

Article

Evaluation of the Egg Predator *Blattisocius tarsalis* (Mesostigmata: Blattisociidae) for the Biological Control of the Potato Tuber Moth *Tecia solanivora* under Storage Conditions

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Abstract: *Tecia solanivora* is the most prevalent pest causing damage to potato crops in fields in the Canary Islands, but even more so in the postharvest storage period. However, currently, there are no authorised chemical insecticides for potato storage facilities. Analysis of the viability of the predator mite *Blattisocius tarsalis* as a biological control agent for this moth was carried out. A study of the temperature effect showed *B. tarsalis* maintains predatory capacity in the range of 10–27 °C. Though predatory activity increases with temperature, no differences in mortality rates were observed between 10 and 20 °C (33.52 ± 2.44 and 40.14 ± 3.54% efficacy rate, respectively), nor between 25 and 27 °C (59.26 ± 4.59 and 75.19 ± 4.64% efficacy rate, respectively). Under microcosm conditions, at low pest infestation (10 eggs), *B. tarsalis* achieved the highest mortality of eggs at a density of 5 mites, with an efficacy rate of 91.67 ± 8.33%. At high infestation levels (50 eggs), maximum mortality was achieved with a density of 10 mites and efficacy of 98.52 ± 1.48%. The choice-assay showed no preference of *B. tarsalis* between *T. solanivora* and *Phthorimaea operculella*, suggesting this mite could be useful in mixed infestations of potato moths. The results show *B. tarsalis* is a very good candidate as a control agent in storage conditions and even in mixed infestations of *T. solanivora* and *P. operculella*.

Keywords: warehouse; natural enemies; predatory mite; non-refrigerated; efficacy; microcosm; *Phthorimaea operculella*



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1. Introduction

Potato crops (*Solanum tuberosum* L.) worldwide suffer important yield losses due to the potato moth complex (Lepidoptera: Gelechiidae). This pest complex consists of three species: the common potato tuber moth, *Phthorimaea operculella* (Zeller 1873); the Andean potato tuber moth, *Symmetrischema tangolias* (Gyen 1913); and the Guatemalan potato tuber moth, *Tecia solanivora* (Povolný 1970) [1]. While *P. operculella* is a cosmopolitan pest, *T. solanivora* and *S. tangolias* have more restricted distributions. In fact, the latter is not present in Europe [2], and neither is it present in the Canary Islands, where *P. operculella* and *T. solanivora* are currently common potato pests.

Tecia solanivora (TCS) is originally from Guatemala (1970), from the area between the isthmus of Tehuantepec in Mexico to northern Honduras and El Salvador. In a relatively short period, through successive shipments of seed potatoes, it has spread to Costa Rica (1970), Panamá (1973), Venezuela (1983), Colombia (1985), and Ecuador (1996). This insect was first detected in Tenerife (Canary Islands) in 1999, probably introduced from Venezuela [3]. In 2015, TCS was found in Galicia (mainland Spain), probably introduced from the Canary Islands, and spread quickly to the neighbouring region of Asturias [4–6].

In the Canary Islands, TCS produces great yield losses in potato crops, where it can damage more than 50% of harvested tubers in the field and 100% during storage, if the pest is not managed [7,8]. Moreover, the Guatemalan tuber moth has led to a reduction in cultivated area and an increase in production costs. If this pest is not controlled, it could cause a collapse in the local potato production, and many local potato varieties could be lost [9,10]. In the Iberian Peninsula, the management of the Guatemalan tuber moth is essential to prevent its spread through the rest of the European continent.

The severity of this pest has led to its declaration as a quarantine organism by the European Union, and eradication programs have been established in Galicia and Asturias. However, in the Canary Islands, TCS is so well established and widespread, it is assumed it cannot be eradicated [11,12].

In the field, the use of phytosanitary treatments to reduce their populations has shown to be ineffective [13]. This moth spends most of its lifetime under soil in a larval state inside the tuber or in pupal stage near the surface. Therefore, contact pesticides have a very limited effect, whereas systemic pesticides would be dangerous for both the environment and consumers' health [14,15]. Consequently, at present its management is based on cultural techniques throughout the growing cycle, and on insect population monitoring using pheromone traps.

However, in storage warehouses, large losses occur due to conditions favouring tuber moth reproduction (i.e., potatoes are exposed without any protection; the typical darkness of these sites benefit adult moths' activity, and the low temperatures promote egg laying, reaching 311 eggs at 15 °C [16,17]). In storage, chemical control of the tuber moth has limited potential, as most insecticides cannot be applied to potatoes shortly before they are marketed, as this would make them unsafe for human consumption. In fact, at present, there are no pesticides authorised for storage facilities [18]. Therefore, research efforts are aimed at the use of natural enemies.

For the use of a biological control, it is very important to pay attention to different storage conditions, as temperature is a vital factor that affects biological efficacy [19,20]. The temperature affects the pest as a natural enemy. Specifically, the development time of *T. solanivora* decreases from 208 days at 10 °C to 37 days at 27 °C [17]. Moreover, the development time of *B. tarsi* decreases from 22 days at 15 °C to 6 days at 27 °C, and it was observed that the predatory activity of mites rises with temperature [21,22]. Therefore, refrigerated and non-refrigerated conditions should be differentiated. Under refrigerated conditions, usual temperatures range between 5 and 12 °C, depending on the variety and destination (fresh consumption, processing, or seed potatoes) of the tubers. Nevertheless, in large refrigerated facilities, higher temperatures can be found, because potatoes are stored between 12 and 16 °C with a relative humidity of 90–95% for two weeks or more to facilitate wound healing and lowering their sugar content [23].

Surveys of natural enemies in potato storage facilities and crops have been conducted, and a selection of natural enemies have been initiated, focusing on organisms that have already been tested on the common tuber moth, PTM [2,14]. The use of predators on the Guatemalan tuber moth has been little studied. Even if some works on natural enemies of TCS have been carried out [24–27], few predators have been found.

Osorio et al. (2001) [26] found two heteropteran, *Lyctocoris campestris* (Parajulee and Phillips, 1993) and *Buchananiella contigua* (White 1880), and some other occasional, generalist predators, and suggested the use of both heteropterans in storage conditions. However, these species are not commercially available, and consequently, they are very difficult to obtain for research and use. In addition, species and strains of other natural enemies have been tested against TCS: entomopathogenic fungi, *Beauveria* spp [28]; bacteria, *Bacillus thuringiensis* (Berliner, 1915) [29]; virus, *Phthorimaea operculella granulovirus* (PhopGV) [30,31]; and the egg parasitoid *Trichogramma lopezandinensis* (Sarmiento 1993) [32].

In a warehouse survey, Piedra-Buena et al. 2019 [27] found two egg-predator mite species from the *Blattisocius* (Keegan 1944) genus, *B. mali* and *B. tarsalis*, which have been tested against the common tuber moth, PTM, showing high efficacy under laboratory

conditions [33–35]. Although these species are not commercially available, the *Blattisocius* genus is a cosmopolitan group, and its species often appear in stored plant products and in laboratory cultures, so it is relatively easy to find [36]. Owing to its relative availability and promising preliminary results in a similar host, the study of these *Blattisocius* species has been considered interesting for IPM control of TCS.

Therefore, in the present work, the predation of *B. tarsalis* on TCS has been studied under different storage conditions in order to assess its viability as a biological control agent in warehouses.

2. Materials and Methods

2.1. Biological Materials and Experimental Conditions

Blattisocius tarsalis mites were identified and obtained from a fortuitous infestation of *Ephestia kuehniella* in the Agricultural Entomology Laboratory at the University of Almería. Subsequently, the primary colony of the mite was sent to the laboratory of the Plant Protection Unit at the Canary Institute of Agrarian Research. The mite colony was maintained and multiplied in 100 mL plastic containers with a layer of vermiculite. The brood was fed three days a week with fresh or frozen TCS eggs, which were provided by BioAgroLogica SL company (San Cristóbal de La Laguna, Spain). The environmental conditions for the brood were 25 ± 1 °C, 70% RH, and total darkness.

2.2. Prey Acceptance Test and Evaluation of the Predatory Potential at Different Temperatures

A “no-choice” bioassay test was carried out, in which prey acceptance and mortality of TCS eggs by *B. tarsalis* was assessed, following the methodology of Gallego et al. (2020) [33]. Pieces of cotton were introduced into glass test tubes (7.0 cm × 1.0 cm in diameter) and moistened with five drops of water. White cardboard strips (5.0 × 0.9 cm) containing five TCS eggs laid 1–24 h before, followed by a female adult mite with 24 h of starvation (no attempt was made to determine their age, nor to synchronise), were then introduced into each test tube. In the control, the same process was carried out with no female mite placed into the test tube. The test tubes were covered with plastic film, and the females were allowed to prey for 48 h in a climatic chamber.

At the end of the predation period, the female mites were extracted, and all eggs were examined under a stereo microscope (10×) to register the number of eggs predated (no present or less than 25%) and/or partially consumed by the mites’ feeding activity (Figure 1). The eggs were allowed to evolve under the previous conditions of temperature until hatching, and the surviving larvae were counted.

The predation of TCS eggs by *B. tarsalis* was evaluated under four temperatures: 10 ± 1 , 20 ± 1 , 25 ± 1 , and 27 ± 1 °C, with 70% RH and in total darkness. The experimental design for each trial was totally random univariate with a single factor at two levels: predatory mite versus the control, with 30 replicates for the mite and 30 for the control at each temperature. The number of surviving larvae and the killed TCS eggs were statistically analysed using a generalised linear model (GLM) with a Poisson distribution and logarithm link function. In turn, the mean values were compared in pairs using the Wald test at $p = 0.05$. In addition, the percentage of partially consumed TCS eggs at every tested temperature were compared using nonparametric Kruskal–Wallis one-way analysis with a level of significance of $p = 0.05$. All the analyses were carried out with IBM SPSS version 25 statistical software.

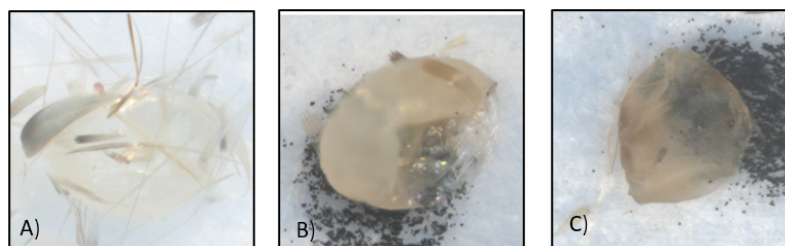


Figure 1. (A) Healthy egg. (B) Partially consumed egg. (C) Totally consumed egg.

Moreover, the effectiveness of TSC egg control by the mite was evaluated using the modified Abbott's equation [37] [$\times 2$]:

$$EP = \left(\frac{M - M'}{100 - M'} \right) \times 100 \quad (1)$$

where EP = efficacy percentage (correcting % efficacy for the natural mortality), M = mortality rate in the treatment (mite), and M' = mortality rate in the check (control).

2.3. Microcosm

Two microcosm bioassays were carried out, following and adapting the methodology of Gallego et al. 2020 [34]. The procedure consisted of infesting potatoes (cultivar: Slaney; size: 75/60 mm) with TCS eggs laid 1–24 h before, simulating two infestation levels: 10 or 50 eggs. The eggs were spread out on the surface of potatoes with a moistened paintbrush, in groups between 5–10 eggs, over the irregularities or “eyes” of the tubers. Cylindrical containers (15.5 cm height, 10.5 cm diameter, and 1 L volume) were prepared, introducing 15 g of vermiculite (pupation substrate) at the bottom. Then, 10 mL of distilled water was added to maintain humidity and facilitate TCS eggs hatching.

Two potatoes were carefully placed in each container, at the corresponding infestation level (10 or 50 eggs), and different densities (0, 5, 10 and 20) of non-sexed adult predatory mites were released in the containers. Finally, the plastic containers were closed with muslin pieces and rubber bands, and placed in a climatic chamber (25 ± 1 °C, 70% RH) for 45 days.

After this period, the surviving individuals, larvae, pupae, and adult moths of each treatment were counted. In both bioassays, the experimental design was univariate and completely randomised, with only one factor, predatory mite density at four levels: 0 (control), 5, 10, and 20 mites released once per container. In each bioassay, there were five replicates per treatment. The values obtained of the number of surviving TCS were analysed statistically through a generalised linear model (GLM) with normal distribution and the identity link function. The average values were compared in pairs through a Wald test at $p = 0.05$. These analyses were carried out with IBM SPSS version 25 statistical software. Finally, the results were corrected for the mortality in the control with the modified Abbott's equation [37].

2.4. Host Choice: *Tecia Solanivora* (Tcs)-*Phthorimaea Operculella* (Ptm)

For the choice bioassay, the same methodology of “no-choice” test was used, with two exceptions: (1) only the standard temperature of 25 °C was tested, and (2) instead of five TCS eggs, three TCS and three PTM eggs were offered simultaneously to each isolated female mite. At the end of the predation period (48 h), female mites were extracted, and the eggs were returned to the climatic chamber (25 ± 1 °C, 70–80% RH, total darkness) until hatching. Finally, the surviving larvae of both species were counted, and the number of killed eggs was calculated. Mite preference for hosts was determined through the Manly preference index (β_2) [38]. In accordance with Chesson (1983) [39], the expression of this index is:

$$\beta = \frac{\ln \frac{n_i - r_i}{n_i}}{\sum_2^1 \ln \frac{n_i - r_i}{n_i}} \quad (2)$$

where (β_2) is the Manly preference index (without replacement); r_i is the number of preyed eggs; n_i is the number of offered eggs; and $i = 1$ or 2 represents the host eggs of each species tested. When the value of the preference index is (β_2) > 0.5 , this means preference; indifference when (β_2) $= 0.5$; and rejection if (β_2) < 0.5 [38]. The values of the Manly index obtained for both moth species were analysed statistically by the Wilcoxon test with IBM SPSS version 25 statistical software.

2.5. Functional Response: Predatory Behaviour at Different Prey Densities

The methodology of Gallego et al. (2020) [34] was followed. Tubes with a piece of moistened cotton were used, analogous to the test of the predatory potential at different temperatures, but in this case different TCS egg densities (1, 2, 3, 6, 9, and 12) were offered to the one female adult mite, and the exposure time to predation was 24 h at 25 °C. The number of replicates was 20 per treatment. The mortality data were collected to fit the type of functional response, and two statistical analyses were performed. In the first one, the data were fitted to the polynomial function used by Juliano (2001) [40]:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2) + P_3 N_0^3}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (3)$$

where N_e is the number of preys eaten; N_0 the initial value of prey offered; and $P_0, P_1, P_2,$ and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively, estimated using the maximum likelihood method. Statgraphics version 18 software was used for the adjustments. P_0 – P_3 parameters were obtained from a logistic regression. If the P_1 coefficient was not significantly different from zero, it corresponded to a type I functional response. It was considered a value to be different from zero when zero was not included in its confidence interval. If the P_1 value was significantly negative, this would demonstrate type II functional response, while a significantly positive P_1 value would demonstrate a type III functional response. A more exact statistical analysis was then conducted, and mortality data were fitted to the equations proposed by Hassell (1978) [41] (Equations (3) and (4)) and Cabello et al. (2007) [42] (Equation (5)) for predators when there is no prey replacement:

- Type I:

$$N_a = N(1 - \exp -a'TP) \quad (4)$$

- Type II:

$$N_a = N \left(1 - \exp \left(-a'P \left(T - T_h \frac{N_a}{P} \right) \right) \right) \quad (5)$$

- Type III:

$$N_a = N \left\{ 1 - \left[\frac{\alpha NP}{1 + T_h (\exp(-\alpha) - 1) N \left(T - T_h \frac{N_a}{P} \right)} \right] \right\} \quad (6)$$

where N_a represents the number of prey attacked, N the number of prey offered, a the attack rate of the predator, T the time length of the assay, P the number of predators used, T_h the handling time of the prey by the predator (capture time and feeding time), and α the predation potential. In this assay, $T = 1$ day and $p = 1$ predator. The statistical software used for the functional response fitting was TableCurve 2D, version 5.

3. Results

3.1. Evaluation of the Predatory Potential at Different Temperatures

Table 1 presents the mean values obtained in the number of surviving eggs, mortality percentage, and efficacy percentages in the 48 h predation period of adult *B. tarsalis* females on TCS eggs in the temperature tests.

Table 1. Average values (\pm SE) of the predatory potential evaluated at 10, 20, 25, and 27 °C.

Temperature °C	Surviving Eggs ($n^{\circ} \pm$ SE)		Mortality (% \pm SE)		Efficacy %
	Treatment	Check	Treatment	Check	
10	3.15 \pm 0.35 a	4.30 \pm 0.39 b	40.00 \pm 2.34	13.10 \pm 1.80	33.52 \pm 2.44
20	2.87 \pm 0.31 a	4.53 \pm 0.18 b	45.33 \pm 4.06	3.45 \pm 1.43	40.14 \pm 3.54
25	1.90 \pm 0.25 a	4.62 \pm 0.40 b	64.67 \pm 4.15	7.33 \pm 1.83	59.26 \pm 4.59
27	1.17 \pm 0.20 a	4.4 \pm 0.38 b	76.67 \pm 4.40	12.41 \pm 2.51	75.19 \pm 4.64

Mean values followed by different letters in the same row show significant differences at $p = 0.05$

In Table 1, the statistical analysis showed a highly significant treatment effect in the number of surviving eggs at the four temperatures tested (10 \pm 1, 20 \pm 1, 25 \pm 1 and 27 \pm 1 °C) using omnibus tests (likelihood ratio chi-squared test = 4.911, d.f. = 1, $p < 0.0001$; likelihood ratio chi-squared test = 14.261, d.f. = 1, $p < 0.0001$; likelihood ratio chi-squared test = 35.384 and d.f. = 1; likelihood ratio chi-squared test = 60.035 and d.f. = 1, $p < 0.0001$, at 10, 20, 25, and 27 °C, respectively).

The number of surviving eggs was significantly lower ($p < 0.05$) in treatments with the female mite than in control treatments without it, at all the temperatures tested. This indicates that the mite can predate on TCS eggs at the four assayed temperatures. Results also show the influence of temperature on mortality caused by *B. tarsalis*. The lowest mortality rates were registered at 10 and 20 °C (40.00 \pm 2.34% and 45.33 \pm 4.06%, respectively), whereas at higher temperatures (25 and 27 °C), the mortality increased markedly (64.67 \pm 4.15% and 76.67 \pm 4.40%, respectively) (Table 1). When Abbott's equation was applied, it was observed that the efficacy percentage also increased with temperature: 33.52 \pm 2.44%, 40.14 \pm 3.54%, 59.26 \pm 4.59%, and 75.19 \pm 4.64% at 10, 20, 25, and 27 °C, respectively.

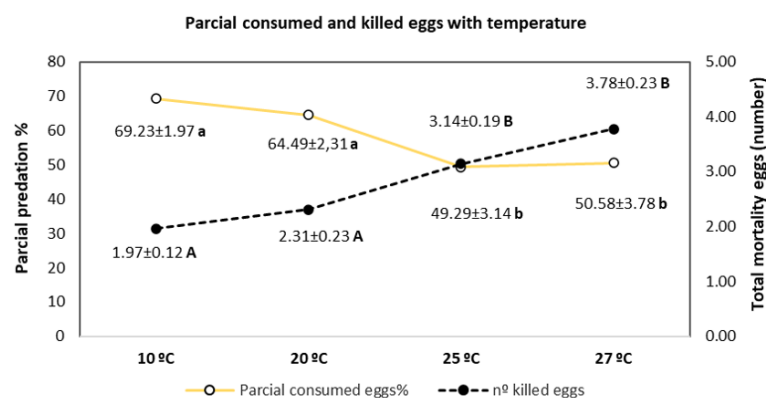


Figure 2. Mean percentage values of partial killed eggs (\pm SE) and number killed eggs (\pm SE) in the predatory potential bioassays. Values with different letters mean significant differences at $p = 0.05$, using the Wald test in the case of number of killed eggs and Kruskal–Wallis test for partially consumed eggs.

In Figure 2 the statistical analysis showed a highly significant effect of temperature in partially consumed eggs using the omnibus tests (likelihood ratio chi-squared test = 154.784, d.f. = 3, $p < 0.0001$). The comparison in pairs using the Wald test at $p = 0.05$ did not reveal

significant differences at temperatures of 10 and 20 °C, nor between 25 °C and 27 °C. Significant differences were only observed with temperatures over 20 °C ($p > 0.05$ in all case). The Kruskal–Wallis test results showed the same pattern for the mean of killed eggs with p -value = 0.05.

3.2. Microcosm

The number of surviving larvae and the mortality percentage of TCS resultant from *B. tarsalis* predatory activity at the two infestation levels of pest (10 and 50 eggs) when exposed to different densities of the predator mite are shown in Figure 3.

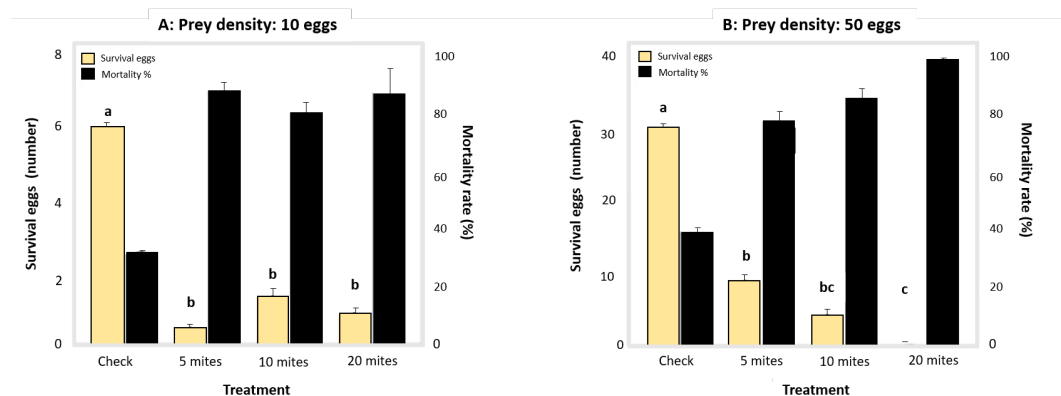


Figure 3. Mean values of surviving eggs (\pm SE) and mortality rate (\pm SE) at the infestation levels of 10 and 50 TCS eggs and doses of 0, 5, 10, and 20. Values with different letters denote significant differences at $p = 0.5$, using Wald test.

In the bioassay at low infestation level (10 eggs), a highly significant effect was observed in the number of surviving eggs with significant variance (omnibus test: likelihood ratio chi-squared test = 17.177, d.f. = 3, $p < 0.001$). The efficacies obtained for the mite densities of 5, 10, and 20 were $91.67 \pm 8.33\%$, $82.29 \pm 17.73\%$, and $81.25 \pm 11.97\%$, respectively.

At a prey density of 50 eggs, statistical analysis of the number of surviving eggs found a highly significant effect (significant variance with omnibus test: likelihood ratio chi-squared test = 41.233, d.f. = 3, $p < 0.0001$). The efficacies obtained for mite densities of 5, 10, and 20 were $67.02 \pm 13.53\%$, $82.29 \pm 5.77\%$, and $98.52 \pm 1.48\%$, respectively.

3.3. Host Choice

The mean values of killed eggs for both moths (TCS and PTM) and the Manly index obtained in the choice test are shown in Table 2.

Table 2. Mean number (\pm SE) of killed eggs in the “choice” trials.

Host	Eggs Mortality	Manly Index
<i>T. solanivora</i>	1.77 \pm 0.43 a	0.57 \pm 0.07 a
<i>P. operculella</i>	1.46 \pm 0.23 a	0.43 \pm 0.07 a

Values with different letters denote significant differences at $p = 0.05$, using Wilcoxon test.

The number of killed eggs in the choice bioassay showed that there was not a significant difference in egg predation of TCS and PTM by the mite ($p = 0.482$; omnibus test: likelihood ratio chi-squared test: 0.495; gl = 1; $p = 0.482$), nor was the difference in the Manly index significant ($p = 0.293$). These results indicate indifference in the hunt of *B. tarsalis* mite between TCS and PTM eggs.

3.4. Functional Response: Predatory Behaviour at Different Prey Densities

Table 3 shows the adjustment parameters to the polynomial function (Equation (3)) of the number of TCS eggs killed by *B. tarsalis* female mites. As mentioned above (Materials

and Methods, Section 2), the P_1 value is significantly negative; therefore, in this previous analysis, this mite shows a type II functional response (when considering a value different from zero, when zero is not included in its confidence interval, as happens in this case).

Table 3. Results of logistic regression analyses of the proportion of *Tecia solanivora* eggs killed by the adult female of *Blattisocius tarsalis* in the bioassay carried out under laboratory conditions.

Parameter	Value	SE	95% Confidence Level		F.R. Type
P_0 (Intercept)	1.1707	0.0598	0.9805	1.3609	II
P_1 (Linear)	−0.1661	0.025	−0.0865	−0.0865	II

The above result is consistent with the results of the fits carried out with the three types of functional responses shown in Table 4. These confirm type II (Equation (5)), because the corrected Akaike information criterion (AICC) shows the lowest value. Therefore, Figure 3 shows the type II functional response of the predatory mite. This figure also shows a comparison of the number of killed eggs, where a highly significant effect was found (omnibus tests: likelihood ratio chi-squared test = 61.361, d.f = 5, $p < 0.0001$), the maximum value of *B. tarsalis* predation was for the offer of nine eggs, without significant differences as to the number of offered eggs increased ($p > 0.05$).

Table 4. Parameters and statistical significance for the functional response equations type I, II, and III when different densities of *Tecia solanivora* eggs were exposed to the adult female of *Blattisocius tarsalis*, during 24 h, under laboratory conditions.

Type	Fit Curve Parameters (\pm SE)			Statistical Parameters		
	a'	T_h	α	d.f.	R^2	AICC
I	0.4999	-	-	6	0.7237	−2.2467
II	2.0502	0.2091	-	5	0.9457	−6.1924
III	-	0.2934	0.7833	5	0.8358	1.8271

4. Discussion

Currently, TCS management in the field relies on cultural practices and population monitoring using pheromone traps, as there are not effective phytosanitary products. As for storage conditions, there are not authorised products for chemical control nor commercial biological control agents available. The use of *B. tarsalis* has already been analysed in laboratory conditions on other storage pests: two pyralids, *Cadra cautella* (Walker) and *Ephestia kuehniella* (Zeller, 1879) [22,43], and most recently, on other gelechiid, PTM, with promising results under non-refrigerated conditions [34,35].

This study reports results of the efficacy and suitability of *B. tarsalis* as an egg predator on TCS in storage, as evaluated in a series of laboratory assays. This is the first attempt to evaluate a natural enemy against TCS in postharvest conditions. It is known that temperature is one of the main factors in the predator–prey relationship [44]. In this sense, the temperature bioassays performed showed that *B. tarsalis* maintained its predatory activity on TCS at the range of 10 to 27 °C. Additionally, it was observed that, while the predator mite increased prey mortality significantly between 20–27 °C, there were no differences at the lowest assayed temperatures (10–20 °C; Table 1). Evaluation at low temperatures is important to determine the viability in refrigerated conditions. At 10 °C, the number of TCS killed eggs by *B. tarsalis* was 1.25 ± 0.11 in 48 h, which is equivalent to 0.63 eggs/day. This result is similar to Nielsen 1999, who also found a mortality rate less than 1 egg/day on *Ephestia kuehniella*, but contrasts with the result of Gavara et al. 2020 [35], who found a higher predatory activity of *B. tarsalis* on PTM, the common moth, with a rate of 1.44 eggs/day. The differences in our results could be explained by difference in size between preys, because it has been observed that a greater size of eggs favours partial consumption, since the greater the size, the earlier the mite would be satisfied

and the greater the survival possibilities of the prey eggs, although other chemical and mechanical factors may be involved [34,36]. The TCS eggs at around 0.41×0.53 mm [12,15,45] are bigger than *E. kuehniella* and PTM, which are approximately 0.57×0.30 [46] and 0.5×0.35 [45], respectively.

This theory was confirmed by our experiment, where we could observe that partially consumed egg percentage remained high at all evaluated temperatures. This value was around 65–70% at low temperatures and was significantly lower, around 50%, at 25–27 °C. Conversely, the number of killed eggs increased with increasing temperature (Figure 2). The mortality rate at 10 °C yields an efficacy of $33.52 \pm 2.44\%$. It is a lower value than that obtained by Gavara et al. 2020 [35] for PTM under the same conditions ($49.66 \pm 5.06\%$). This result is interesting for large, refrigerated facilities during the recommended period of 12–16 °C mentioned in the introduction.

In addition, the results obtained at the temperature range of 20–27 °C are promising for most small farmers, who keep their harvest in small storage facilities at room temperature. At 25 °C (no difference with 27 °C (Table 1), our results showed surviving egg mean values of between 1.17 ± 0.20 and 1.90 ± 0.25 in 48 h (Table 1). This value is lower than that found by Gallego et al. 2020 [34], who reported a mean value of 0.05 ± 0.05 for PTM eggs under the same conditions. Efficacies were $59.26 \pm 4.59 - 75.19 \pm 4.64\%$ for TCS against 98.86% for PTM [35]. These results contrast with those obtained in the microcosm bioassay (Figure 3), where we obtained mortality rates for TCS similar to those reported for PTM under the same conditions [35]. As *B. tarsi* mainly preys on the egg stage [36], differences in acceptance tests but similar results in microcosm assays could be partially explained by differences in egg stage duration between the two moths. While TCS eggs in our experimental conditions (25 °C, 70%HR) hatch between the sixth and seventh day [11,47], PTM eggs hatch between the fourth and fifth day. This means additional time for predation activity by *B. tarsi* on TCS eggs against PTM eggs. The effect of this additional time could not be observed in the acceptance assay because the predation period was interrupted at 48 h.

At a low infestation level in the microcosm assay (10 eggs; Figure 3A), higher mortality was reached at minimum predator density, with an efficacy of $91.67 \pm 8.33\%$. At high infestation level (50 eggs; Figure 3B), mortality increased with mite density until the highest efficacy, $98.52 \pm 1.48\%$, when 10 mites were released. These values show the same pattern reported by Gavara et al. 2020 [35] on *P. operculella* with similar efficacy values, $96.97 \pm 3.03\%$ and $92.31 \pm 2.74\%$, at low and high infestation levels, respectively.

Similar exposed efficacies of *B. tarsi* against TCS and PTM are consistent with the results observed in the host-choice assay, in which *B. tarsi* did not show any preference for the eggs of one or the other of the moths. The mite did not present any differences in the number of predated eggs, and the values obtained to apply the Manly index indicate indifference in selection (Table 2). However, a preference of the mite to lepidopteran eggs has already been observed by Haines 1981 [36] and Riudavets & Quero 2002 [48], who found a clear preference of *B. tarsi* for moths' eggs with respect to different coleopteran eggs species present in storage conditions. However, no preference prey studies among tuber moths have been found. Therefore, the indifference result in the predatory efficacy of the mite against both tuber moths is very interesting for potato growers in the Canary Islands, as mixed infestations by TCS and PTM in potatoes are frequent.

For predators, the most commonly found response is Holling's type II, with a negatively accelerating rise to an upper limit, above which further increases in prey density do not influence attack rate [49]. This is the case of *B. tarsi*, who presents a type-II functional response at 25 °C using TCS eggs as prey (Figure 4). This type of functional response has been previously corroborated for *B. tarsi* with eggs of several species of insects, such as *Lasioderma serricornis* (Coleoptera: Ptinidae), *Plodia interpunctella* (Lepidoptera: Pyralidae), and *P. operculella* [34,50].

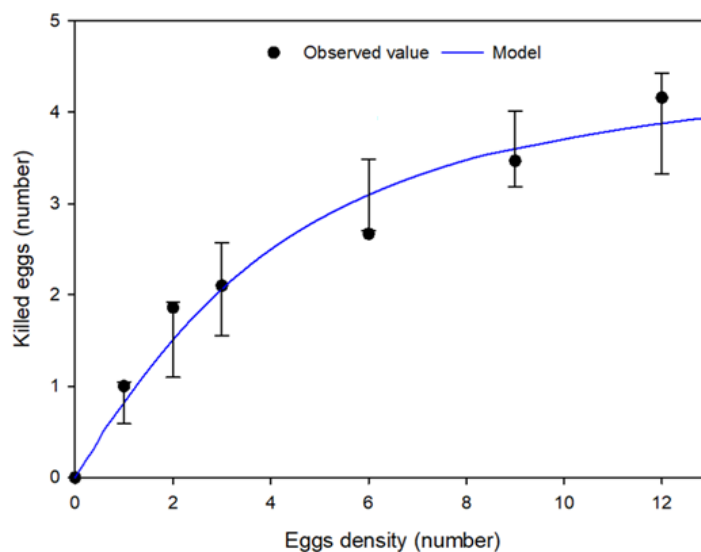


Figure 4. Functional response (number of pest eggs killed), type II, of the adult female of *Blattisocius tarsalis* when different densities of *Tecia solanivora* eggs were offered as prey for 24 h under laboratory conditions (whisker plot: 95% confidence intervals).

B. tarsalis has shown a consumption rate (a') in TCS of 2.0502 days^{-1} . These values are higher than those reported for *L. serricornis* and PTM eggs (1.032 days^{-1} and 1.776 days^{-1} , respectively), and similar to those obtained by Gallego et al. (2020) [34] with PTM eggs as prey ($a' = 2.1258 \text{ days}^{-1}$).

Regarding handling time (T_h), with TCS eggs ($T_h = 0.2091 \text{ days}^{-1}$), it was higher than values previously reported for PTM and *L. serricornis* eggs ($T_h = 0.1014 \text{ days}^{-1}$ and $T_h = 0.1626 \text{ days}^{-1}$, respectively) [34,50]. By contrast, the values were lower than those reported for *P. interpunctella* ($T_h = 0.3467 \text{ days}^{-1}$) [50]. Changes in T_h intrinsically involve several biological parameters, such as digestion time, attack time, gut content, hunger threshold, prey weight, and proportion of prey eaten, in place of a single parameter [49]. Thus, differences in predators' T_h may be due to one or more of these causes at the same time. For example, larger prey egg size may be related to higher food content, leading to earlier satiation of *B. tarsalis*, which would imply an increase in T_h . In this case, the egg size of TCS is larger than the species mentioned above ($0.41 \times 0.53 \text{ mm}$, compared to $0.50 \times 0.35 \text{ mm}$ for PTM, $0.45 \times 0.27 \text{ mm}$ for *P. interpunctella* and $0.38 \times 0.20 \text{ mm}$ for *L. serricornis*) [51–54], which seems to support the results obtained for PTM and *L. serricornis*. However, it would not explain the values for PTM. Therefore, in addition to size, differences could be attributed to other causes, for example: the hardness and/or thickness of the chorion, which would imply a longer time to access the egg's content; the presence of chemical compounds with protective functions (unpalatable or toxic) against predators [55]; and even differences in the nutritional value of the egg.

Some works suggested predatory mites could be used as biological control agents for lepidopteran pests (Nielsen 1999 [22], Thomas et al. 2010 [56], Ghoneim 2014 [57]). In the case of the potato moth complex (*P. operculella*, *T. solanivora*, and *S. tangolias*), recent works in Spain reported the effectiveness of *B. mali* and *B. tarsalis* against *P. operculella*, the common potato moth, in storage conditions (Gallego et al. 2019 [33], Gallego et al. 2020 [34], Gavara et al. 2021 [35]). Similar results have been obtained in this work on the effectiveness of *B. tarsalis* against *T. solanivora*, the Guatemalan potato moth, also present in Spain. Our work completes the evaluation of *B. tarsalis* as a biological control agent for both potato moths under laboratory conditions and, considering the promising results obtained, encourages proceeding with evaluation in real storage conditions.

5. Conclusions

The experiments have shown the potential use of the predator mite *B. tarsalis* as a biological control agent of TCS under storage conditions in the range of 10–27 °C. By contrast, the results show low and insufficient efficacy of *B. tarsalis* at 10 °C; for this reason, it would not be effective under the usual refrigerated conditions (5–10 °C). Thus, the mite achieves greatest control of tuber moths under non-refrigerated storage conditions, even against high infestation levels. It has also been observed that the mite could maintain its efficacy in the case of mixed infestation of TCS and PTM. For these reasons, future studies should proceed on the evaluation of *B. tarsalis* under non-refrigerated semi-storage and storage conditions.

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