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CROP PRODUCTION

Spatial and temporal distribution and bioinsecticides control of olive leaf moth in an olive orchard in Southwest of Paraná, Brazil

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ABSTRACT: We verified the spatial and temporal distribution of the olive leaf moth (*Palpita forficifera* Munroe, 1959) in a new potential region for olive cultivation and evaluated the bioinsecticides effectiveness to control this pest. The experimental orchard composed by Koroneiki and Arbequina cultivars was planted in Pato Branco, Southwest region of Paraná, Brazil. In the field, larvae counts were performed weekly on 25 plants of each cultivar, for 12 months, to verify the spatial and temporal distribution. Furthermore, in laboratory conditions, second instar larvae of *P. forficifera* were fed with olive leaves treated with *Azadirachta indica* oil, *Bacillus thuringiensis, Metarhizium anisopliae* and *Beauveria bassiana*, and the effectiveness in the larvae mortality was used to compare four bioinsecticides and control. The *P. forficifera* larvae occurred mainly during the three summer months, had a host preference for the 'Arbequina' (68.9% of total larvae) and occurred at random distribution patterns throughout the orchard. The *A. indica* oil and *B. thuringiensis* both caused almost 100% larvae mortality under laboratory conditions.
Key words: *Olea europaea* L., *Palpita forficifera*, population fluctuation, biological control.

Distribuição espacial e temporal e controle com bioinseticidas da lagarta-da-oliveira em um pomar da região Sudoeste do Paraná, Brasil

RESUMO: Os objetivos deste trabalho foram verificar a distribuição espacial e temporal da lagarta-da-oliveira (*Palpita forficifera* Munroe, 1959) em uma nova região com potencial de cultivo de oliveira e avaliar a eficiência de controle desta praga com bioinseticidas. O pomar experimental com as cultivares Koroneiki e Arbequina foi implantado em Pato Branco, região Sudoeste do Paraná, Brasil. No pomar foram realizadas contagens semanais de lagartas em 25 plantas de cada cultivar, durante 12 meses, para verificar a distribuição espacial e temporal. Além disso, em condições de laboratório, larvas de segundo instar foram submetidas à alimentação com folhas de oliveira tratadas com óleo de neem (*Azadirachta indica*), *Bacillus thuringiensis*, *Metarhizium anisopliae* e *Beauveria bassiana,* e a efetividade de mortalidade das larvas foi utilizada para comparação entre os quatro bioinseticidas e a testemunha. As lagartas de *P. forficifera* ocorreram concentradas durante os três meses de verão, tiveram preferência hospedeira pela cultivar Arbequina (68,9% do total de larvas registradas), e ocorreram em padrão de distribuição aleatório ao longo do pomar. A aplicação de óleo de neem (*A. indica*) ou de *B. thuringiensis* causaram quase 100% de mortalidade das larvas em condições de laboratório.
Palavras-chave: *Olea europaea* L., *Palpita forficifera*, flutuação populacional, controle biológico.

INTRODUCTION

The olive tree, *Olea europaea* L. (Lamiales: Oleaceae) (APG IV, 2016), is widely cultivated in countries of the Mediterranean climate, which has dry and hot Summer and cold Winter, needed to stimulate flowering and fruiting. In Brazil, the olive cultivation area is expanding from 43 to 1,894 ha between 2011 to 2020 (FAOSTAT, 2020), mainly in highlands areas (> 800 m) from Minas Gerais, São Paulo, Santa Catarina and Rio Grande do Sul States. Besides that, Brazil is the world's second-largest importer of olive oil and olives (IOC, 2020), demonstrating the potential of olive cultivation, aiming to reduce imports.

The official reccomended cultivating areas are available only to Rio Grande do Sul and São Paulo states (MAPA, 2022). However, cultivation tests are being developed, to identify potential new grow frontier areas, such as Southwest of Paraná. Orchard tests in Santa Catarina State demonstrated good olive productivity (DA CROCE et al., 2016), in four areas near and with edaphoclimatic conditions similar to Southwest of Paraná.

The most serious pests of olive trees are leaf moths (*Palpita* sp., Lepidoptera: Pyralidae), such as *P. vitrealis* (Rossi) and *P. unionalis* (Hübner), mainly in Mediterranean countries, and *P. forficifera* (Munroe) in subtropical regions (NOORI & SHIRAZI, 2012; GHONEIM, 2015), including in Brazil (RICALDE et

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al., 2014). The damage of leaf moth in olive orchards is mainly caused by feeding on young shoots and leaves (HAYDEN & BUSS, 2012) and, consequently, reducing flowering and olive production in the next cycle, causing mild or severe loss rate of yield up to 30% depending on pest population density (RICALDE et al., 2014; GHONEIM, 2015).

In the Southern region of Brazil, *P. forficifera* was detected in some olive orchards, in which the population fluctuation and biological cycle of the pest were verified (RICALDE et al., 2014, SCHEUNEMANN et al. 2018). However, further studies are essential, such as demonstrating the potential for controlling this pest with bioinsecticides. After all, there is none insecticide registered to *Palpita* control in Brazil (AGROFIT, 2022). This research verified the spatial and temporal distribution of the olive leaf moth (*P. forficifera*) in a new potential region for olive cultivation and evaluated the bioinsecticides effectiveness to control this pest.

This research was carried out in a seven-year-old experimental olive orchard in Pato Branco, Paraná, Brazil (26º10'36" S, 52º41'22" W, 764 m a.s.l.). The soil is classified as 'Latossolo Vermelho distroférrico típico' based on the Brazilian soil system classification (EMBRAPA, 2018), and the climate is humid subtropical *Cfa*-type, according Köppen (ALVARES et al., 2014). The orchard comprised 80 plants from Koroneiki and 40 plants from Arbequina cultivars, spaced 6.0 x 3.0 m.

This experimental olive orchard was the first in the Southwest region of Paraná, but olive leaf moths (*Palpita* sp.) occurred and caused injuries since the second year of cultivation, even without nearby olive orchards. To identify *Palpita* sp. occurring in this orchard, adult moths were collected in the same olive plantation where the study was carried out, using an entomological net, mounted and preserved according to recommendations for Lepidoptera species. The samples of moths were sent to the Uiraçu Institute (Camacã, Bahia, Brazil), which are identified as *Palpita forficifera* Munroe, 1959 (Lepidoptera: Pyralidae) and deposited in the institution's entomological collection by Dr. Vitor Osmar Becker.

Considering that it is a little-known insect pest in Brazil, the morphological characteristics of each stage of life and the damage caused by the early phase were registered (Figure 1). Besides, to define the temporal distribution, the population of the pest was monitored for 12 months from September, throughout weekly counting of larvae from all instars directly on eight branches in each tree (the same branches in all counts), at the upper part of trees and distributed around all canopy in 25 trees of each cultivar (Koroneiki and Arbequina). The spatial distribution pattern of olive larvae occurrence in the orchard was assessed using the 'inverse distance weighting' technique, based on weekly counting data and cartesian coordinates of each tree inside the orchard (WEBER et al., 2018).

The control of olive larvae was compared by following bioinsecticides treatment, using the dosage recommended for Lepidoptera control by manufacturers: 1) neem oil, *Azadirachta indica* 0.014 mL L^{-1} (Óleo

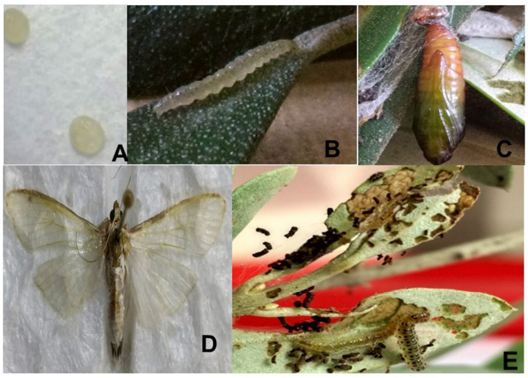

Figure 1 - Biological phases of *Palpita forficifera* (Munroe, 1959) (Lepidoptera: Pyralidae) and damages in leaves observed in the experimental olive (*Olea europea* L.) orchard, in Pato Branco, Paraná, Brazil. A) Eggs. B) Larvae. C) Pupa. D) Moth. E) Larvae feeding olive leaves.

vegetal®); 2) *Bacillus thuringiensis* var. kurstaki 1.5 g L^{-1} (Dipel WP®); 3) *Metarhizium anisopliae* 3.2 g L^{-1} (Metharril WP®); and 4) *Beauveria bassiana* 2.0 g L^{-1} (Boveril WP®). Apical branches with 5.0 cm in length of the cultivar Arbequina were collected in the field. This collected material was taken to the laboratory and sprayed using 10 mL of four insecticidal solutions or distilled water as the control on its entire surface with a manual spray pump. It was later supplied to the larvae as a food source. Following the adapted methodology from YILMAZ & GENÇ (2012), four olive branches were treated and 10 second-instar larvae (3 to 4 mm) were placed in a plastic box (11.0 x 11.0 x 3.5 cm), in 10 replications for each treatment. These boxes were kept in acclimatized chambers (25 ± 1 ºC, relative humidity 75 ± 5%, and 12 hours of photophase). Every 72 hours, two untreated olive branches were placed in the boxes to provide food *ad libitum* for surviving larvae. The number of dead larvae was counted daily up to 21 days after treatments, and it was analyzed by non-linear regression, using the Weibull model, to determine the lethal exposure time for 50% larvae mortality (LE_{50}). The analyzes were performed in the R language (R CORE TEAM, 2020).

The beginning of *P. forficifera* infestation in the orchard occurred in late December and was predominant in the summer. A total of 1,203 larvae was counted from all instars, with 40.4% in January, 46.2% in February and 12.4% in March, only 1% in December and April and no larvae were reported between May and November (Figure 2). Similar to our data, in the Rio Grande do Sul, another South Brazilian State, the majority of damage of *P. forficifera* in olive trees occurred from January to March and the oviposition starts in November (RICALDE et al., 2014). In a Mediterranean country (Iran), the population of *P. unionalis* larvae also reaches its peak concentration during the summer months in olive trees, decreasing gradually to zero in the autumn and winter months (NOORI & SHIRAZI, 2012). This temporal pattern showed that higher temperatures are a key factor in the multiplication of leaf moth in the olive orchards. Conversely, when temperatures are lower, from April to November in the South of Brazil, it also reduces young leaves development from olive trees and *P. forficifera* probably migrates nearby to the other Oleacea, such as *Ligustrum lucidum* Aiton, that is one of the main hosts of olive leaf moth (GHONEIM et al. 2015; SCHEUNEMANN et al., 2018), that were present nearby at olive orchard in our study.

Arbequina presented 68.9% of the total number of larvae counted, evidencing the preference of *P. forficifera* to host this cultivar, instead Koroneiki. This preference could be explained due to the emission of a greater amount of kairomones, volatile compounds that are associated with greater attractiveness of insects in vegetables and which are variable in amount among olive cultivars (DURSUN et al., 2017). However, this should be confirmed by future research.

The spatial distribution of *P. forficifera* throughout the orchard followed a random pattern, without association with a group of plants and with a few concentrate points in specific sites (Figure 3).

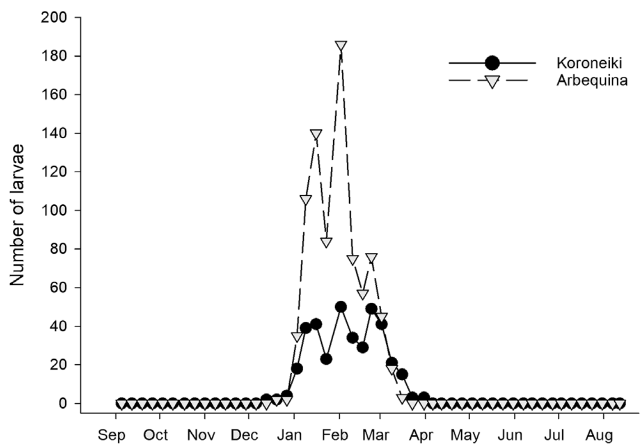

Figure 2 - Temporal occurance of *Palpita forficifera* (Munroe, 1959) (Lepidoptera: Pyralidae) larvae for 12 months in the experimental olive (*Olea europea* L.) orchard of Koroneiki and Arbequina cultivars, in Pato Branco, Paraná, Brazil.

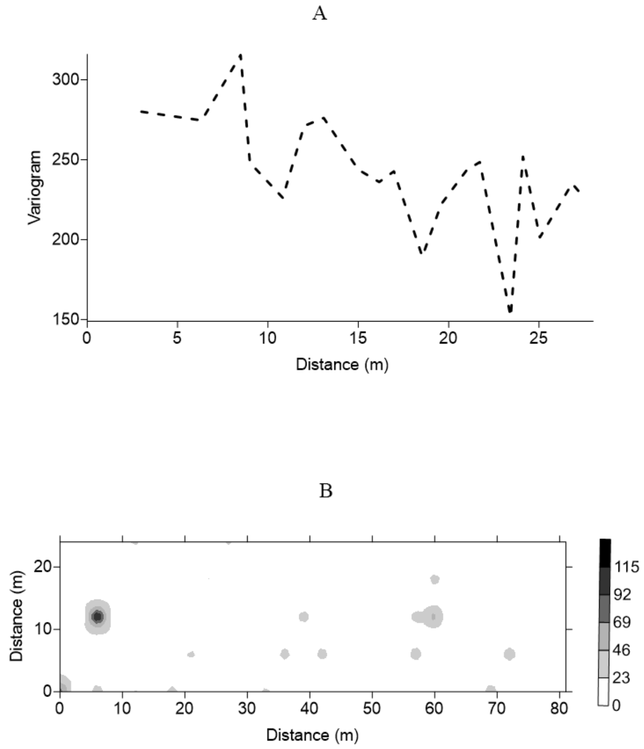

Figure 3 - Spatial distribution of *Palpita forficifera* (Munroe, 1959) (Lepidoptera: Pyralidae) larvae in the experimental olive (*Olea europea* L.) orchard of Koroneiki and Arbequina cultivars, in Pato Branco, Paraná, Brazil. A) Semivariogram; B) spatial distribution graph (darker grey and larger circles represent higher larvae number).

This result means that the number of larvae observed in one plant may vary significantly with the other plant located very close. In contrast, plants distant from each other may present a similar number of larvae (WEBER et al., 2018). This allows us to infer that both monitoring and control of this pest must be carried out over the entire orchard due to random distribution.

The application of neem oil (*A. indica*) and *B. thuringiensis* (*Bt*) increased the mortality of second instar of *P. forficifera*, both caused almost no larvae surviving. Neem oil caused mortality more quickly since the lethal time for a 50% reduction (LE_{50}) was 10.9 hours and for *Bt* the LE_{50} was 34 hours (Figure 4). A possible explanation for these effects is due to molecules presented in *A. indica* leaves acting as anti-feeding (ALMEIDA et al., 2010; BEZZAR-BENDJAZIA et al., 2017). The rapid larval mortality observed with the application of neem oil can be mainly related to chemical changes on the surface of the leaves used as a food substrate, inducing an anti-feeding action and avoidance in the insect larvae. Thus, considering the great nutritional demand in the early stages, it may have caused the insect death (BEZZAR-BENDJAZIA et al., 2017). In turn, the causes of larvae mortality attributed to the *Bt* act are the effect on the columnar cells of the insect's mid gut, as occurs for other Lepidoptera and can be extended to *P. forficifera*, under the action of endotoxins contained in the protein crystal of the bacterium *Bt* (KHALIL et al., 2021; NASCIMENTO et al., 2022).

The bioinsecticide based on *B. bassiana* was not efficient in causing the mortality of *P. forficifera* larvae, as was similar to control. Conversely, the bioinsecticide based on *M. anisopliae* had an increase in control efficiency in relation to the control only after 360 hours, reaching 93% of larval mortality, even at a lower rate than neem oil and *Bt* (Figure 4). The late action of *M. anisopliae* to induce larvae mortality may be due to the slower multiplication of this entomopathogenic fungus since larvae mortality

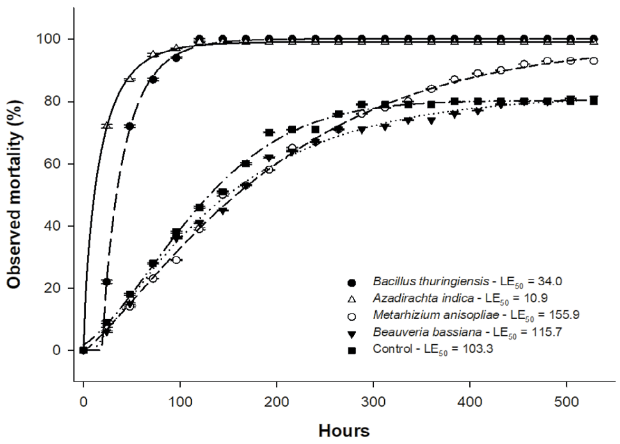

Figure 4 - Lethal exposure to 50% (LE_{50}) of larvae mortality of *Palpita forficifera* (Munroe, 1959) (Lepidoptera: Pyralidae) after hours of bioinsecticides application.

increases significantly with increasing of conidium multiplication and toxins release along time after application (EL HUSSEINI, 2019). Additionally, the result of Abbott's correction to larvae mortality based in control (ABBOTT, 1925) were 100%, 95%, 65% and 10% to *Bt*, *A. indica* oil, *M. anisopliae* and *B. bassiana*, respectively, in the last evaluation (21 days after bioinsecticides application).

The results of this research indicated that control of olive leaf moth can be concentrated from December to March in this olive orchard using neem oil and *Bt*. However, these bioinsecticides were tested in laboratory conditions, and they can have low persistence under environmental conditions (OSMAN et al., 2015; CHAUDHARY et al., 2017). Therefore, the efficiency against *P. forficifera* in the field should be investigated, including precise application practices, reapplication intervals and spray number tests.

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DECLARATION OF CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to the manuscript.

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