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Population dynamics of *Palpita forficifera* Munroe, 1959 (Lepidoptera: Crambidae) and associated parasitoids in olive orchards

Dinâmica populacional de *Palpita forficifera* (Lepidoptera: Crambidae) em oliveira e parasitoides associados

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Abstract

Palpita forficifera Munroe, 1959 (Lepidoptera: Crambidae) is the main pest of the olive tree (*Olea europaea* L., Oleaceae) in Brazil and its management has been difficult, as there are few products recommended for its control. This study aimed to evaluate the population dynamics of *P. forficifera* and the entomofauna of parasitoid eggs and larvae in three olive orchards under different cultivation systems, in Rio Grande do Sul state, Brazil. The study was carried out from October to May, in the 2017/18 and 2018/19 harvests, in olive orchards of different cultivars in the municipalities of Pelotas and Rio Grande, state of Rio Grande do Sul, Brazil. Population dynamics of *P. forficifera* varied according to the agricultural season, the months sampled, and the orchards (sites) evaluated. The highest infestation reached around 60% of the shoots in some months, depending on the orchard evaluated. During the pest infestation period, the occurrence of the egg parasitoid *Trichogramma foersteri* Takahashi, 2021 (Hymenoptera: Trichogrammatidae) was observed along with three larval parasitoids belonging to the genera *Dolichogenidea* Viereck, 1911, *Hymenochaonia* Dalla Torre, 1898 (Hymenoptera: Braconidae) and *Temelucha* Förster, 1869 (Hymenoptera: Ichneumonidae). *Temelucha hilux* Gauld, 2000 is recorded for the first time in association with a host, and its distribution in the country is extended to the southern region, from Brazil. Due to the scarcity of information on pest management, the natural occurrence of natural enemies in crops is of paramount importance in helping to manage *P. forficifera* in the field. The population dynamics of *P. forficifera* is also influenced by the parasitoid community, made up of at least four species of parasitic Hymenoptera. Therefore, strategies aimed at managing *P. forficifera* must be well developed in order to maintain and increase natural biological control in the field.

Keywords: Braconidae, Ichneumonidae, olive caterpillar, Trichogrammatidae.

Resumo

Palpita forficifera Munroe, 1959 (Lepidoptera: Crambidae) é a principal praga da oliveira (*Olea europaea* L., Oleaceae) no Brasil e seu manejo é difícil, pois há poucos produtos recomendados para seu controle. O objetivo do estudo foi avaliar a flutuação populacional de *P. forficifera* e avaliar os parasitoides de ovos e lagartas associados à praga em três olivais conduzidos sob diferentes sistemas de cultivo no estado do Rio Grande do Sul, Brasil. O estudo foi realizado entre outubro e maio, nas safras de 2017/18 e 2018/19, em pomares de oliveira de diferentes cultivares nos municípios de Pelotas e Rio Grande, Rio Grande do Sul, Brasil. A dinâmica populacional de *P. forficifera* variou em função da safra agrícola, dos meses amostrados e dos pomares (locais) avaliados. A maior infestação alcançou cerca de 60% das brotações em alguns meses, dependendo do pomar avaliado. Durante o período de infestação da praga, foi observada a ocorrência do parasitoide de ovos *Trichogramma foersteri* Takahashi, 2021 (Hymenoptera: Trichogrammatidae) juntamente com três parasitoides larvais pertencentes aos gêneros *Dolichogenidea* Viereck, 1911 e *Hymenochaonia* Dalla Torre, 1898 (Hymenoptera: Braconidae) e *Temelucha* Förster, 1869 (Hymenoptera: Ichneumonidae). *Temelucha hilux* Gauld, 2000 é registrado pela primeira vez em associação a um hospedeiro e sua distribuição no país é estendida para a região Sul do Brasil. Devido à escassez de informações sobre o manejo da praga, a ocorrência natural de inimigos naturais nos cultivos agrícolas é de suma importância para auxiliar no manejo da *P. forficifera* a campo. A dinâmica populacional de *P. forficifera* é influenciada também,

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pela comunidade de parasitoides, formada por no mínimo quatro espécies de himenópteros parasitoides. Assim, estratégias direcionadas para o manejo da *P. forficifera* devem ser bem elaboradas para manter e aumentar o controle biológico natural a campo.

Palavras-chave: Braconidae, Ichneumonidae, lagarta-da-oliveira, Trichogrammatidae.

1. Introduction

Olive growing is an agricultural activity that has been intensified in Brazil. Currently, the country has a production area of approximately 10,000 hectares, and the state of Rio Grande do Sul is responsible for 60% of the national production (Ibraoliva, 2022). The great interest in this agricultural activity is because Brazil is the fifth-largest importer of olive oil (68.000 tons) and the second-largest importer of olives (100,000 tons) (IOC, 2022), which represents an opportunity to fill this market niche. However, for this to happen and for there to be good productivity, the olive tree needs to be correctly adapted to the national scenario and this involves developing and implementing management systems for the main phytosanitary problems.

The olive caterpillar, *Palpita forficifera* Munroe, 1959 (Lepidoptera: Crambidae), is among the main pests found in producing regions. This species is native to South America and widely distributed in Brazil and Uruguay`s olive groves (Paullier, 2013; Ricalde et al., 2015). Damage is caused by larvae which, in their first instars, feed on the apical bud and/or abaxial part of the leaf. However, as they progress to larger instars (> third instar), they consume whole leaves and shoots, even consuming fruit when there are high infestations (Ricalde and Garcia, 2013; Scheunemann et al., 2020). Even so, the production chain produces olive oils comparable to the best in the world. To optimize the methods already used by producers, it is essential to know the organisms responsible for maintaining the natural biological control that occurs in orchards. This knowledge will help maintain, and in the future increase their populations through potential releases of species that can be multiplied in the laboratory.

In countries close to the Mediterranean, which traditionally grow olive trees, several species of natural enemies of *Palpita unionalis* (Hübner, 1796) (Lepidoptera: Crambidae) were recorded, with egg parasitoids standing out, such as *Trichogramma bourarachae* Pintureau & Babault, 1988 and *Trichogramma cordubensis* Vargas & Cabello, 1985 (Hymenoptera: Trichogrammatidae), which occur naturally in olive groves in Egypt and are also considered promising for flooding crops in the region (Hegazi et al., 2007). In Turkey, parasitism of *P. unionalis* by *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) and *Apanteles brunnistigma* Abdinbekova, 1969 (Hymenoptera: Braconidae) was observed in eggs and larvae, respectively (Kaçar and Ulusoy, 2012). In addition, *Trichogramma* Westwood, 1833 was observed in olive groves in Portugal, Greece and Tunisia, being collected on exposed eggs of *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae) and *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) (Herz et al., 2007). Larval parasitoids, on the other hand, have been little studied in olive growing, but are promising for controlling lepidopteran pests. Given the prospects for the expansion of olive growing in Brazil and the need to develop control methods, especially biological ones, studies that seek to verify the population dynamics of the pest and its associated natural enemies are considered the first steps in the implementation of Integrated Pest Management (IPM) strategies. This study aimed to evaluate the population dynamics of *P. forficifera* and the entomofauna of egg and larvae parasitoids in three olive orchards under different cultivation systems, in Rio Grande do Sul state, Brazil.

2. Material and Methods

The study was carried out in three non-commercial olive orchards, with no standard cultivars, during the 2017/2018 and 2018/2019 harvests. The orchards were located in the municipalities of Pelotas [Orchard A - Embrapa Temperate Climate Experimental Station (31°37'16"S, 52°31'40"W) and Orchard B - Cascata Experimental Station (31°40'53.16"S, 52°26'23.60"W)] and Rio Grande [Orchard C - Horto Municipal do Povo Novo (31°57'04"S, 52°18'35"W)], in the state of Rio Grande do Sul, Brazil. Orchards A, B, and C had an area and age of approximately 1.19 ha and 15 years, 0.18 ha and 12 years, and 0.67 ha and 40 years, respectively. The orchards were formed with various cultivars, predominantly Arbequina, Koroneiki, and Arbosana. Despite the location, orchards A and C were surrounded by native vegetation, with predominant grass species, while orchard B was surrounded by remnants of the Atlantic forest. Orchard management was carried out with little human intervention. In orchards A and C, chemical inputs were used for fertilization and control of insect pests and diseases. Orchard B was cultivated in the agroecological system, without the use of chemical inputs. The climatic conditions of this region according with Köppen system are of the Cfa type, a subtropical characteristic marked by high humidity (ranging from 75 to 85%) in all seasons, rainfall in all months of the year, and temperatures above 22 °C in summer (usually January) and above 3 °C during winter (usually July) (Kottek et al., 2006).

2.1. Population fluctuation of P. forficifera

Visual assessments of the presence of *P. forficifera* larvae on olive tree shoots were carried out every 15 days from October to May in the 2017/2018 and 2018/2019 agricultural seasons. This period was defined considering information on the presence of the pest obtained from olive growers. In each orchard, 20 plants were evaluated during the study period. The plants were identified using red nylon ribbons. On each plant, two branches on opposite sides of the middle of the plant were visualized and the presence of larvae was assessed. The infestation of *P. forficifera* in the orchards was determined by the percentage of branches where the pest was present.

2.2. Parasitoids associated with P. forficifera

2.2.1. Egg parasitoids

Filter paper discs (11 cm in diameter) with approximately 200 eggs (up to 12 hours old) of *P. forficifera* obtained from adult rearing cages (16 cm × 22 cm) in the laboratory (Scheunemann et al., 2019) were separated and used to capture the possible egg parasitoids in the field. To do this, the discs containing the eggs were placed individually inside a plastic tube (16 cm × 10.5 cm) with the two openings protected with micro-tulle fabric (2 mm) to allow only parasitoids to enter and, consequently, prevent predators, especially ants, from entering. Evaluations were carried out every two weeks between October and May of each crop year. Five plastic tubes containing egg disks were randomly distributed in each orchard. The tubes were randomly fixed to the branches of the plants at a height of 1.5 m from the ground using a nylon thread and left there for 48 hours. After this time, the tubes were collected and transported to the laboratory. The discs containing the eggs were individualized in glass containers (500 mL). Each container was closed with a paper towel secured with a rubber tie to prevent the possible parasitoids from escaping when they emerged. The containers were placed in an air-conditioned chamber (temperature 25 ± 1 °C, relative humidity 60 ± 10%, and 14h photophase). Observations were made daily to check for parasitism and emergence. When they emerged, the parasitoids were removed and kept in another tube (16 cm × 22 cm) where they were fed with a 10% honey solution and used to multiply the individuals in *P. forficifera* eggs. When the brood was established, around 30 parasitoids were isolated for identification. To do this, the insects were placed in 5 mL glass tubes filled with 70% alcohol. The specimens were then sent for taxonomic identification, considering morphological and molecular analyses. The slides of the specimens were prepared in Hoyer's medium (Querino and Zucchi, 2011).

The *Trichogramma* species were identified using illustrated keys depicting characteristics of the male genitalia (Querino and Zucchi, 2011, 2019). To complement this, the specimens were subjected to DNA extraction and analysis, and submitted to a PCR reaction to amplify the mitochondrial cytochrome c oxidase I (COI) gene. The COI sequences were compared with reference sequences deposited in GenBank (National Center for Biotechnology Information) using the Basic Local Alignment Search Tool (BLASTN), and the sequences with the highest similarity patterns (greater than 90%) were selected. The candidate sequences were compared with the samples by multiple alignments using the ClustalW tool. For the phylogenetic analysis, the genetic distance matrix (Hasegawa-Kishino-Yano model) implemented in the MEGAX software (version 10.1.8) was obtained. The tree was obtained using the Maximum Likelihood method with node consistency obtained after 1000 bootstrap repetitions.

2.2.2. Larval parasitioids

Olive tree shoots with symptoms of olive caterpillar attack were collected every two weeks (approximately 4 shoots from 10 plants) from the time the shoots emerged until the end of the harvest, which corresponded to October to May. The samples were duly labeled with the date and location, packed in plastic bags (5 L), and kept in thermal boxes (50 L) until they were transported to the laboratory. In the laboratory, the collected shoots were sorted, and the caterpillars found were individualized in glass tubes (3 cm × 8.5 mm). Subsequently, the larvae were fed on olive shoots of the Koroneiki cultivar fixed in a 2% water-agar solution (25 mL), according to the methodology described by Scheunemann et al. (2019). The tubes were then capped with absorbent cotton and kept in an air-conditioned room (temperature 25 ± 1 °C, relative humidity 60 ± 10%, and 14h photophase). The development of the caterpillars was observed daily, and fresh food was provided as needed until the pupal stage. In the pupal stage, if parasitoids emerged, they were counted and separated by morphospecies and sex and stored in glass tubes (5 mL) containing 70% alcohol for later identification. The parasitoids belonging to the Braconidae were identified using the identification keys for genera in Wharton et al. (1997) and those belonging to the Ichneumonidae species using the taxonomic key in Gauld (2000). The data obtained was used to determine the parasitism index, the percentage of parasitoid occurrence and the population fluctuation of parasitoid species over time.

3. Results

The percentage of olive shoots infested by *P. forficifera* larvae varied depending on the period sampled the crop year and the location of the orchard (Figure 1). When analyzing the infestation in the different groves, grove B was the least infested for both agricultural seasons, with the highest infestations between 30 and 40%. For orchards B and C, there was generally an increase in pest infestation in the warmer months (Figure 1). In contrast, for orchard A, the highest infestation occurred at the beginning of the

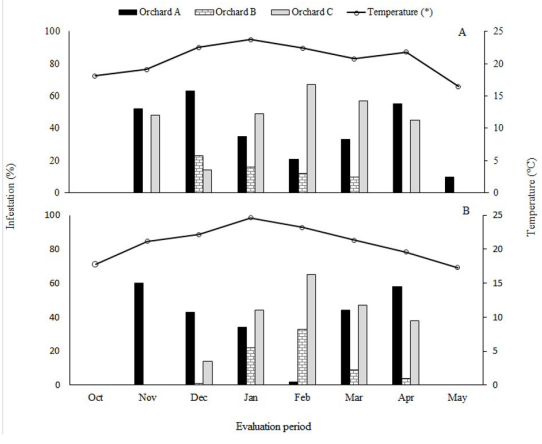

Figure 1. Population fluctuation of *Palpita forficifera* (Lepidoptera: Crambidae) in two agricultural seasons, 2017/2018 (A) and 2018-2019 (B), from October to May in three orchards located in the south of Rio Grande do Sul state, Brazil.

evaluation, in November and December, decreasing in the warmer months (January and February) and increasing again between March and April (Figure 1).

Regarding the presence of natural enemies, egg and larvae parasitoids of *P. forficifera* were obtained. The egg parasitoids were identified as *Trichogramma foersteri* Takahashi, 2021 (Hymenoptera: Trichogrammatidae) (Figure 2) and the larval parasitoids as *Dolichogenidea* sp., *Hymenochaonia* sp. and *Temelucha hilux* Gauld, 2000. Of the larval parasitoids found in olive orchards, *Dolichogenidea* sp. was the most abundant, with values above 61%, when considering the year of collection and the location, followed by *T. hilux* (values varying from 7 to 37%) and *Hymenochaonia* sp. (varying from 2 to 7%) (Figure 3).

The presence of parasitoids was recorded in all three orchards and, in both agricultural seasons, except for egg parasitoids which were only collected in the second season (2018/2019) (Figure 2A). The presence of the hymenopteran parasitoids was not recorded in all the fortnightly evaluations, and they were distributed differently throughout the evaluation period. The presence of the egg parasitoid *T. foersteri* was recorded from March to May (Figure 2A). Of the larval parasitoids, the braconids (*Dolichogenidea* sp. and *Hymenochaonia* sp.) were recorded from November onwards, while the ichneumonid *T. hilux* was collected from January onwards (Figure 2B).

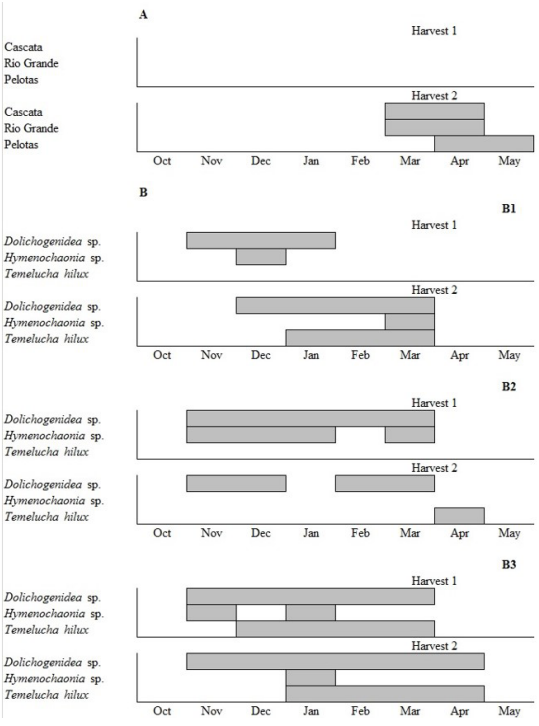

Figure 2. Distribution of egg parasitoids (*Trichogramma foersteri*) (A) and larvae parasitoids (B) of *Palpita forficifera* (Lepidoptera: Crambidae), collected in olive groves over the period from October to May, in the 2017/2018 (Harvest 1) and 2018/2019 (Harvest 2) agricultural seasons in three orchards in the south of Rio Grande do Sul sate, namely: Pelotas (B1), Pelotas (B2) and Rio Grande (B3), in the Brazil.

Considering the three larval parasitoids, the percentage of parasitized larvae reached values close to 60% (Figure 4). In orchard A, located in the experimental area of Embrapa Clima Temperado, the peak parasitism of approximately 60% (2017/2018 season) and 40% (2018/2019 season) occurred in December (Figure 4). For orchard B, located at Embrapa Clima Temperado's Cascata Station, there was the highest parasitism rate (ranging from 30 to 49%) between November and January in the 2017/18 season. In the 2018/2019 season, the highest parasitism rate (approximately 30%) occurred from January to March (Figure 4). For orchard C, the highest parasitism rate in the 2017/18 season occurred from December to February (ranging from 25 to 33%). However, in the 2018/2019 season, *P. forficifera* parasitism peaked in January, with approximately 60% of the larvae parasitized.

4. Discussion

The results obtained indicate that the infestation of *P. forficifera* in olive trees has an irregular population dynamic during the period evaluated, being recorded from November to May. This irregular behavior is clearest in Orchard A, which is probably influenced by management practices where the olive caterpillar is controlled with insecticides. In addition, at this location, there was a windbreak barrier formed by ligustrum plants (*Ligustrum lucidum* Ait., Oleaceae) which are hosts for *P. forficifera*, as demonstrated by Scheunemann et al. (2019). This may have triggered a form of shelter for the pest during the winter months. In the other areas evaluated, *P. forficifera* control was not carried out at any time, so the highest population level of the pest occurred from January to April. During this period, *P. forficifera* was favored by the higher temperatures in January (average temperatures of 25 °C) and tended to decline in the following months. In addition, olive plants produce shoots from September to April, which were mainly favored by the constant rainfall and high temperature of the region (average temperatures of 18 to 25 °C). These shoots are essential for the development of first-instar larval caterpillars, which need tender plant tissue to feed on (Scheunemann et al., 2020).

The presence of *T. foersteri* parasitizing P. *forficifera* eggs is considered the first record of an egg parasitoid on this host. Recently, this parasitoid species was described and related to *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae), the main defoliator of the soybean crop (Takahashi et al., 2021). The authors recorded a parasitism of 113 eggs in the first three days of life. They also reported that other species of lepidopteran provided the development of *T. foersteri*. In *P. forficifera* the parasitoid was recorded in one agricultural season and, in the three sites evaluated, indicating that this species may have other hosts, since inside the orchards and around the study area there is native grassland with a great diversity of undergrowth and trees. Furthermore, considering the locations of the collections made in Paraná (Takahashi et al., 2021) and Rio Grande do Sul, it is clear that the species prefers milder temperatures.

Trichogramma have been recorded parasitizing the eggs of various lepidopteran pests in a wide variety of crops (Querino

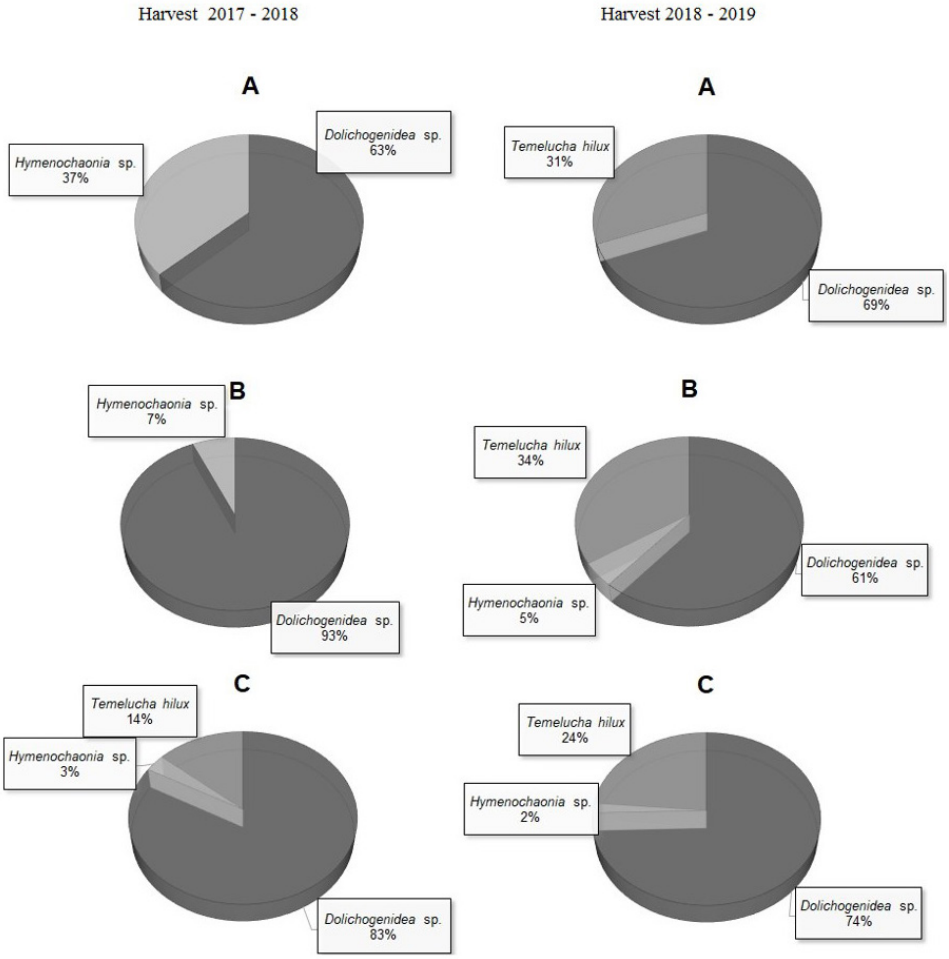

Figure 3. Relative distribution of parasitoid species on caterpillars of *Palpita forficifera* (Lepidoptera: Crambidae) collected during the 2017/2018 and 2018/2019 harvests, in olive orchards in the municipalities of Pelotas (A and B) and Rio Grande (C), Rio Grande do Sul state, Brazil.

and Zucchi, 2011, 2019). Hegazi et al. (2007) found *P. unionalis* egg parasitism rates of 91% with native trichogrammatids species in Egypt, and Herz and Hassan (2006) identified species native to the Mediterranean region as the most effective in controlling *P. unionalis* in olive groves in Egypt.

Amond the larval parasitoids obtained, this is also the first record of *Dolichogenidea* sp., *Hymenochaonia* sp., and *T. hilux* on *P. forficifera*. In Brazil, *Dolichogenidea* was reported by Nava et al. (2005) as frequent in the attack of the avocado borer, *Stenoma catenifer* Walsinghan, 1912 (Lepidoptera: Depressariidae), in the state of Minas Gerais. *Dolichogenidea* sp. reported on *P. forficifera* as a species with gregarious habit, whose characteristic is to parasitize the larval stage and emerge during the pre-pupa stage. According to Fernandez-Triana et al. (2019), *Dolichogenidea* is a cosmopolitan genus with more than 200 described species, of which 12 species occur in the Neotropics and none registered for Brazil. The authors point out that there are still a significant number of unknown species. Most of the species from the Neotropical region are associated with Depressariidae larvae.

Hymenochaonia is a cenobiont endoparasitoid of Lepidoptera larvae (Noctuidae, Tortricidae, Pyralidae, Sesiidae, Tineidae, Oecophoridae and Gelechiidae) (Shaw and Huddleston, 1991), including Crambidae, the family that includes *P. forficifera*. In Brazil, this genus has been reported on *S. catenifer* infesting avocado (Nava et al., 2005) and on *Gymnandrossoma aurantianum* Lima, 1927 (Lepidoptera: Tortricidae) infesting citrus (Milano et al., 2015). *Hymenochaonia* and *Dolichogenidae* are subfamilies that exclusive attack Lepidoptera. Both apparently prefer attacking larvae with concealed habits, with is reflected in their relatively log ovipositors (Fernandez-Triana et al., 2019; Wharton, 1997) the collected specimens of *Hymenochaonia* exhibit solitary habits, although there are record indicating that species of this genus can be polyembryonic (Wharton, 1997).

Temelucha hilux was originally described from specimens from Costa Rica, and was later recorded in Mexico, and Brazil in coffee plantations in the interior of São Paulo, Brazil (Gauld, 2000; Pérez-Urbina et al., 2018; Fernandes et al., 2020). This is the first record of this species association

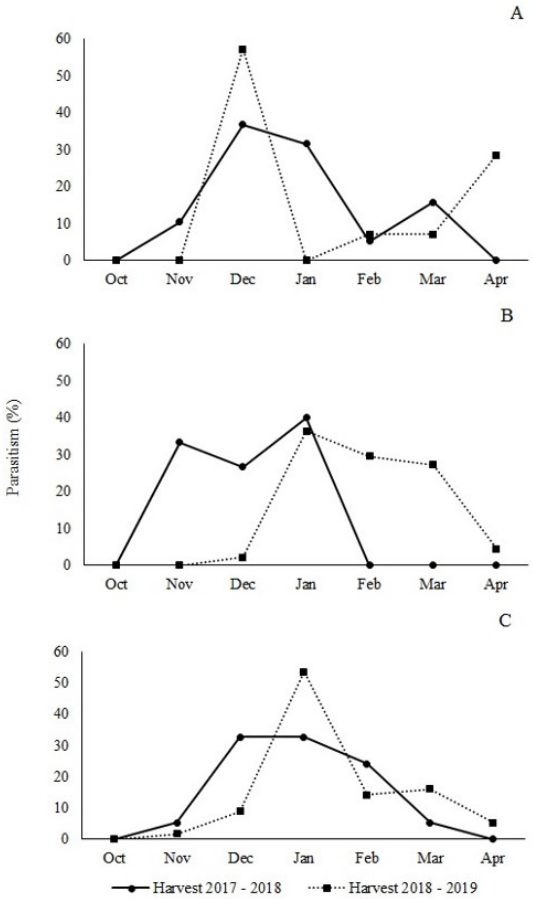

Figure 4. Total parasitism of *Palpita forficifera* (Lepidoptera: Crambidae) larvae collected in olive orchards throughout the production cycle, in the 2017-2018 (Harvest 1) and 2018-2019 (Harvest 2), in the Pelotas (A), Cascata (B), and Rio Grande (C) áreas, in the Rio Grande do Sul state, Brazil.

with a host (*P. forficifera*), and its distribution has now been extended to the south of the Brazil, now the southernmost record of this species' known distribution. In Turkey, in the Mediterranean region, Kaçar and Ulusoy (2011) recorded *Temelucha anatolica* (Sedivý, 1968) and *Temelucha decorata* (Gravenhorst, 1829) parasitizing *Palpita unionalis* (Hübner, 1796) in olive plantations.

These high parasitism rates in *P. forficifera* to *Dolichogenidea* can also be observed in Orchard A, located at the Embrapa Temperate Climate Experimental Station, where chemical insecticides were used to manage *P. forficifera*. In addition, its greater ability to search for the host may also be related to its adaptation to the meteorological factors as well as those of the landscape surrounding the orchards, as shown in Figures 2 and 3.

The parasitism rates of *P. forficifera* larvae can be considered high, especially during the months when the pest is most heavily infested. Certainly, the phytosanitary management practices carried out in the orchards, especially concerning the control of olive larvae and mealybugs, do not interfere with parasitism.

The abundance, constancy, and frequency among the genera of larval parasitoids may be caused by interspecific competition between the insects and, most likely, by the best adaptation to the climatic conditions of each harvest. The most promising species is *Dolichogenidea* sp., which was present in all harvests in all three areas. Therefore, more study is needed into its biology and behavior in order to provide mass release. The same is true of the egg parasitoids. As mentioned above, the *Trichogramma* has several records in olive groves in the Mediterranean, the cradle of olive growing, and is used commercially to control the European olive caterpillar (*P. unionalis*). Therefore, studies on the biology and percentage of parasitism, as well as the selection of promising strains is an important step to be taken in order to provide the viability of applied biological control of *P. forficifera* in Brazilian olive growing.

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