

Life cycle and population parameters of *Aceria malherbae* in field bindweed: a laboratory approach

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Abstract

The weed *Convolvulus arvensis* L. (Solanales: Convolvulaceae) was introduced and is native to the Mediterranean region of Europe; it is currently widely distributed throughout the world. Eriophyid mites, in the case of weeds, are considered to have great potential as biological control agents; these attributes are due to their monophagous or oligophagous habit, which makes them highly specific. Within the mites associated with this plant, the species *Aceria malherbae* Nuzzaci (Acar: Eriophyidae) has been reported, a gall mite that inhabits the midribs of the leaves and causes their deformation. The high degree of specificity of the mite in feeding on *C. arvensis* makes it an optimal candidate for controlling this weed. The objective of this research was to determine the life cycle and population parameters (Ro, rm, TG, t2 and #) of *Aceria malherbae* on plants of field bindweed, *Convolvulus arvensis* (Solanales: Convolvulaceae). The experiment was conducted from June 2023 to June 2024; the collection took place in agricultural fields where the weed had previously been infested by members of the Local Board of Plant Health of the Yaqui Valley in Ciudad Obregón, Sonora. The samples were transferred to the Department of Agricultural Parasitology of the Antonio Narro Autonomous Agrarian University, and a mother colony of *A. malherbae* was established under laboratory conditions. *Aceria malherbae* completed its cycle in 12.29 days. The fecundity rate was 23.83 eggs laid per female on average in 13 days (e/f/d); likewise, the population parameters Ro, rm, and # were 18.87, 0.54, and 1.72, respectively. The development time of the second generation (TG) was 5.4, and the population doubling time (t2) was 1.27. The population parameters and life cycle determined in this study confirm the rapid growth of the populations of the mite *A. malherbae*; these metrics explain why it became an important biological control agent. The values obtained allowed us to understand the impact of its activity in the field when it encounters favorable conditions for its multiplication and development. This positions it as an important alternative for controlling weed *C. arvensis* and highlights the relevance of continuing research on its biology in order to promote its use.

Keywords:

Aceria malherbae, *Convolvulus arvensis*, population, weeds.

Introduction

Weeds are one of the main biotic factors affecting agricultural productivity as they compete for essential resources and negatively alter the crop environment through mechanisms such as allelopathy (Khamare *et al.*, 2022; Horvath *et al.*, 2023). Although the use of herbicides is a common strategy for their control, these products present environmental problems and have generated resistance in various species, which has led to the search for more sustainable alternatives, such as biological control (Weyl *et al.*, 2019; Alcántara-de la Cruz *et al.*, 2021).

Biological weed control (BWC) uses living organisms, such as pathogenic microorganisms or phytophagous arthropods, that are capable of suppressing populations of invasive species in a specific and effective way (Navarro *et al.*, 2015; Zimdahl, 2018; Sotelo#Cerón *et al.*, 2023). *Convolvulus arvensis* L. (field bindweed) is an invasive weed of Eurasian origin, with high adaptability to poor soils, drought, and pH variations, which has favored its global expansion and makes it a serious problem in agricultural and urban environments (Barreto *et al.*, 2017; Sosnoskie *et al.*, 2020; Guzmán-Mendoza *et al.*, 2022).

A promising biological control agent for this species is the eriophyid mite *Aceria malherbae* (Acar: Eriophyidae), which is highly specific and capable of producing galls that interfere with host plant development (Skoracka *et al.*, 2010; Marini *et al.*, 2021; Desnitskiy and Chetverikov, 2022). This study aimed to determine the life cycle and population parameters of *A. malherbae* on *C. arvensis* under laboratory conditions.

Materials and methods

The experiment was conducted at the Acarology Laboratory of the Department of Parasitology of the Antonio Narro Autonomous Agrarian University in Buenavista, Saltillo, Coahuila from June 2023 to June 2024.

Collection and establishment of biological material

The colony was established from biological material provided by the Local Board of Plant Health of the Yaqui Valley in Ciudad Obregón, Sonora, through the Center for the Reproduction of Beneficial Organisms of the Yaqui Valley (CREROB), by its Spanish acronym. To establish the colony, samples of infested galls collected in the field were placed on healthy field bindweed plants at the vegetative development stage (approximately four months); subsequently, small cuts were made at the level of the central vein of the leaf to facilitate feeding and ensure infestation. The development and maintenance of the colony were carried out in a Biotronette® chamber under controlled conditions of 25 ± 2 °C, relative humidity of 60-70%, and a photoperiod of 12:12 h (light-dark).

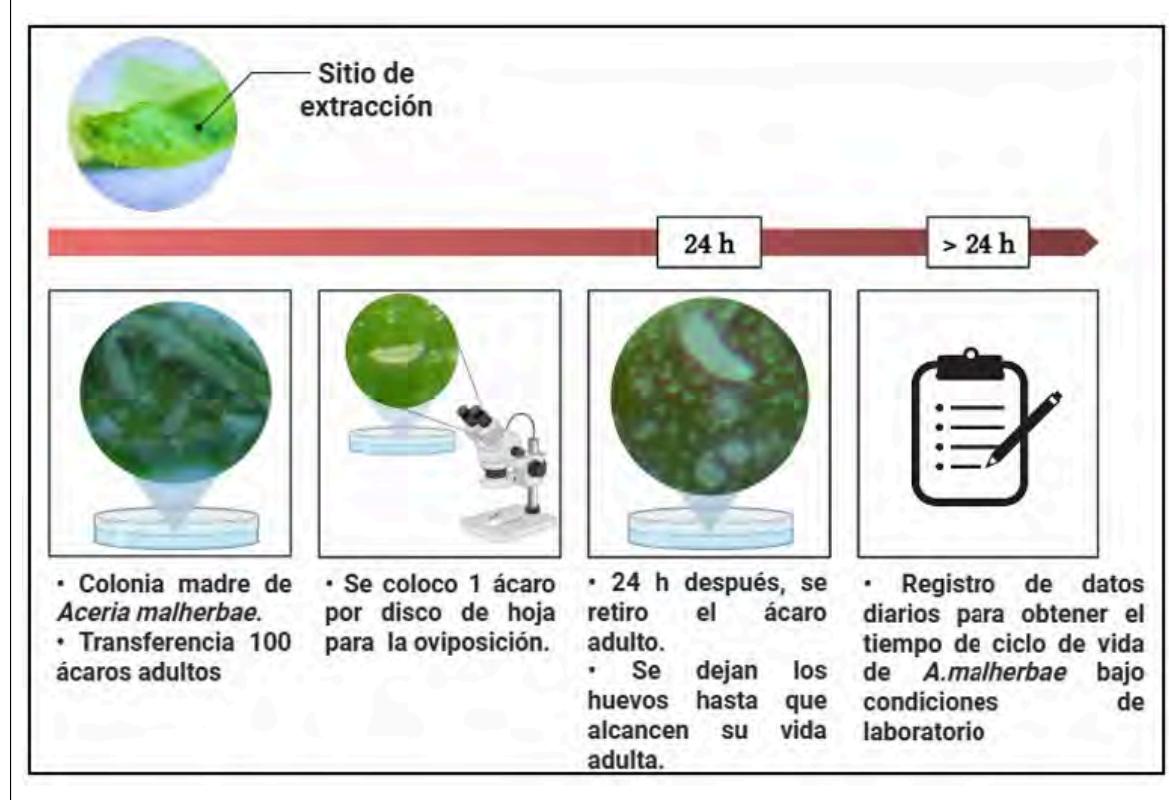
Determination of the life cycle of *Aceria malherbae*

To evaluate the life cycle, a modified methodology of the leaf-sand method was used, placing individuals on fresh leaves to record the developmental phases (Abou-Setta and Childers, 1987). To establish the experimental units, fresh and tender leaves of *Convolvulus arvensis* were placed in plastic Petri dishes with a diameter of 5 cm; at the bottom of these, a cotton pad moistened with distilled water to saturation was placed to keep the leaf turgid, thus allowing the specimens to develop. Likewise, the dishes had a hole in the lid, which was covered with organza fabric to allow ventilation.

To begin the evaluations, a leaf with distinctive signs of galls caused by the mite was selected and cut longitudinally to expose the mites. Subsequently, adult specimens were transferred using a micropin measuring 0.1 mm in thickness and 12 mm in length, which is commonly used for insect micromounting. Subsequently, an AmScope stereo microscope was used at a magnification of 90x to select 100 adult specimens; after 24 h, they were removed, and only one egg was left on the leaf to evaluate its development.

The dishes were labeled with the specimen number and date, and each individual was considered an experimental unit; they were placed in a Biotronette® Mark III Environmental Chamber under the aforementioned conditions and observations were made every 24 h to determine the development time of each mite stage (Figure 1).

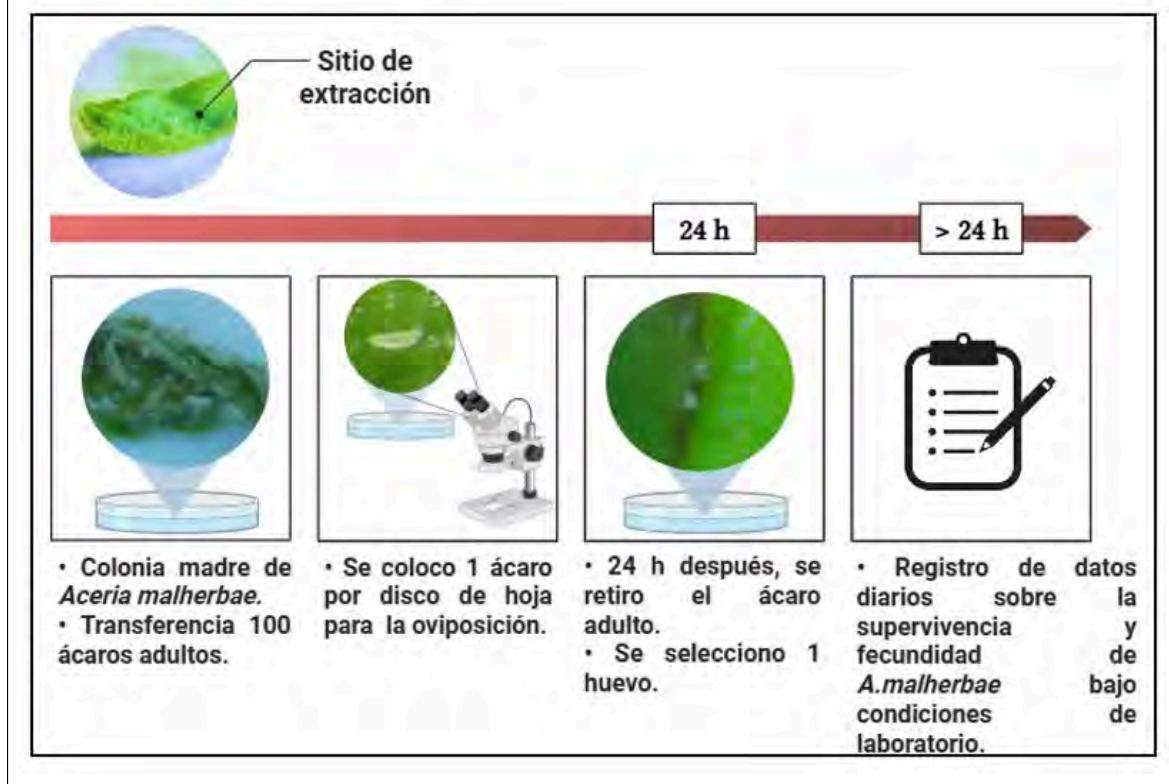
Figure 1. Methodology for the life cycle of *Aceria malherbae* under laboratory conditions.



Estimation of population parameters

To record mite survival, the modified methodology of the leaf-sand method was also employed, placing individuals on fresh leaves to record the phases of development. A total of 100 specimens in the egg stage were taken, which were placed individually in fresh field bindweed leaves, so that each experimental unit consisted of one specimen per leaf. From this moment on, daily records were kept of the survival and death of each individual that made up the offspring; these observations continued until the last adult specimen died (it was considered death when the mite under observation did not show movement). To determine the fecundity of each female, the number of eggs deposited on the leaf of each experimental unit was recorded daily (Figure 2).



Figure 2. Methodology for estimating population parameters of *A. malherbae*, under laboratory conditions.

Statistical analysis

The data on population parameters of *A. malherbae* were analyzed through Birch's formulas for measuring the natural intrinsic rate of population growth (Birch, 1948), which were analyzed using Excel software. The parameters to be evaluated were fecundity (m_x), which is defined as the total number of eggs deposited by a female; the net reproduction rate (R_o), which refers to the number of females a female produces in a generation where the individuals used in the fertility tests were assumed to be female, based on the fact that they oviposited during the experiment.

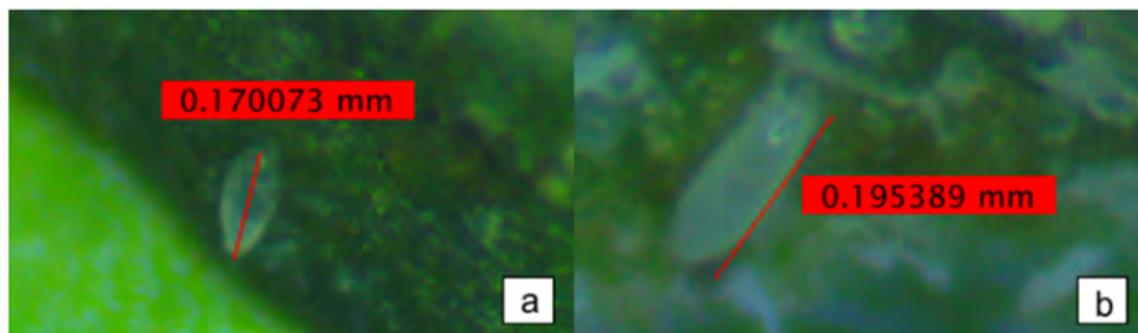
This approach is consistent with previous demographic studies in Eriophyidae, where direct identification of sex is not always feasible and is inferred from the presence of oviposition (Leiva, 2016; Revynthi *et al.*, 2023); the intrinsic growth rate (r_m), defined as the ability of a population to multiply over the span of one generation; the generation time (t_2), which is the average time between two successive generations; and the finite rate of reproduction (λ), which is the number of individuals per day. Additionally, a survival curve was developed only for female mites, based on the assumption that the mites evaluated corresponded to ovigerous females, responsible for the offspring recorded during the experiment.

Results and discussion

Life cycle of *Aceria malherbae* under laboratory conditions

The life cycle of *Aceria malherbae* consists of egg, larva, nymph and adult (Krantz and Walter, 2009); however, in the present project, a considerable variation in the size of the nymphs was observed, so two data were taken for this value and they were classified as 'nymph 1' and 'nymph 2', where nymph 1 measured from 0.15 to 0.17 mm and nymph 2 from 0.18 to 0.2 mm (Figure 3).

Figure 3. a) nymph 1= 0.15 to 0.17 mm; and b) nymph 2= 0.18 to 0.20 mm. Of *A. malherbae*, under laboratory conditions.



Egg-to-adult development under controlled laboratory conditions (25 ± 2 °C, 60-70% RH, and a 12:12 light-dark photoperiod) was completed in 12.29 ± 0.3 days. The incubation period of the egg was 1.77 days; the larval stage lasted 2.27 days; the nymph stages 1 and 2 lasted 2.3 and 2.44 days, respectively; to finish the complete cycle, the adult stage lasted 3.78 days (Table 1).

Table 1. Average life cycle duration of *Aceria malherbae*.

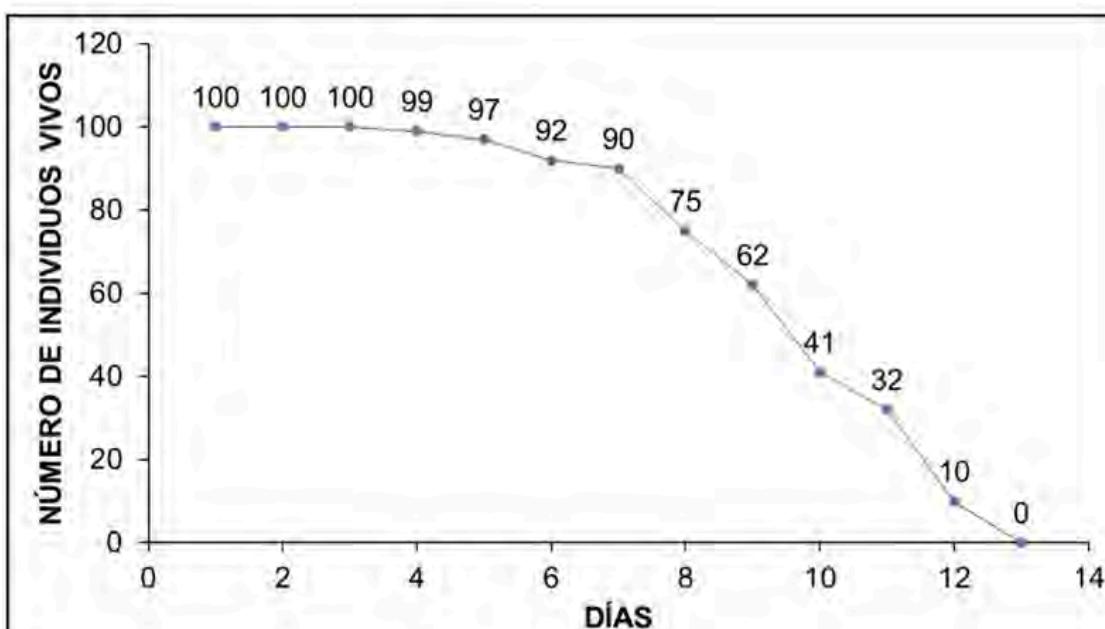
Stage	No. of individuals	Days (mean)	Mean \pm SE	Standard deviation	Minimum	Median	Maximum
Egg	100	1.77	0.644	0.644	3	4	5
Larva	100	2.27	0.06	0.609	4	6	6
Nymph 1	100	2.3	0.095	0.957	6	8	9
Nymph 2	100	2.44	0.088	0.88	9	10	11
Adult	100	3.78	0.045	0.456	12	12	13
Total		12.29					

On *C. arvensis* leaf discs under laboratory conditions (25 ± 2 °C, 60-70% RH and a photoperiod of 12:12 h light-dark).
SE= standard error.

Survival and mortality of *Aceria malherbae*

At the beginning of the survival and mortality testing, a total of 100 experimental units were established with a total of 100 live specimens (day 0). This value remained constant until the third day, so it was determined that 100% survival occurred only in this period. Subsequently, the progressive decrease in total individuals began to occur from the fourth day; on the eighth day, there was already a mortality of 25% with 75 individuals still alive; the trend continued similarly, so that on the tenth day, mortality reached 59% and on day 13, it reached 90%; the evaluations ended on day 13 when all individuals died. The mortality curve for mites was generated (Figure 4).

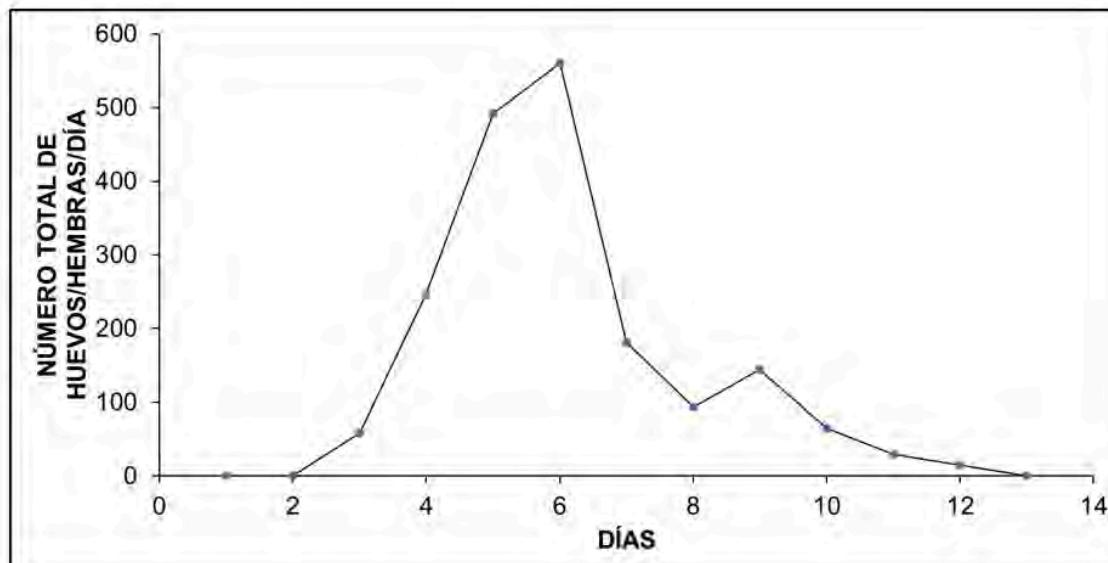


Figure 4. Survival and mortality of *Aceria malherbae* under laboratory conditions.

Fecundity by specific age

Regarding fecundity by specific age, the highest value was observed on day six, where a total of 560 eggs/female/day were obtained among the 100 experimental units. Fecundity continued to decrease; on day 11, there was a sum of 29 e/f/d, and it was not until day 13 that there was no response from the mites. Based on the above data, it was determined that females have reproductive potential, where they can deposit between 15 and 21 eggs in a period of 13 days (Figure 5).



Figure 5. Fecundity of *Aceria malherbae* eggs/females/day.


Population parameters

The results of the population parameters were calculated based on the survival and fecundity records obtained from the observations made every 24 h (Table 2).

Table 2. Formulas of Birch (1948) and results of population parameters of *A. malherbae*.

Symbol	Definition	Formula	Results
GRR	Gross reproductive rate: total females born/ mother across all X's	$\sum mx$	23.83
Ro	Net reproductive rate	$\sum lxmx$	18.87
rc	Approximation to the intrinsic growth rate	$\ln Ro/TC$	0.48
rm	Intrinsic growth rate	$\sum e^{-rmx} lx mx = 1(1) e^{rmt}$	0.54
λ	Infinite rate of growth	rmt	1.72
Tc	Cohort duration time	$\sum (lx mx X / \sum lx mx)$	6.07
TG	Generation time (one generation)	$\ln Ro / rm$	5.4
t2	Doubling time	$\ln 2 / rm$	1.27

The fecundity rate was 23.83 eggs laid per female on average in 12.29 ± 0.3 days (e/f/d). The population parameters Ro , rm and λ were 18.87, 0.54 and 1.72, respectively. In the case of the parameter regarding the time of formation of a generation (TG), it was 5.4, and the doubling time of the population (t2) was 1.27 (Table 3).



Table 3. Table of the life of females of *Aceria malherbae* on *C. arvensis* leaves

X (days)	nx	Ave daughters	Ix	Mx	IxmX	IxmX* X	r negative *x	EXP -rx	IxmX*EXP
1	100	0	1	0	0	0	-0.54	0.58	0
2	100	0	1	0	0	0	-1.09	0.34	0
3	100	58	1	0.58	0.58	1.74	-1.63	0.2	0.11
4	99	247	0.99	2.49	2.47	9.88	-2.18	0.11	0.28
5	97	492	0.97	5.07	4.92	24.6	-2.72	0.07	0.32
6	92	560	0.92	6.09	5.6	33.6	-3.26	0.04	0.21
7	90	182	0.9	2.02	1.82	12.74	-3.81	0.02	0.04
8	75	94	0.75	1.25	0.94	7.52	-4.35	0.01	0.01
9	62	145	0.62	2.34	1.45	13.05	-4.89	0.01	0.01
10	41	65	0.41	1.59	0.65	6.5	-5.44	0	0
11	32	29	0.32	0.91	0.29	3.19	-5.98	0	0
12	10	15	0.1	1.5	0.15	1.8	-6.53	0	0
13	0	0	0	0	0	0	-7.07	0	0
		1887		23.84	18.87	114.6			1

Under controlled laboratory conditions of 25 ± 2 °C, 60-70% RH, and a photoperiod of 12:12 h (light-dark). Age (x); Number of individuals at the beginning of X (nx); proportion of living individuals on each X (Ix); average daughters/mother/X (mx).

The results obtained provide specific and detailed observations on the biological cycle of *A. malherbae* under laboratory conditions. Similar studies conducted on other mite species of the family Eriophyidae show similarities in the length of the stages and the life cycle with the results obtained for *A. malherbae*. Leiva (2016) mentioned that the complete life cycle of *Aceria oleae* is 11.4 ± 0.4 days under controlled conditions of 25 ± 2 °C, 70% RH, and 14 h of light.

When comparing the fertility of *A. malherbae* with that of some species of eriophyids, it is determined that there are similar behaviors. Leiva (2016) mentions that the females of *Aceria oleae* in olive crops can deposit between 7 and 18 eggs in a period of 11 days. The population parameters and life cycle obtained in this study confirm the rapid growth of the populations of this mite and explain why it is an important biological control agent (Smith *et al.*, 2010; Cortat *et al.*, 2024). The values obtained for the generation time (TG) and doubling time (t2) demonstrate the biological capacity of *A. malherbae* and allow us to understand the impact of its activity in the field when it finds favorable conditions for its multiplication and development.

Conclusions

The results obtained in this study confirm that *Aceria malherbae* exhibited rapid development and high reproductive potential under controlled laboratory conditions, which supports its suitability as a biological control agent for *Convolvulus arvensis*.

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