

E-ISSN: 2320-7078 P-ISSN: 2349-6800 www.entomoljournal.com JEZS 2023; 11(5): 172-177 © 2023 JEZS Received: 26-05-2023 Accepted: 28-06-2023

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# Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com



### Biology and life table of *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* (Hemiptera: Aphididae) on *Brassica oleracea* var. *acephala*

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#### DOI: https://doi.org/10.22271/j.ento.2023.v11.i5c.9245

#### Abstract

The aphid Brevicoryne brassicae is a key pest of brassicas (cabbage). However, Lipaphis pseudobrassicae is increasingly common in brassica areas, and as it is better adapted to high temperatures, it can become the main pest in warmer seasons or in regions with higher temperatures. Biological research has aided in the management of aphids in different crops, reducing the use of insecticides. However, to accomplish this and ensure agricultural sustainability, we need to understand the biological parameters of insect pests associated with crops. Therefore, this study aimed to evaluate the biological parameters and population development of L. pseudobrassicae and B. brassicae in the laboratory. Nymphs at 24 h of age were maintained on cabbage (Brassica oleracea L. var. acephala) seedlings (25±2 °C, RH: 60% and 12 h photophase) and monitored at 24 h intervals until death. The immature phase was similar between B. brassicae (6.46 days) and L. pseudobrassicae (6.57 days) with high mortality of 1st and 2nd instar B. brassicae nymphs. Adults of B. brassicae lived longer in the preand post-reproductive periods, but a shorter time in the reproductive period. Time interval of each generation (T), innate ability to increase in number ( $r_m$ ), and finite rate of increase ( $\lambda$ ) were similar between species. The specific fertility of B. brassicae was superior to that of L. pseudobrassicae. However, the net reproduction rate (Ro) of L. pseudobrassicae (17.62) was higher than that of B. brassicae (9.98). The time required for the population to double the number of individuals (TD) was shorter for L. pseudobrassicae (3.62 days) when compared to B. brassicae (4.37 days). These results indicate that L. pseudobrassicae may become a more relevant aphid for brassicas in Brazil, compared to B. brassicae.

Keywords: Aphids, population dynamics, growing parameters, pest management

#### Introduction

The aphid *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae) is an important pest of the Brassicaceae. The damage caused by *B. brassicae* occurs from feeding, causing wilting and wrinkling of leaves (Zawadneak *et al.*, 2015)<sup>[41]</sup> and virus transmission (Jesus & Mendonça, 2012)<sup>[21]</sup>. Although *B. brassicae* is the most important aphid associated with Brassicaceae in Brazil, the entomofauna of insect pests associated with a crop may change. Indeed, changes do occur as a result of adaptation of native species (Botton *et al.*, 2009)<sup>[11]</sup> and introduction of exotic species (Lopes *et al.*, 2016)<sup>[24]</sup>. In 2006, *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae) was recorded in kale areas of Brazil (Resende *et al.*, 2006)<sup>[31]</sup>. *L. pseudobrassicae* originates from the Palearctic region, and, like *B. brassicae*, it also causes damage when feeding, as a vector of several plant viruses (Blackman & Eastop, 2007)<sup>[10]</sup>.

The control of aphids in cabbage is based on chemical insecticides, such as organophosphates and neonicotinoids. However, abusive use of insecticides has caused negative effects on the environment and nontarget organisms (Almeida *et al.*, 2007) <sup>[2]</sup>. The registration of *L. pseudobrassicae* may increase the use of insecticides in cabbage plantations. In order to reduce the use of chemical insecticides, it is necessary to employ strategies based on Integrated Pest Management (IPM) (Deguine *et al.*, 2021) <sup>[15]</sup> which will enable agricultural sustainability and reduced use of chemical insecticides (Barzman *et al.*, 2015) <sup>[6]</sup>. However, to ensure successful implementation of an IPM program, the biological parameters of insect pests associated with particular crops must be established (Conte *et al.*, 2010) <sup>[14]</sup>.

Life and fertility tables make it possible to understand biological parameters of insects (Taghizadeh, 2019)<sup>[37]</sup>. These tables provide information on reproduction, development, longevity, fecundity, survival and population growth of pests (Chi & Hy, 2021)<sup>[12]</sup>, allowing an understanding of their population dynamics in a given host under given environmental conditions (Silveira Neto et al., 1976) [34]. While research on the biology of aphids has provided important information for the implementation and improvement of IPM strategies in different cultures (Bernardi et al., 2012; Conte et al., 2010; Ouereshi & Michaud, 2005)<sup>[8,</sup> <sup>14, 29]</sup>, the biological parameters of insect pests associated with particular crops must still be established. Therefore, in order to contribute to the development of effective IPM strategies in the management of aphids in Brassicaceae, we evaluated the biological parameters and population development of L. pseudobrassicae and B. brassicae in the laboratory.

#### Materials and Methods

#### Production of kale (Brassica oleracea L. var. acephala)

Seedlings were obtained from seeds not treated with insecticides and fungicides, sown in commercial substrate (Biomix<sup>®</sup>) and kept under protected cultivation. Upon reaching two true leaves, the seedlings were washed in running water to remove excess substrate from the roots and placed in glass bottles (10 mL) containing sterilized distilled water. To fix the seedling and seal the flask, a cotton plug wrapped in the hypocotyl was used. Subsequently, the aerial part underwent superficial disinfection (Pacheco *et al.*, 2017) <sup>[27]</sup>.

## Brevicoryne brassicae and Lipaphis pseudobrassicae rearing

The rearing of *B. brassicae* and *L. pseudobrassicae* started from insects collected in areas of cultivation of Brassicaceae (27°41'08.9" S: 48°32'37.5" W). The insects were transported to the laboratory, and after the elimination of natural enemies, they were transferred to cages containing kale plants and kept at  $25\pm2$  °C, Relative Humidity (RH):  $60\pm10\%$  and 12 h photophase.

### Biology of Brevicoryne brassicae and Lipaphis pseudobrassicae

The biology was verified by inoculation of adult aphids in kale seedlings. Seedlings inoculated with an adult *B. brassicae* or *L. pseudobrassicae* were isolated in cages (500 mL) and kept at  $25\pm2$  °C, RH:  $60\pm10\%$  and 12 h photophase. After 24 h, the females were removed, keeping one nymph up to 24h per seedling.

Inspections were carried out at 24 h intervals until the death of the insect. During the immature phase, the development of each instar was evaluated through the collection of exuvia and dead nymphs. After aphids reached adulthood, the prereproductive, reproductive and post-reproductive periods, number of daily offspring and total number of off spring were evaluated. After counting, the nymphs were removed from the leaves to prevent recounting in the next evaluation. The experiment was carried out in a completely randomized design. For each aphid species, 100 repetitions were performed. Each replicate was formed by a cabbage seedling with an aphid.

#### Analysis

Fertility life table parameters were based on Andrewartha and

Birch (1954). Population growth parameters (Ro, T,  $r_m$ ,  $\lambda$  and TD) were calculated based on the Jackknife method (Meyer *et al.*, 1986) <sup>[24]</sup>. Comparisons of population growth parameters of *B. brassicae* and *L. pseudobrassicae* were performed using the one-sided *t*-test, using the tabvida program (Penteado *et al.*, 2010) <sup>[28]</sup>.

Average duration of each instar, total period of development of the nymphs, mortality of each instar, total mortality of nymphs, duration of pre-reproductive, reproductive and postreproductive periods, longevity of adults, number of nymphs/female, total biological cycle and total longevity were all submitted to normality and homoscedasticity analysis. Subsequently, means of the biological parameters were submitted to Student's *t*-test or to the non-parametric Mann-Whitney test (U-test), using the Minitab 18.0 program.

#### Results

Four instars each developed for *B. brassicae* and *L. pseudobrassicae*. The mean duration of each instar and duration of the juvenile period were similar between species with a total developmental period of 6.46 days for *B. brassicae* and 6.57 days for *L. pseudobrassicae* (Figure 1). No significant differences were observed in the duration of each instar or total time of nymphal development between the species (Figure 1).

The mortality of 1<sup>st</sup> instar nymphs was 73.6% higher for *B. brassicae* when compared to *L. pseudobrassicae* (Table 1), which, for immatures, was 69.7% in the 2<sup>nd</sup> instar, 47.4% in the 3<sup>rd</sup> instar, and 24.0% in the 4<sup>th</sup> instar, albeit no significant differences. However, mortality that accumulated in the different developmental instars significantly influenced the total mortality of nymphs during the juvenile phase. At this stage, mortality of *B. brassicae* (Table 1).

Adults of *B. brassicae* had a pre-reproductive period (2.5 days) 48% longer than that observed for *L. pseudobrassicae* (1.3 days) (Table 2). *L. pseudobrassicae* had a reproductive period of 18.9 days, 32.3% longer than that observed for *B. brassicae* (12.8%) (Table 2). The post-reproductive period, where adults remain alive, but do not generate offspring, was 33.3% longer for *B. brassicae*, which is longer than the post-reproductive period for *L. pseudobrassicae* (Table 2). Adult longevity, total number of nymphs/female and total life cycle of *B. brassicae* and *L. pseudobrassicae* did not show significant differences, indicating a similar biological period between species (Table 2).

The reproductive period of *L. pseudobrassicae* started between the 5<sup>th</sup> and 6<sup>th</sup> day of life, and for *B. brassicae*, it started between the 6<sup>th</sup> and 7<sup>th</sup> day (Figure 2). The maximum rate of increase (maximum specific fertility peak) of *B. brassicae* occurred on the 13<sup>th</sup> day with an increase of 1.83 female/female, and for *L. Pseudobrassicae*, the maximum value was 1.74 female/female on the 8<sup>th</sup> day (Figure 2). The mean specific fertility of *B. brassicae* was 1.68 female/female, and that of *L. pseudobrassicae* was 1.35 female/female.

The net reproduction rate (Ro) was significantly higher for *L. pseudobrassicae*, indicating that this species has a theoretical capacity to increase 17.62 times from one generation to another with a mean time between generations (T) of 15 days (Table 3). The net reproduction rate (Ro) of *B. brassicae* was 9.98 times with a mean time between generations (T) of 14.52 days. The innate capacity for growth ( $r_m$ ) shows how a population develops under constant environmental conditions,

expressing the biotic potential of a species because the higher the value of  $r_m$ , the more successful the species will be. Here, the  $r_m$  was positive for both species, indicating that both have growth potential. The  $r_m$  of *L. pseudobrassicae* (0.19) was greater than that of *B. brassicae* (0.15) (Table 3). The values obtained for finite increase ratio ( $\lambda$ ), which is the multiplication factor of the original population at each unitary time interval, were 1.18 females/day to be added to the population of *B. brassicae* and 1.21 female/female/day for *L. pseudobrassicae*, and no differences were found between the two means. The time taken for the aphid population to double in number (TD) was 4.37 for *B. brassicae* and 3.62 for *L. pseudobrassicae*. Thus, it can be inferred that *B. brassicae* and *L. Pseudobrassicae* can, in approximately four days, manage to double their populations (Table 3).

#### Discussion

The rapid development of *B. brassicae* and L pseudobrassicae nymphs is a characteristic of the Aphididae. The period for aphids to reach adulthood is influenced by biotic and abiotic factors, such as nutritional quality of the host (Lazzari & Zonta de Carvalho, 2009)<sup>[23]</sup>, with the plant and its phenological stage influencing the development of B. brassicae and L. pseudobrassicae (Aslam et al., 2011; Fatemeh et al., 2014; Rana, 2005; Yue & Liu, 2000) [4, 16, 30, <sup>40]</sup>. The mean time for *B. brassicae* to reach adulthood was shorter in cauliflower (8.9 days) when compared to mustard (9.5 days), broccoli (9.8 days), canola (10.2 days) and cabbage (10.4 days) (Ulusov & Olmez-Bayhan, 2006)<sup>[39]</sup>. For L. pseudobrassicae, the developmental period in canola varieties ranged from 7.13 to 8.91 days (Taghizadeh, 2019) <sup>[37]</sup>, and in cabbage varieties, it ranged from 6.1 to 7.0 days (Yue & Liu, 2000)<sup>[40]</sup>. In this context, the rapid development of B. brassicae and L. pseudobrassicae in kale may indicate the nutritional quality of the host, which, in addition to enabling rapid development, can provide greater progeny (Lazzari & Zonta-de-Carvalho, 2009)<sup>[23]</sup>.

High mortality characterized the 1<sup>st</sup> and 2<sup>nd</sup> instar nymphs of *B. brassicae*. High mortality in the first stages of development is common in insects (Gallo *et al.*, 2002) <sup>[17]</sup>. To achieve higher survival rates in environments with scarce or limiting resources, aphids generate few individuals with more adipose reserves and, in optimal environments, they generate more nymphs with less vitality (Lazzari & Zonta-de-Carvalho, 2009) <sup>[23]</sup>, depending on external alimentation (Tsai & Wang, 2001) <sup>[38]</sup>. With the succession of instars, the mortality of *B. brassicae* decreased, which may indicate better use of food resources (Blackman, 1978; Bermingham & Wilkinson, 2009) <sup>[9, 7]</sup>. The mortality of *L. pseudobrassicae* nymphs was lower than that of *B. brassicae*. The low mortality could be attributed to a lower responsiveness of *L. pseudobrassicae* to the host such that its biology was less affected compared to

#### that of *B. brassicae* (King *et al.*, 2006)<sup>[22]</sup>.

The aphid *L. pseudobrassicae* had a shorter pre- and post-reproductive period and a longer reproductive period. Preand post-reproductive, as well as reproductive, phases vary considerably in aphids, depending on the influence of the host, in addition to species biology (Blackman & Eastop, 2007) <sup>[10]</sup>. A plant can be defined as a host because it allows the insect to complete its biological cycle (Souza *et al.*, 2022) <sup>[36]</sup>. However, each plant species has variations in nutritional quality, as well as distinct morphological and biochemical characteristics (Agarwala *et al.*, 2009; Aziz *et al.*, 2016) <sup>[1, 5]</sup> that can favor or harm the insect.

The population development of *L. pseudobrassicae* and *B. brassicae* was similar. Although population development parameters, such as T,  $r_m$ ,  $\lambda$  and TD, did not differ significantly, *L. pseudobrassicae* had the highest net reproduction rate (Ro). Ro indicates the average number of females born in the lifetime of each female, and if this value is greater than one (> 1.0), population growth is occurring (Horm, 1988) <sup>[20]</sup>. Ro is used to evaluate the artificial creations of insects since it is an innate characteristic of the population (Garcia *et al.*, 2006) <sup>[18]</sup>.

The aphid L. pseudobrassicae may become a key pest of Brassicaceae in Brazil. Even though B. brassicae and L. pseudobrassicae presented a similar total of nymphs/female, 1<sup>st</sup> and 2<sup>nd</sup> instar nymphs of *B. Brassicae* suffered higher mortality. The higher survival of L. pseudobrassicae could be attributed to its biology since it is less affected by the host (King *et al.*, 2006)<sup>[22]</sup>, generating a net reproduction rate (Ro) 43% higher than that observed for *B. brassicae*. Furthermore, temperature may favor the development of L. pseudobrassicae. L. pseudobrassicae and B. brassicae reach the highest growth rates at 25 °C (Cividanes, 2003; Godoy & Cividanes, 2002; Satar et al., 2005; Soh et al., 2018) [13, 19, 33, <sup>35]</sup>; however, *L. pseudobrassicae* is better adapted to high temperatures and able to cause more damage in the hottest times of the year and in regions with higher temperatures (Ronquist & Ahman, 1990)<sup>[32]</sup>.

The increase in global temperature will likely change the phytosanitary scenario of agriculture. As it is more adapted to high temperatures, climate change may, therefore, favor *L. pseudobrassicae*. Although *B. brassicae* is the main aphid that attacks brassicas in Brazil, *L. pseudobrassicae* is considered a more severe pest in other countries because it maintains high biotic potential, even under stress conditions (King *et al.*, 2006) <sup>[22]</sup>. Brazil is a tropical country with high temperatures, and since the biology of *L. pseudobrassicae* is less affected by the host (King *et al.*, 2006) <sup>[22]</sup> and has fewer natural enemies, such as parasitoids (Oliveira *et al.*, 2013) <sup>[26]</sup>, studies should be carried out with the objective of making aphid management strategies more effective in brassicas, focusing on *L. pseudobrassicae*.

 Table 1: Mortality (±SEM) at each developmental instar and throughout the immature period of *B. brassicae* and L. *pseudobrassicae*.

 \*Significant differences by the nonparametric Mann-Whitney test (p-value <0.05).</td>

Species	Mortality (%)					
	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	Total	
B. brassicae	17.8 (±5.8)	16.2 (±6.1)	9.7 (±5.4)	7.1 (±5.0)	42.2 (±7.4)	
L. pseudobrassicae	4.7 (±3.2)	4.9 (±3.3)	5.1 (±3.6)	5.4 (±3.8)	18.6 (±6.0)	
U-test	2129.5*	1547.5	1128,0	9933.0	2231.0*	
p-value	0.05	0.10	0.47	0.78	0.01	

 Table 2: Mean duration (±SEM) of different biological parameters of *B. brassicae* and *L. pseudobrassicae*. \* Significant differences by *t*-test for independent samples (p-value <0.05).</th>

<b>Dialactical management</b>		4	p-value	
Biological parameters	B. brassicae	icae L. pseudobrassicae		
Pre-reproductive period (days)	2.5 (±0.3)	1,3 (±0.1)	3.68	0.001*
Reprodutive period (days)	12.8 (±1.6)	18.9 (±1.5)	2.76	0.008*
Post-reproductive period (days)	8.1 (±1.6)	5.4 (±1.8)	1.13	0.037*
Adult Longevity (days)	19.5 (±2.1)	23.1 (±2.1)	1.22	0.227
Total nymphs/female	23.2 (±4.9)	25.5(±2.6)	0.41	0.682
Biological cycle (days)	26.0 (±2.1)	29.6 (±2.1)	1,21	0.231

**Table 3:** Net reproductive rate (Ro), mean interval between generations (T), intrinsic growth rate  $(r_m)$ , finite growth rate  $(\lambda)$ , and time for population to double (TD) for *B. brassicae* and *L. pseudobrassicae* kept in kale (*Brassica oleracea* var. *acephala*). \* Significant differences by *t*-test for independent samples (p-value <0.05).

Aphid	Parameters						
	Ro	Т	r <sub>m</sub>	λ	TD		
B. brassicae	9.98 (±0.07)	14.52 (±0.02)	0.15 (±0.00)	1.18 (±0.00)	4.37 (±0.00)		
L. pseudobrassicae	17.62 (±0.51)	15.00 (±0.43)	0.19 (±0.01)	1.21 (±0.03)	3.62 (±0.10)		
<i>t</i> -test	9.99*	0.72	0.42	0.21	2.18		
Significance	> 0.001	0.49	0.629	1.000	1.068		



**Fig 1:** Mean duration (±SEM) of each developmental instar and total developmental period of the immature phase of *B. brassicae* and *L. pseudobrassicae* in kale (*Brassica oleracea* var. *acephala*).





Fig 2: Life expectancy up to age x (l<sub>x</sub>) and specific fertility (m<sub>x</sub>) of *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* kept on kale (*Brassica oleracea* var. *acephala*).

#### Acknowledgments

CAPES – Research funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (nº 88882.345259/2019-01) and Maria Aparecida Cassilha Zawadneak, professor at the Federal University of Paraná, for identifying the aphid species.

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