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Original Article

Quadrastichus mendeli (Hymenoptera: Eulophidae) parasitizing *Leptocybe invasa* (Hymenoptera: Eulophidae) on *Eucalyptus* spp. Seedlings

Quadrastichus mendeli (Hymenoptera: Eulophidae) parasitando *Leptocybe invasa* (Hymenoptera: Eulophidae) em mudas de *Eucalyptus* spp.

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Abstract

Galls by *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) reduce the productivity of *Eucalyptus* spp. plantations and *Quadrastichus mendeli* Kim & La Salle (Hymenoptera: Eulophidae) parasitizes this pest. This study aimed to evaluate, under laboratory conditions, two biological control strategies against *Leptocybe invasa* using the parasitoid *Quadrastichus mendeli*: (i) inundative biological control (referred to here as applied biological control), consisting of the release of five mated females per seedling, and (ii) natural biological control, representing the action of naturally occurring populations of the parasitoid already established in the environment. Ninety-day-old seedlings of the clone 3025 (*Eucalyptus grandis* × *E. camaldulensis*), naturally infested by *L. invasa*, were placed individually in cages under controlled conditions (25 ± 2 °C; 70 ± 10% RH; 12 h photoperiod) in a completely randomized design with 22 replicates per treatment. The number of insects was adjusted using zero-inflated mixed nonlinear regression models. The emergence of *L. invasa* adults ($p>0.05$) was similar between treatments, but that of *Q. mendeli* greater with the release of five females of this natural enemy per seedling compared to the applied biological control. The controlled release of *Q. mendeli* reduced *L. invasa* damage on *Eucalyptus* seedlings, increasing the importance of applied biological control to manage this pest in cultivated forests.

Keywords: bioassay, biological cycle, efficiency, galls, release.

Resumo

Galhas formadas por *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) reduzem a produtividade de plantações de *Eucalyptus* spp., e *Quadrastichus mendeli* Kim & La Salle (Hymenoptera: Eulophidae) parasita essa praga. Este estudo teve como objetivo avaliar, em condições laboratoriais, duas estratégias de controle biológico contra *Leptocybe invasa* utilizando o parasitoide *Quadrastichus mendeli*: (i) controle biológico inundativo (denominado aqui como controle biológico aplicado), consistindo na liberação de cinco fêmeas acasaladas por muda, e (ii) controle biológico clássico, representando a ação de populações naturalmente ocorrentes do parasitoide já estabelecidas no ambiente. Mudas com 90 dias de idade do clone 3025 (*Eucalyptus grandis* × *E. camaldulensis*), naturalmente infestadas por *L. invasa*, foram colocadas individualmente em gaiolas sob condições controladas (25 ± 2 °C; 70 ± 10% UR; fotoperíodo de 12 h) em delineamento inteiramente casualizado, com 22 repetições por tratamento. O número de insetos foi ajustado por meio de modelos de regressão não linear mistos com inflação de zeros. A emergência de adultos de *L. invasa* ($p>0{,}05$) foi semelhante entre os tratamentos, mas a de *Q. mendeli* foi maior com a liberação de cinco fêmeas desse inimigo natural por muda, em comparação ao controle biológico aplicado. A liberação controlada de *Q. mendeli* reduziu os danos causados por *L. invasa* em mudas de *Eucalyptus*, ressaltando a importância do controle biológico aplicado para o manejo dessa praga em florestas cultivadas.

Palavras-chave: bioensaio, ciclo biológico, eficiência, galhas, liberação.

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1. Introduction

Galls on the central vein and petiole and on new shoots and branches characterize damage by *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) on *Eucalyptus* spp. plants (Sarmento et al., 2021) an important culture for the wood and cellulose industry in Brazil (Pinkard et al., 2010; Silva et al., 2019; Mota et al., 2022). All developmental stages (egg, larva, and pupa) occur within the gall, with the larvae feeding and developing inside these structures, reducing growth and causing drying of branches and main shoots of eucalyptus plants (Kavitha-Kumari et al., 2010; Dittrich-Schröder et al., 2020). This insect reproduces by thelytoky parthenogenesis with its females laying eggs soon after emerging from the galls with overlapping generations. The life cycle of this pest spans 132.6 ± 8.1 days with five instars (Mendel et al., 2004). Its adults are brown with a metallic green to blue with 1.1–1.4 mm long (Mendel et al., 2004). *Leptocybe invasa* was first detected in Brazil in nurseries and in the field on hybrid clones of *E. camaldulensis* × *E. grandis* in 2008 in the Bahia state and spread to other regions of this country (Wilcken and Berti Filho, 2008).

The protection of *L. invasa* larvae inside galls reduces the effectiveness of chemical control and specific parasitoids and resistant eucalyptus genotypes are the most efficient methods to manage this pest (Zheng et al., 2014; Gevers et al., 2021b; Mphahlele et al., 2021). The parasitoid *Quadrastichus mendeli* Kim & La Salle (Hymenoptera: Eulophidae), originally from Australia, was reported in Brazil in 2019 in *Eucalyptus* plantations infested by *L. invasa* (Puretz et al., 2022).

The life cycle of the parasitoid *Q. mendeli* (from larva hatching to adult) is about 30 days in *L. invasa* larvae. Adults are yellow, with two to four dark brown transverse stripes on the gastric tergite, and measure 1.15–1.35 mm in length (Kim et al., 2008; Sangtongpraow and Charernsom, 2019). The antennae are geniculate, with segments adapted to locate host larvae, and the cylindrical, pointed ovipositor facilitates parasitism on larvae within the galls (Kim et al., 2008). *Quadrastichus mendeli* reproduces through thelytoky parthenogenesis induced by the symbiotic bacterium *Rickettsia*, abundant in its mature eggs and ovaries (Gualtieri et al., 2017). Field parasitism rates of *L. invasa* by *Q. mendeli* have been reported to range from 7.9% to 84.0% in Israel and from 0.0% to 100.0% in Italy, depending on the site, season, and host plant genotype (Kim et al., 2008; Nugnes et al., 2016), highlighting the high potential of this parasitoid for pest management.

The objective was to evaluate the parasitism of *L. invasa* larvae by *Q. mendeli* on seedlings of the hybrid clone 3025 (*E. camaldulensis* x *E. grandis*), with or without the release of this natural enemy. The hypothesis tested is that the controlled introduction of the parasitoid *Q. mendeli* increases its population and reduces that of *L. invasa* on *Eucalyptus* spp. plants infested by this pest.

2. Material and Methods

2.1. Seedlings of the hybrid clone 3025 (E. camaldulensis x E. grandis), infested by L. invasa and naturally parasitized by Q. mendeli

The development of *Q. mendeli* was evaluated in twenty-eight galls containing third-instar larvae of *L. invasa* per seedling of the hybrid clone 3025 (*E. camaldulensis* × *E. grandis*), 90 days old and naturally infested and with parasitism by that natural enemy (Mendel et al., 2004) in the nursery of the Biological Control of Forest Pests Laboratory (LCBPF) in the Department of Plant Protection at the Faculty of Agricultural Sciences, Botucatu Campus, São Paulo State, Brazil (28°85’25”S, 48°43’17”W). The third instar was visually confirmed by gall dissection and larval observation, characterized by larger body size, evident segmentation, and more developed mandibles. The seedlings were micropropagated in Carolina Soil® substrate, irrigated as needed, and exposed to *L. invasa* infestation and *Q. mendeli* parasitism under outdoor conditions. Each replicate consisted of one seedling containing an average of 38 galls with third-instar larvae, which were selected for the experiment (Mendel et al., 2004).

2.2. Bioassay

Seedlings of the hybrid clone 3025 (*E. grandis* × *E. camaldulensis*) were placed in a room at a temperature of 25 ± 2 °C, relative humidity of 70 ± 10%, and a 12-hour photoperiod using, respectively, an air conditioner, an Arsec® 250 D dehumidifier, and a photoperiod controller. They were irrigated with potable water three times per week.

The experiment included two treatments. In the first one (T1), five *Q. mendeli* females, 48 hours old and previously fed pure honey, were released onto the plants. Females of this parasitoid, reared in the laboratory colony maintained at the Laboratory of Biological Control of Forest Pests (LCB PF) mentioned above, were released onto plants with galls and enclosed with Voil fabric to prevent their escape. The parasitism rate was determined by dividing the total number of emerged parasitoid adults by the total number of emerged insects (parasitoids + hosts), following the methodology used for this natural enemy in field studies in Italy (Nugnes et al., 2016). The second treatment (T2), without parasitoid release, represented the natural biological control by *Q. mendeli*. All seedlings used in both treatments had a similar initial infestation level, averaging 38 galls per plant containing third-instar larvae, to ensure comparability between treatments.

A total of 22 seedlings were used in the experiment, corresponding to 11 plants per treatment. Each plant was considered an experimental unit. The seedlings were individually covered with Voil fabric from the seventh day onward to prevent insect escape. Adults of *Q. mendeli* and *L. invasa* began emerging on the tenth day after the experiment started, and individuals were collected daily using a manual aspirator and identified based on morphological characteristics.

2.3. Data analysis and statistics

The effect of treatments T1 (with release of *Q. mendeli*) and T2 (without release of *Q. mendeli*) on the parasitism rate (percentage of emerged parasitoid adults over the total number of emerged insects) and on the total number of emerged adults of *L. invasa* and *Q. mendeli* was modeled using a generalized linear model with negative binomial distribution and logarithmic link function (Nelder and Wedderburn, 1972; Ovruski et al., 2004). The *genmod* procedure (from the SAS statistical program – Free Statistical Software, SAS University Edition) was used, and data between treatments were compared using the Tukey–Kramer test (Westfall et al., 2011).

The total number of adults emerged per treatment (*L. invasa* and *Q. mendeli)* were adjusted over time sing zero-inflated mixed nonlinear regression models, considering it as a covariate (Nelder and Wedderburn, 1972; Min and Agresti, 2005). The components of the zero-inflated models were: a logistic (binomial) component modeling the presence-absence of adults of the insects and a Poisson component modeling non-zero numbers. A linear effect with repeated measures over time was considered for the binomial model and a quadratic exponential effect with repeated measures of time and random intercept for the Poisson model. These models were adjusted using a nlmixed procedure (from the SAS statistical program – Free Statistical Software, SAS University Edition).

A significance level of 5% was considered for the zero-inflated, and Poisson models and their goodness-of-fit assessed using standardized Pearson residuals, Q-Q plots, and deviation plots.

The expected mean number of *L. invasa* and *Q. mendeli* adults over time was estimated using a quadratic exponential function with this choice based on the expectation that the number of emergent adults would follow a non-linear pattern, increasing initially, reaching a peak, and decreasing over time in a quadratic form. The quadratic term was used to represent the expected trend of adult emergence describing the average number of emerging ones over time and considering the data variability. The estimated mean number of adults was given by Equation 1:

$$\hat{\mu} = exp\left(\hat{\beta}_0 + \hat{\beta}_1 t + \hat{\beta}_2 t^2\right) \tag{1}$$

Where $\hat{\beta}_0, \hat{\beta}_1$ and $\hat{\beta}_2$ are the estimated parameters of the model. The negative sign in the quadratic term indicates a Gaussian-like curve with a shape of the normal probability distribution allowing to identify a single peak in the emergence. Additionally, a random intercept was included in the model to account for individual variations reflecting the unmeasured heterogeneity between eucalyptus seedlings that could affect the results of emergence rates.

The period to estimated maximum mean number (T_{max}) and the value of emerged insects (N_{max}) are given by Equations 2 and 3:

$$T_{max} = -\frac{\hat{\beta}_1}{2\hat{\beta}_2} \tag{2}$$

$$N_{max} = exp\left(\hat{\beta}_0 + \hat{\beta}_1\left(-\frac{\hat{\beta}_1}{2\hat{\beta}_2}\right) + \hat{\beta}_2\left(-\frac{\hat{\beta}_1}{2\hat{\beta}_2}\right)^2\right) \tag{3}$$

The effect of treatments and period on parasitism rate was analyzed using a generalized linear model with a gamma distribution and logarithmic link function (Nelder and Wedderburn, 1972), utilizing the genmod procedure (from SAS - Free Statistical Software, SAS University Edition). The generalized equation (Generalized Estimating Equations- repeated statement in the genmod procedure) was estimated due to repeated measures. The means between treatments were compared using the Tukey-Kramer test (Nelder and Wedderburn, 1972).

3. Results

The total number of *Q. mendeli* adults emerged was greater in the applied (29.58 ± 3.63) than in the natural (7.41 ± 1.40) ($p > 0.05$) biological control treatment (Table 1).

The total number of adults per *L. invasa* female was similar between treatments ($p < 0.0660$) in the natural (49.22 ± 4.70) and applied (34.65 ± 5.46) ($p > 0.05$) biological control (Table 1).

The number of *L. invasa* and *Q. mendeli* adults emerged followed a quadratic curve over time in the applied and natural biological control treatments, emerging from the tenth to the 72nd day after releasing the female parasitoids, increasing to a peak followed by a decrease in both ones (Figure 1). The early emergence (from day 10) observed in both treatments was due to the presence of parasitized galls containing pupae or late-instar larvae of *Q. mendeli* at the start of the experiment. Day 10 was adopted as the baseline for comparison across treatments, as it marked the onset of adult emergence. This standardized starting point allowed for consistent analysis and direct comparison of the results.

To describe and compare the temporal dynamics of adult emergence between treatments, the data were fitted to a zero-inflated mixed nonlinear regression model. The parameters β_0, β_1, and β_2 describe the shape of the emergence curve, while T_{max} and N_{max} indicate the timing and magnitude of peak emergence, respectively. These metrics allow assessment of treatment effects beyond total emergence counts. The parameters β_0, β_1 and β_2 of the fitted

Table 1. Total number of adults (mean ± standard error) of *Leptocybe invasa* (Hymenoptera: Eulophidae) and *Quadrastichus mendeli* (Hymenoptera: Eulophidae) adults emerged from eucalyptus seedlings under treatments simulating applied and natural biological control (Temp.: 25 ± 2 °C, RH: 70 ± 10%, and 12-hour photoperiod).

Treatment	*Leptocybe invasa*	*Quadrastichus mendeli*
Applied biological control	34.65 ± 5.46 a	29.58 ± 3.63 a
Natural biological control	49.22 ± 4.70 a	7.41 ± 1.40 b

Means followed by the same lowercase letter per column do not differ by the Tukey-Kramer test ($p<0.05$).

Gaussian model, and the estimated maximum number of *L. invasa* adults emerged, were lower in the applied (3.73 individuals) than in the natural (6.12 individuals) biological control treatment. (Table 2).

The maximum number of *Q. mendeli* adults emerged was higher in the applied (5.08 adults) than in the natural (1.10) biological control, as indicated by their $\hat{\beta}_0, \hat{\beta}_1$ and $\hat{\beta}_2$ (Table 2).

The estimated parameters and p-values (in parentheses) for the intercept and the time period coefficient in the fitted binomial component of the zero-inflated nonlinear mixed model were -5.9068 (<0.0001) and 0.1151 (<0.0001) for natural biological control, and 2.5732 (0.0065) and -0.1314 (0.0002) for applied biological control.

The parasitism rate of *L. invasa* by *Q. mendeli* was similar between treatments from the 10 to 20 th and from the 20 to 30 th days, higher in the applied biological control from the 30 to 40th days and 40 to 50 th days, and constant from 50 to 60 th days (Table 3).

4. Discussion

The greater number of *Q. mendeli* adults in the applied biological control treatment confirms the successful establishment of this parasitoid, with satisfactory adaptation in the laboratory, greenhouse, and field (Bush et al., 2017). The use of applied biological control increases the population of this parasitoid on eucalyptus seedlings with host galls. However, no statistically significant reduction in *L. invasa* was observed during the evaluation period, despite the higher number of *Q. mendeli* in the treated plants. This suggests that the impact on the pest population may require longer evaluation periods to be detected. The mass-rearing of *Q. mendeli*, similarly to *Selitrichodes neseri* (Kelly & La Salle) (Hymenoptera: Eulophidae), facilitates its use in applied biological control of *L. invasa* (Gualtieri et al., 2017; Le et al., 2018). Additionally, the parasitoid's potential for rapid spread and adaptability to different environments enhances its promise as a biocontrol agent. Nevertheless, further studies are needed to determine the effectiveness of this strategy under varying conditions and time frames (Mendel et al., 2017; Gevers et al., 2021b).

The similar total number of *L. invasa* adults between treatments is attributed to the short evaluation period of 62 days, which was sufficient only to observe parasitism by *Q. mendeli* during a single generation on eucalyptus seedlings, as reported for its development in Israel (Mendel et al., 2004). This period was defined based on the biological cycle of *L. invasa* and the time required to complete one generation of *Q. mendeli* under the experimental conditions. Additionally, the release rate of five *Q. mendeli* females per plant was based on preliminary studies and protocols adopted in similar experiments reported in the literature, aiming to simulate a realistic initial inoculation scenario. However, the high number of *L. invasa* individuals, even with natural biological control, highlights the potential need for higher release doses to increase the effectiveness of this method (Huang et al., 2018). The spread and adaptation of *L. invasa* under different climatic conditions and with multiple lineages in some regions (Otieno et al., 2019; Zhang et al., 2021) increase the importance of integrated management with this parasitoid to reduce damage by this pest (Dittrich-Schröder et al., 2014; Lin et al., 2021; Wondafrash et al., 2021).

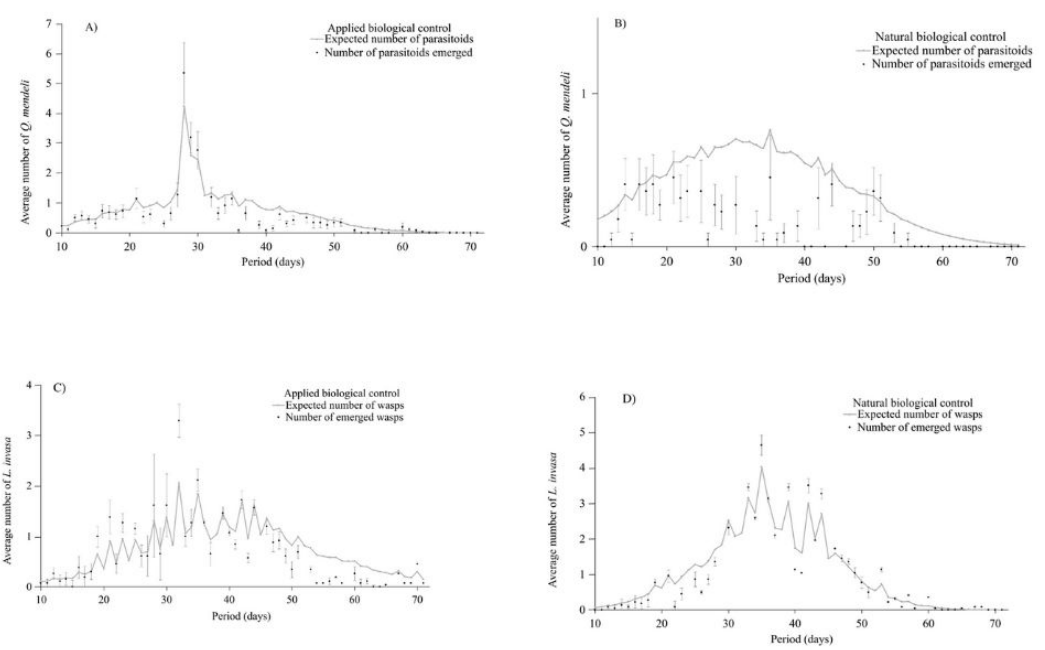

Figure 1. Numbers of expected and emerged *Quadrastichus mendeli* (Hymenoptera: Eulophidae) and *Leptocybe invasa* (Hymenoptera: Eulophidae) adults over time in the applied (A and C) and natural (B and D) biological control.

Table 2. Estimates, standard error of the estimate (Est./SE), and 95% confidence intervals (Int. Conf.) for the parameters of the zero-inflated mixed nonlinear regression model over time, and mean time (days) for the maximum and average maximum number of *Leptocybe invasa* (Hymenoptera: Eulophidae) and *Quadrastichus mendeli* (Hymenoptera: Eulophidae) adults emerged in the applied and natural biological control treatments (Temp.: 25 ± 2 °C, RH: 70 ± 10%, and 12-hour photoperiod).

Parameters	Treatment	Est/ SE	Confidence Intervals
		Leptocybe invasa	
β_0	Applied Biological Control	3.67800 ± 0.3046	(-4.3054; -3.0505)
	Natural Biological Control	-5.8780 ± 0.5573	(-7.0369; -4.7191)
β_1	Applied Biological Control	+0.20590 ± 0.01603	(+0.1729; +0.2389)
	Natural Biological Control	+0.3955 ± 0.02716	(+0.3390; +0.4520)
β_2	Applied Biological Control	-0.00268 ± 0.000247	(-0.00320; -0.00220)
	Natural Biological Control	-0.00555 ± 0.000328	(-0.00624; -0.00487)
Time(2)	Applied Biological Control	33.77 ± 1.5505	(30.58; 36.96)
	Natural Biological Control	35.86 ± 0.5617	(33.75; 37.98)
Number(3)	Applied Biological Control	3.73 ± 0.4922	(2.71; 4.74)
	Natural Biological Control	6.12 ± 0.5617	(4.95; 7,29)
		Quadrastichus mendeli	
β_0	Applied Biological Control	-3.3498 ± 0.3351	(-4.0399; -2.6597)
	Natural Biological Control	-3.0522 ± 0.5857	(-4.2702; -1.8342)
β_1	Applied Biological Control	+0.2637 ± 0.01943	(+0.2237; +0.3037)
	Natural Biological Control	+0.1607 ± 0.03533	(+0.08728; +0.2342)
β_2	Applied Biological Control	-3.3498 ± 0.000311	(-0.00502; -0.00374)
	Natural Biological Control	-0.00244 ± 0.000537	(-0.00355; -0.00132)
Time(2)	Applied Biological Control	28.69 ± 0.7741	(27.10; 30.29)
	Natural Biological Control	30.14 ± 1.6440	(26.72; 33.56)
Number(3)	Applied Biological Control	5.08 ± 0.7850	(3.46; 6.70)
	Natural Biological Control	1.10 ± 0.1268	(0.84; 1.36)

(2) Average period (days) for the maximum number of *Leptocybe invasa* and *Quadrastichus mendeli* adults; (3) Maximum emergence of *Leptocybe invasa* and *Quadrastichus mendeli* adults.

Table 3. Parasitism rate (%) of *Leptocybe invasa* (Hymenoptera: Eulophidae) by *Quadrastichus mendeli* (Hymenoptera: Eulophidae) and emergence of this parasitoid per period from 10 to 70 days under applied and natural biological control (Temp.: 25±2 °C, RH: 70±10%, and 12-hour photoperiod).

Period (Days)	Applied Biological Control	Natural Biological Control
10 – 20	63.1 ± 6.03 Aa (0.0; 100)	52.3 ± 9.34 Aa (0.0; 100)
20 – 30	61.2 ± 4.36 Aab (12.5; 100)	30.2 ± 5.89 Ab (0.0; 100)
30 – 40	37.8 ± 5.64 Ab (0.0; 100)	4.1 ±1.75 Bc (0.0; 100)
40 – 50	36.3 ± 5.86 Ab (0.0; 100)	11.1 ± 2.75 Bc (0.0; 37.5)
50 – 60	34.8 ± 8.22 Ab (0.0; 100)	24.2 ± 9.02 Ac (0.0; 44.44)
60 - 70	19.2 ± 12.31 b (0.0; 100)	-

Means followed by the same uppercase letter per row (between treatments) or lowercase letter per column (between periods) do not differ by the Tukey-Kramer.

The number of *L. invasa* and *Q. mendeli* adults, adjusted to a quadratic curve in the applied and natural biological control treatments, with emergence from the tenth to the 72nd day after releasing female parasitoids, indicates more than one generation for this natural enemy. The increase in the number of *L. invasa* and *Q. mendeli* individuals to a peak followed by a decline in both treatments confirm at least two generations of this parasitoid on hosts in the same eucalyptus plant, with a biological cycle of 27 to 30 days (Kim et al., 2008; Sangtongpraow and Charernsom, 2019). Additionally, the shorter biological cycle, compared to its host *L. invasa*, may increase the population of *Q. mendeli* and, consequently, its efficiency in the biological control reducing populations of this pest and its damage (Mendel et al., 2004; Huang et al., 2018; Sangtongpraow and Charernsom, 2019).

The lower values of the parameters β_0, β_1 and β_2, of the fitted Gaussian model, and the estimated maximum number of *L. invasa* adults emerged in the applied biological control, are due to the release of the parasitoid *Q. mendeli*, simulating this method. This is similar to the reduction in the number of individuals of this pest after releasing the parasitoids *Aprostocetus gala* Walker (Hymenoptera: Eulophidae), *Megastigmus* sp., and *Megastigmus dharwadicus* Narendran & Vastrad (Hymenoptera: Megastigmidae) in greenhouses and/or field in India (Ramanagouda and Vastrad, 2015; Gevers et al., 2021a; Lazaro et al., 2023), and *Megastigmus* spp. Dalman (Hymenoptera: Megastigmidae) in Indonesia (Tavares et al., 2023). The reduction in the maximum number of *L. invasa* adults after the release of the parasitoid *Q. mendeli* on eucalyptus seedlings in the nursery, simulating applied biological control, confirms a decrease in the pest population and, consequently, its damage, as reported in other regions of the world (Huang et al., 2018; Sangtongpraow and Charernsom, 2019; Yousuf et al., 2020). Releases of parasitoids are important because their populations and those of their hosts vary with edaphoclimatic conditions and genetic materials of *Eucalyptus* (Guo et al., 2020, 2021; Gevers et al., 2021a).

The higher number of *Q. mendeli* adults in the applied biological control is due to simulating inundative releases (Huang et al., 2018), reinforcing this strategy, even with the presence of the parasitoid, to reduce damage by the pest *L. invasa*. Mass rearing the parasitoid *Q. mendeli* under controlled conditions allows its application and population increase in regions with *L. invasa* damage (Masson et al., 2017; Oates et al., 2020). The rapid reproduction rate and short generation contribute to the population increase of this parasitoid in the field (Huang et al., 2018).

The parasitism rate of *L. invasa* by *Q. mendeli* in the applied and natural biological control stabilized on eucalyptus seedlings after a certain period, but it was higher using the first method. This confirms the importance of parasitism by *Q. mendeli* in the integrated management of *L. invasa*, as reported in China, India, Italy, and Tanzania (Nugnes et al., 2016; Zheng et al., 2016; Garonna et al., 2018).

5. Conclusion

The hypothesis that the controlled introduction of the parasitoid *Q. mendeli* would reduce the *L. invasa* population was only partially supported. Although the applied biological control treatment resulted in a numerical decrease in the mean number of *L. invasa* adults compared to the natural biological control, this difference was not statistically significant. The similar emergence of *L. invasa* adults between the two methods suggests that other factors, such as overlapping generations or variation in gall development stages, may have influenced the population dynamics of this insect. The total number of *Q. mendeli* adults was higher in the applied biological control, following the release of this parasitoid. Additionally, the quadratic fit over time for the emergence of adults of both species indicates the occurrence of multiple generations of this parasitoid in the same host. The greater effectiveness of applied biological control confirms that this method is promising to reduce *L. invasa* populations and its damage to eucalyptus plants.

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Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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