being released in the field.

Anastrepha fraterculus

Adults were kept in wooden cages (45 cm x 30 cm x 30 cm) covered with voile. Water was provided by capillaries through the tissue strips Spontex Resist® with the base submerged in a plastic tube. The adult feed was composed of granulated sugar, beer yeast and yeast hydrolyzate Biorigin® (in the ratio 3: 1: 1) provided in petri dishes (Ø 5 cm).

The oviposition substrate of the fruit flies took the form of a bag of 30 cm x 30 cm, made with blue voile coated with silicone and on one end had an opening with a cover, through which it was possible put in water. The substrate was on the top of the cage, and the fruit flies were able of oviposit through the tissue. The eggs were immersed in water, which was replaced daily.

The eggs were placed above the larvae food, as described by Meirelles (2011), distributed in polystyrene trays (15 cm x 15 cm x 2 cm), wrapped in newspaper and placed in a climate chamber for seven days. After this period, the trays were taken to a climatic chamber and deposited in plastic containers (50 cm x 30 cm x 5 cm) on a layer of sterilised sand and covered with voile. Some larvae were removed to serve as hosts for the *D. longicaudata* the others spent two more days in sand and were used for maintenance of the fruit flies creation.

Field Experiment

The field experiments were carried out in the Estação Experimental Agronômica (EEA) of UFRGS (30°06'16.54"S e 51°39'58.40"O), in Eldorado do Sul, RS. Releases of *D. longicaudata* were made in loquat (0.15 ha), peach (1.17 ha) and persimmon orchards (0.74 ha). These orchards are collections containing more than one variety and/or cultivar of each fruit tree. The study also used an area of 0.3 ha of strawberry guava trees, naturally present in the EEA and which were not planted with the intention of forming an orchard.

The strawberry guava trees area is located north of the study site. The persimmon orchard, is located to the east, 20 meters away from the strawberry guava trees. The loquat headquarters was 100 meters south of persimmons. The peach trees were 20 meters east of the loquat. Between the southern most tip of the peach orchard and the northern end of the strawberry guava trees orchard was 400 meters away.

The areas of the loquats, peaches and persimmons were mowed as needed in times of pruning, harvest and hand thinning the fruit. No herbicides were used. Strawberry guava trees do not require any management or phytosanitary treatment.

The peach orchard, established in 1999, received a *Grapholita molesta* control (Busck) (Lepidoptera: Tortricidae) with phosmet (500 Imidan WP®) once per season and the fruit flies with toxic bait [50 g dimethoate (Nortox®500 EC) + 100 L water + 5 kilograms are sugar] sprayed weekly on orchard trees. Applications started from the second half of September.

The loquat orchard, established in 2003, received only the application of the toxic bait for fruit-flies weekly, from September to the end of the season, in order to prevent the insects from damaging the peaches. The persimmon plantation, installed in 1994, received only spray applications of sulfur during the vegetative growth stage of the crop.

In the first year of experiment (2011/2012), which was considered as a pre-release, ripe fruit of all species was collected weekly on the ground under the canopy of trees, with the exception of the loquats which were picked mature, directly from the trees. The fruits were taken to the laboratory, counted, weighed, washed and packed in plastic trays with vermiculite in the background, capped with voile which stored at room temperature. After seven days, the vermiculite was screened to make the separation of pupae, which were kept in plastic pots (140 ml) until emergence. The fruits were placed back in the trays and the procedure was repeated weekly until no puparia was collected. Insects that emerged were killed and identified with the aid of keys (ZUCCHI, 2000) and the reference collection BIOECOLAB. Confirmations of the identifications of parasitoids were made by Dr. Miguel Francisco de Souza Filho and Valmir Antonio Costa, of the Biological Institute in São Paulo.

In the second year (2012/2013 harvest), approximately 1,700 parasitoids /ha at a ratio of one male to one female, were released every two weeks (Table 1). The releases began when the loquats reached the semi-mature stage (first half of August) and followed until the end of Persimmon seasonal harvest (second half of March), totaling 10 releases. No releases in the off seasonal harvest were held.

The releases were carried out in the morning, near the tops of the plants which had fruit. The cages with adults were held by hand, with the door open and walked around the area until the all parasitoids had escaped. In the second year, in addition to the releases, fruit samples were taken, repeating the methodology employed in the year of pre-release.

In the third year (2013/2014 harvest), considered as a post-release, releases were not made, however, fruit was collected from the four fruit trees following the same method as the pre-release year

of the procedure.

Data Analysis

The rates of parasitism and infestations were calculated for three years and for the four fruit trees. The infection was calculated by dividing the number of pupae obtained by the number of fruits sampled. The apparent parasitism index, or successful parasitism, took into account only the emerged insects and was calculated as follows: apparent parasitism = $[\text{N}^{\circ} \text{ parasitoids} / (\text{N}^{\circ} \text{ parasitoids} + \text{N}^{\circ} \text{ flies})] \times 100$.

The species of flies and parasitoids of the four species of fruit were recorded.

Statistical analysis was used BioEstat 5.0® program. infestation of data (number of pupae/kg of fruit) and rate of parasitism, before and after the releases (increase in parasitism) were compared using the chi-square test of heterogeneity ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Anastrepha fraterculus was the most collected specie of fruit fly. This predominance in the majority of the fruit trees, as this specie is native (KOVALESKI et al., 2000) adapted in the ambient environment (Table 2). The result corroborates to the results of Gatteli et al. (2008), who collected strawberry guavas, guavas, surinam cherry, feijoas and oranges in the Cai Valle (RS), and of Nunes et al. (2012), who collected strawberry guava, surinam cherries, guavas, feijoas, *Eugenia pyriformis*, wild cherries, loquats, peaches and persimmons in Pelotas (RS), both registered the same specie of fly as more abundant.

The lowers infestations were observed in persimmon, in which *Ceratitis capitata* was the predominant frugivorous specie (Table 2), suggesting that *A. fraterculus* is less adapted to this host. It is worth noting that, despite having occurred, these fruit infestations were low, lower than observed in peaches (< 8 pupae/kg of fruit). Schliserman et al. (2003), in Argentina and McQuate et al. (2005), in Hawaii, registered the predominance of *C. capitata* in persimmons. According to Zanardi et al. (2011), the persimmon is a less adequate host, when compared with peaches. According to the authors, the infestations can be influenced by the insect physiology, the environment, by farming practices and non-nutritional substances present in the fruit, such as tannins, acids, carotenoids and anthocyanins. According to the authors, the infestations can be influenced by insect physiology, by the ambient environment, by farming practices

and not by nutritional substances present in the fruit, such as tannins, acids, carotenoids and anthocyanins. Another hypothesis which could explain the low infestation in this fruit tree is the fact that the persimmon fruits appear between the end of March and the beginning of May. In this period of time the persimmon competes temporarily as host for *A. fraterculus* with other native fruit trees which evolved together with in the same ambient environment with the the South American fruit fly, strawberry guavas which were also present in the area of the research. Thus, it is possible that flies prefer to oviposit in a more adequate host for the development of its offspring

In loquats, peaches and guavas, specimens of the Lonchaeidae family were collected. There is divergence as to the capability of the insects of this family to cause harm to the fruits. In fact, they are frugivorous flies and feed on pulp of fruits, like the tephritids, and some researchers authors to the advocate in the idea that some species can become primary pests, causing damage to healthy fruits (STRIKIS; PRADO, 2005). However, the results of the present study do not allow us to state that lonchaeids are potential pests for commercial fruit production in RS, as flies of this family were collected in fruits that were infested with Tephritidae specimens. Its occurrence can only be registered in the collected fruits.

There was no significant difference in the infestation intensity (pupae/kg of fruits) in three years for peaches ($\chi^2 = 3,381$, gl = 2, p = 0,1844), strawberry guavas ($\chi^2 = 2,094$, gl = 2, p = 0,351) and persimmon ($\chi^2 = 0,227$, gl = 2, p = 0,8929). In loquats, however, the infestation was statistically lower in the year following the release ($\chi^2 = 6,848$, gl = 2, p = 0,0326) (Table 2).

The small quantity of pupae collected in peaches, when compared with loquat and strawberry guavas, can be related to toxic bait sprayed weekly, aiming to reduce the quantity of adults in the orchard. Thus, by lowering the adult population, the quantity of larvae in the interior of the fruit can be reduced as well. Härter et al. (2010), assessing peach orchards in the region of Pelotas (RS), verified that the populations of fruit flies were significantly lower in orchards which had toxic bait applied hidrolased protein + malathion) than in orchards which received insecticides in total area.

Despite the effect of the toxic bait, variations in the populations of fruit flies may occur due to factors such as microclimate and the ambient structure in the same region. A study done in two counties in the north of Argentina, Schliserman et al.

(2010) verified quite different infestations in peach orchards not treated with insecticides, there was 8,55 pupae/kg of fruit in Laharrague and 45,33 pupae/kg of fruit in Montecarlo, nearby cities (distant about 16 km) in the province of Misiones.

No parasitism in fruits of persimmon were recorded, which could have occurred because the host was not recognized by parasitoids or due to the low infestation observed in this fruit tree.

The highest indexes of parasitism in the years before and after release were registered in strawberry guava trees. In this fruit tree the highest richness and abundance of native parasitoids were observed, besides being the only place where *D. brasiliensis* was observed (Table 3). An important aspect to be considered is the fact that the native parasitoids and the fruit trees of the Myrtaceae family evolved together in the same ambient environment (GUIMARÃES et al., 2004). It is likely that the reach and parasitism abilities of these native organisms are not yet well adapted to the exotic fruit trees.

These results corroborate to the ones obtained by Nunes et al. (2012), who observed higher indexes of parasitism, abundance and richness of native parasitoids in species of Myrtaceae, including the collecting of strawberry guavas, compared to exotic fruit trees. Schliserman et al. (2010) highlights that the species of this family have a great importance in the north east of Argentina to the multiplication and maintenance of the populations of parasitoids of fruit flies.

Given that the species of native parasitoids registered in the previous year's release were verified in the year of the release and the following the release, thus, it is considered there was no deletion. This indicates that the introduction of *D. longicaudata* in the location of the study did not harm the other native parasitoids in that environment, ratifying the results obtained in Bahia, Brazil (CARVALHO, 2004; BOMFIM et al., 2010) and Minas Gerais, Brazil (ALVARENGA et al., 2005), after the releases of *D. longicaudata*.

The native species, which compete for the same resources, in this case the fruit fly larvae, specialize themselves so they can use their hosts in different locations of the plant, and explore different phases of the cycle of their hosts (SIVINSKI et al., 1998). Thus, it is possible that the introduction of *D. longicaudata* caused no injury to the populations of native parasitoids as each specie has a specific niche or a competition strategy. Sivinski et al. (1998) reported that the parasitoids respond to many factors in the environment according to the intrinsic characteristics of each specie, what can induce

the interspecific competition. According to these authors, aspects of the morphology of the parasitoid and characteristics of the fruit are responsible for the strategy of parasitism and for the niche of each specie.

The species registered in this study, *D. areolatus*, *U. anastrephae* and *A. pelleranoi*, have characteristics that give them advantages in the parasitism, or possibilities to compete with one another. According to Sivinski et al. (2000), *A. pelleranoi* has the ability to enter their hosts in openings or cracks in the fruits in the soil. Thus, this parasitoid can search larvae inside the fruits, where the larvae have entered deeper into the fruit, fleeing the parasitism of other species. *Utetes anastrephae*, has in its larval phase, stronger and bigger jaws than the other parasitoids and a robust body, it is capable of competing and eliminating the larvae of other species, such as *D. areolatus*, inside the body of its host (ALUJA et al., 2013). Regarding *D. areolatus*, it usually parasitizes larvae of third instar. However, in situations of competition, it can also search hosts of lower instars and parasitise in periods of time of colder temperatures, what gives it advantages when compared to other species, including in relation to *D. longicaudata* (SIVINSKI et al., 1998). Another advantage that *D. areolatus* and *U. anastrephae* may have when competing with *D. longicaudata*, is the fact that these native parasitoids can search hosts in unripe fruits (CARVALHO, 2004).

The indexes of parasitism were higher in the release year, in loquats ($\chi^2 = 138,307$, $gl = 2$, $p < 0,0001$), peaches ($\chi^2 = 40,278$, $gl = 2$, $p < 0,0001$) and strawberries, guavas ($\chi^2 = 293,044$, $gl = 2$, $p < 0,0001$), due to the presence of *D. longicaudata* (Tables 2 and 3). The number individuals of this specie collected in the years that the releases were made, show that the participation of this insect was a determinant for the increase in the indexes of parasitism (Table 3) and, in general, *D. longicaudata* was responsible for more than 88% of parasitism of fruit flies. The increase in the indexes of parasitism or reduction of the population of fruit flies were verified in many places where *D. longicaudata* was introduced, such as Florida (BARANOWSKI et al., 1993) and Mexico (MONTROYA; CANCINO, 2004).

The time elapsed between the releases and the recovery of individuals of *D. longicaudata* fluctuated depending on the fruit tree (Table 1). When the fruit harvests are done shortly after the releases, the potential of parasitism of this specie may be underestimated. According to Carvalho (2004), the harvest of fruits after the releases must occur after a sufficient period of time of exposition of the fruits to

the exotic parasitoids. The author states that, harvests of fruits from the trees as well as those that have recently fallen to the ground may underestimate the parasitism.

In loquats, the first capture of parasitoids occurred one week after the first release (Table 1), and 15 days after the introduction, 150 specimens were recovered.

The last sampling of loquats in the year of the liberation, on September 25 of 2012 no individuals of *D. longicaudata* were detected. On the first peach harvest this parasitoid was not observed as well. It is important to observe that the lowest temperatures between 20 and 28 of September of 2012 were below 10°C (EEA-UFRGS, 2014). In the laboratory, Meirelles (2011) verified that the base temperature for the development of *D. longicaudata* is 12,5°C, when created with *A. fraterculus*. Thus, it is possible that the population of the exotic parasitoid could be hampered by the low temperatures registered in EEA in Colorado do Sul, during the period of study.

In peaches, only in the fourth collection after two releases, only 14 individuals of *D. longicaudata* were found. Between the first release and the first record, 7 days had passed during which the temperatures were below 12,5°C, an occurrence of rain, a fact that probably could have hampered the survival and activity of the parasitoid in the environment. The scarcity of hosts in that period is another explanation to what was observed, since no native fruit tree was identified in the perimeter of the peach orchard. However, after the first peach harvest, specimens of *D. longicaudata* were found in all the following harvests.

According to Montoya and Cancino (2004), the parasitoids releases, even when the pest population is low, are important to the maintenance of the agent of biological control. The continuous releases should increase the chances of encounters between the *D. longicaudata* and its hosts. Thus, the results show that to increase the participation of the parasitoid, in the parasitism indexes and decrease the population of fruit flies, the releases must be more frequent and continuous than the ones conducted in this study. To keep a high parasitoid population in field Montoya and Cancino (2004) stated that, the releases should not cease from one crop to another, a constant activity throughout the biological control program.

In strawberry guavas, before the first release of *D. longicaudata* in this fruit tree, some individuals were already found (Table 1). This may imply that this parasitoid was capable of persisting in this environment, after being released in other crops

and had migrated to find the host in other fruit trees, considering that between the last peach harvest and the first of strawberry guava harvest 60 days had passed.

The complexity and heterogeneity of the environment, where the biological control of fruit flies was to be conducted, must be considered to calculate the quantity and frequency of the releases of *D. longicaudata*. The area of this study is situated on the along properties, most of which are of small size (below 5 ha), with pasture areas and grain crops, interspersed by small areas of native vegetation. Thus, these aspects may be propitious to the exotic parasitoid, by providing shelter and hosts between seasonal harvests.

An important aspect to be noted is that *D. longicaudata* is able to identify and parasitize the larvae of fruit flies in native and exotic hosts. This parasitoid is attracted to the oviposition sites by volatile compounds originating from the decomposition process, and substances produced by fungi that may grow on the fruits during their decay (GREANY et al., 1977). In the fruit, visual clues, such as oviposition, marks or surfaces marked by the development of the fly larvae, help to identify the region where the host is (STHUL et al., 2011). To specify the place where the eggs are laid, the female of this specie uses vibrations caused by feeding larva inside the fruit (LAWRENCE, 1981). This indicates that *D. longicaudata* can adapt to different kinds of fruit since it uses generic tracks, common to decomposing fruit, marks of oviposition activity or development of tephritids in general.

It is very important to identify the success of this organism in parasitizing directly in the field, as the results obtained in laboratory tests cannot be observed in the field. According to Leyva et al. (1991), the preferred result of *D. longicaudata* for oviposition in a given test under controlled conditions, may not be the favorite in the field, where many variables interact (volatile, shell thickness, prior experience, location in the plant, color and fruit size, for example), making this a more complex choice.

No occurrence of *D. longicaudata* post-release year was recorded, indicating that this species could not resist the winter. During June, July and August, in the Rio Grande do Sul, it is common that the average daily temperatures (EEA-UFRGS, 2014) are below the lower temperature threshold of this insect, which is 12.5 °C to *A. fraterculus* as host and 7.83 °C with *C. capitata* as a host (Meirelles, 2011), which would prevent the development of this parasitoid. Furthermore, the quantity of fruits

available at this time is reduced, which also hinders the meeting attendants.

Despite that, the parasitoid did not survive over the winter, its effect during the period when it was in the field with favorable temperatures could be one of the factors that reduced the population of fruit flies in loquats, as the significantly lower log in the post-release year (Table 2). This may indicate

that, with releases continuing in good times, *D. longicaudata* can be an important biological control agent in Rio Grande do Sul. However, it is important that there will be releases in other regions of the state, to observe the performance of this organism in the control of fruit flies and the feasibility of implementing a biological control program in southern Brazil.

TABLE 1- Dates of first and last releases and the first and last captures, number and average interval between releases *Diachasmimorpha longicaudata* of the Estação Experimental Agronômica, UFRGS, Eldorado do Sul, RS, Brazil (30°06'16.54"S e 51°39'58.40"O) (2012/2013).

| Fruits | First release | N° of releases | Interval Between releases (days) | Last release | First capture | Capture |
|------------------|---------------|----------------|----------------------------------|--------------|---------------|------------|
| Loquat | 07/08/2012 | 3 | 16,3 | 25/09/2012 | 14/08/2012 | 06/09/2012 |
| Peach | 19/10/2012 | 3 | 13,6 | 29/11/2012 | 19/11/2012 | 06/12/2012 |
| Strawberry guava | 12/02/2013 | 2 | 15 | 27/02/2013 | 07/02/2013 | 27/02/2013 |
| Persimmon | 04/03/2013 | 2 | 16 | 20/03/2013 | - | - |

TABLE 2- Number of fruits, fruit weight, number of pupae, infestation (pupae / kg of fruit), number of flies, viability of flies, parasitism rate and species of parasitoids in loquats, peaches, Strawberry guavas and persimmons, in pre-release years (Year 1) (2011/2012), release (Year 2) (2012/2013) and post-release (Year 3) (2013/2014) of *Diachasmimorpha longicaudata* the Estação Experimental Agronômica (30 ° 06'16.54 "S and 51 ° 39'58.40 "W), in Eldorado do Sul, RS, Brazil.

| | Loquat | | | Peach | | | Strawberry guava | | | Persimmon | | |
|---|---------|-------|-------|-------------------|--------|-------|---------------------|--------|-------|--------------------|-------|-------|
| | Years | | | Years | | | Years | | | Years | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Fruits | 893 | 1971 | 1505 | 692 | 762 | 555 | 884 | 1365 | 1433 | 443 | 393 | 332 |
| Weight (kg) | 22,17 | 41,93 | 28,11 | 48,81 | 66,15 | 32,95 | 4,19 | 9,14 | 7,79 | 51,58 | 38,73 | 35,89 |
| Puparia | 2024 | 3762 | 1424 | 389 | 484 | 167 | 1488 | 7747 | 5159 | 18 | 15 | 15 |
| Puparia/kg | 91,2 A* | 89,7A | 50,7B | 7,9 ^{NS} | 7,3 | 5,1 | 355,5 ^{NS} | 847,7 | 662,7 | 0,34 ^{NS} | 0,38 | 0,41 |
| Flies | 1192 | 2240 | 962 | 261 | 235 | 94 | 751 | 5323 | 2874 | 5 | 6 | 5 |
| <i>Anastrepha fraterculus</i> | 1187 | 2163 | 939 | 241 | 205 | 89 | 750 | 5322 | 2872 | 0 | 1 | 0 |
| <i>Ceratitis capitata</i> | 0 | 30 | 20 | 17 | 17 | 2 | 0 | 0 | 0 | 5 | 5 | 5 |
| Lonchaeidae | 5 | 47 | 3 | 3 | 13 | 3 | 1 | 1 | 2 | 0 | 0 | 0 |
| Viability of the flies (%) | 58,9 | 59,5 | 67,5 | 67,1 | 48,5 | 56,1 | 50,4 | 68,7 | 55,6 | 27,7 | 40 | 33,3 |
| Parasitoids Rate of the parasitism (%) | 8 | 221 | 12 | 4 | 50 | 3 | 18 | 969 | 107 | 0 | 0 | 0 |
| | 0,6 A | 9,2 B | 1,2A | 1,5 A | 21,2 B | 3,2A | 2,4 A | 15,4 B | 3,6A | 0 | 0 | 0 |

* Different capital letters differ in the same line, between years in the same specie of fruit, by the chi-square test ($\alpha = 0.05$).

^{NS} Not significant difference.

TABLE 3 - Total number of parasitoids, parasitism and relative frequency (%) of parasitoids in three species of fruit in the years pre-release (2011/2012) (Year 1), release (2012/2013) (Year 2) and post-release (Year 3) *Diachasmimorpha longicaudata* in the Estação Experimental Agronômica (30 ° 06' 16.54 "S and 51 ° 39' 58.40" W), in Eldorado do Sul, RS, Brazil.

| | Loquat | | | Peach | | | Strawberry guava | | |
|---|--------|------|-----|-------|------|------|------------------|------|------|
| | Years | | | Years | | | Years | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Total of Parasitoids | 8 | 221 | 12 | 4 | 50 | 3 | 18 | 969 | 107 |
| Rate of parasitism (%) | 0,6 | 9,2 | 1,2 | 1,5 | 21,2 | 3,2 | 2,4 | 15,4 | 3,6 |
| Frequency of each specie (%) (%) | | | | | | | | | |
| <i>Diachasmimorpha longicaudata</i> | 0 | 99,1 | 0 | 0 | 94 | 0 | 0 | 88,5 | 0,0 |
| <i>Doryctobracon areolatus</i> | 100 | 0,9 | 50 | 0 | 0 | 66,6 | 11,1 | 1,5 | 31,8 |
| <i>Doryctobracon brasiliensis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 5,6 | 0 | 1,9 |
| <i>Utetes anastrephae</i> | 0 | 0 | 50 | 0 | 0 | 33,3 | 27,8 | 9,9 | 31,8 |
| <i>Aganaspis pelleranoi</i> | 0 | 0 | 0 | 100 | 6 | 0 | 55,6 | 0 | 34,6 |

CONCLUSION

Based on the results obtained, it is considered that *D. longicaudata* is able to complete its cycle in the environmental conditions found in Eldorado do Sul during the months of August to May and contributed positively to the increase in parasitism of fruit flies registered in loquats, peaches and strawberry guavas, without negatively impacting the native populations of parasitoids.

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