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Potential for the Postharvest Biological Control of *Phthorimaea operculella* (Lepidoptera, Gelechiidae) by *Blattisocius tarsalis* (Mesostigmata, Blattisociidae)

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Abstract: *Phthorimaea operculella* is one of the most important pests causing damage to stored potatoes. In this work, the effect of temperature (at 10, 20 and 30 °C) on the predation of pest eggs by *Blattisocius tarsalis* was studied in the laboratory. In addition, the effect of three predatory release rates on two pest densities was studied under microcosm conditions. The results showed that *B. tarsalis* maintains its predatory capacity at low temperatures (10 °C), obtaining an efficiency of 49.66 ± 5.06% compared to the control. In turn, at 20 °C, a maximum efficacy of 78.17 ± 4.77% was achieved, very similar to that presented at 30 °C (75.57 ± 4.34%). Under microcosm conditions and at low pest density (10 eggs/container), the mortality due to the mite was 96.97 ± 3.03%, 81.82 ± 8.84%, and 84.85 ± 8.30%, respectively, for the three predatory release rates (5, 10 or 20 mites/container). At the high infestation level, the pest control ranged from 61.54 ± 9.21% to 92.31 ± 2.74%, depending on the predatory release rate. The results obtained show that *B. tarsalis* could be a relevant control agent against *P. operculella* under non-refrigerated potato storage conditions, as well as in the first stages of their storage under refrigerated conditions.

Keywords: potato tuber storage; non-refrigerated; pest; predatory mite; release rate; microcosm; efficiency

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1. Introduction

The importance of the potato as a key food source in the global diet is evident both by the quantity produced and by its nutritional value [1,2]. Potatoes are produced throughout the world and are grown both during the summer in temperate areas of the northern hemisphere and during the winter in subtropical areas such as in the Mediterranean region, northern India, and southern China [3]. In most countries, there is only one growing cycle per year and therefore fresh tubers are only available for 2 to 3 months, making storage necessary [4].

Storage requirements vary depending on how the potatoes are used. First, potatoes are vegetatively propagated through the tubers; for this reason, the "seed potatoes" (tubers) are stored at low temperatures (2–4 °C) for the year prior to planting. However, potatoes stored and processed for consumption are in demand throughout the year, which means that both short and long-term storage is required. Potatoes are stored at higher temperatures (8–12 °C) to reduce the build-up of sugars. In short-term storage (3–4 months), non-refrigerated storage methods in the growing areas themselves are inexpensive and a common alternative, especially in tropical and subtropical countries, for potato crops [4,5].

One of the most cosmopolitan and harmful pests in the world is the potato tuberworm moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera, Gelechiidae) [6]. This pest species attacks all the vegetative parts of the plant causing mines within the plant tissue due to larval feeding. When the potato plant is young, PTM can cause considerable damage, even killing the plant outright, although the greatest economic importance caused by the pest is the damage caused to the tubers [7]. Feeding galleries in the tubers can also lead to the entry of secondary pests and pathogens, further reducing the quality and market value of the production [8].

Furthermore, as the potato tubers can be exposed to several generations of the pest, they can be destroyed under non-refrigerated storage conditions within a few months if the tubers are not treated against PTM [9,10]. In contrast, another way of storing potato tubers is to use large refrigerated facilities. For this purpose, the tubers are subjected to a slow reduction in temperature. This facilitates the curing and cicatrisation of the wounds, as well as the reduction of the sugar content (e.g., this is the case of potatoes to be pre-fried). In this case, the usual recommendation is to keep the temperature between 12–16 °C and the relative humidity (RH) between 90–95% for two weeks or more [11]. During this period, damage may be caused by PTM. It is known that the development rate of all the pest stages is significantly reduced at temperatures below 11 °C [12]; therefore, it has been suggested that significant PTM larval development might take place during this period [9].

Even though several integrated pest management methods have been developed, the use of chemical insecticides remains the principal control method worldwide [13,14]. However, the chemical control of PTM in tuber storage has limited potential since most insecticides cannot be safely applied to potatoes shortly before they are marketed for human consumption [15]. Under these conditions, both microbiological agents (granulovirus, *Bacillus thuringiensis* and nematodes) and macrobiological agents (*Chelonus blackburni* (Cameron), *Copidosoma koehleri* (Blanchard), *Trichogramma chilonis* Ishii and *T. evanescens* Westwood) have been applied with different levels of success [8,9,15–17]. More recently, research has begun on the potential of using two species of predatory egg mites from the Blattisociidae family, *Blattisocius mali* (Oudemans) and *B. tarsalis* (Berlese), to control PTM [18,19].

Scant research has been carried out into the biology of the many Blattisociidae family species [20] although *B. tarsalis* is the most studied species regarding its potential as a storage control agent for pests such as *Ephestia kuehniella* Zeller and *Cadra cautella* (Walker) (Lepidoptera, Pyralidae) [21–23]. *B. tarsalis* is a common predator of moth eggs (mainly) and has been reported in numerous areas; thus, it appears to be cosmopolitan [24,25]. The behaviour of this species at low temperature seems to be adequate for it to be used under potato storage conditions. Nielsen [22] determined that the females of this species were still active and predating at temperatures around 9 °C. On the other hand, at much higher temperatures (25 and 27 °C) [25,26], a consumption rate was reported of 1.4 eggs/day and 3.54 eggs/day by *B. tarsalis* females on the species *Plodia interpunctella* (Hübner) (Lepidoptera, Pyralidae) and *C. cautella*, respectively.

Based on the wide range of temperatures at which *B. tarsalis* has demonstrated feeding activity, this work aims to study its predation behaviour on *P. operculella* eggs at different temperatures. In addition, we studied the role of possible intraspecific competition when different mite release rates are used at two levels of pest infestation (low and high).

2. Materials and Methods

2.1. Biological Materials and Experimental Conditions

B. tarsalis mites were identified and obtained from a serendipitous infestation of *E. kuehniella* in the Agricultural Entomology Laboratory at the University of Almeria in September 2018, using the keys of Nesbitt [27], Chant [24] and Haines [28]. Specimens were reared in the same laboratory for 15 months prior to the start of the trials. The colonies of *B. tarsalis* were maintained following the methodological indications of Nielsen [22], with slight modifications. Plastic containers (500 mL) filled with a layer of vermiculite were

used; the relative humidity (R.H.) was 75%, achieved by means of a saturated aqueous NaCl solution [29]. The mites were fed 0.20 gr of *E. kuehniella* eggs every 3 days. The containers were kept in a climatic chamber with ambient conditions of 25 ± 1 °C and 16:8 h of photoperiod of light: darkness (L:D).

The PTM population was reared in the same laboratory and started with specimens supplied by the Almeria Plant Health Laboratory (Junta de Andalucia, Spain). The methodology described by Fenemore [30] was followed, using small potatoes ("baby" type) as food for the larvae. To do this, filter paper disks with moth eggs—obtained from the mating and oviposition chambers of adults (approximately 20 pairs of adults confined in 1000 mL plastic containers sealed with surgical gauze and an elastic band)—were placed in contact with the tubers, which had previously had their surfaces perforated to facilitate neonate larvae penetration. In addition, a layer of vermiculite was placed at the bottom of said containers to favour the formation of pupae. Once larval development was completed, the substrate was sieved to remove the pupae and place them again in the mating and oviposition chambers until the adults emerged. The environmental conditions for the brood were 25 ± 1 °C, 60–80% R.H. and a 16:8 h L:D.

2.2. Evaluation of the Predatory Behaviour at Different Temperatures

Three bioassays were performed in which the predation of the PTM eggs by *B. tarsalis* was evaluated at different temperatures. For this, the methodology of Gallego et al. [18] was followed, using female mites individually placed in glass test tubes (7.0 cm in length and 1.0 cm in diameter). Next, white cardboard strips (the dimensions of 0.9 × 5.0 cm) containing 5 PTM eggs and a piece of sponge (the dimensions of 0.5 × 0.5 cm) moistened with water were introduced into each test tube. The test tubes were covered with cotton and the females were then allowed to prey for 48 h in a climatic chamber. The environmental conditions were the following: temperature: 10 ± 1; 20 ± 1 and 30 ± 1 °C; R.H.: 80–90%; and a 16:8 h L:D. As a control (check), the aforementioned process was carried out with the exception that the adult mite female was not introduced into the test tube.

The experimental design for each trial was completely randomized univariate with a single factor—predatory mite versus the control (check). The number of repetitions was 30 for the mite and 30 for the control at each temperature tested. At the end of the tests, the eggs were examined under a binocular microscope and the number of killed eggs (totally or partially consumed by the mite's feeding activity) was counted. In addition, the eggs were incubated for 7 days to determine the hatchability of the PTM larvae.

The values corresponding to the number of PTM eggs that survived were statistically analysed using a generalized linear model (GZLM) with the Poisson distribution and the logarithm link function; in turn, the mean values were compared in pairs using the Wald test at $p = 0.05$. To carry this out, IBM Statistical Package for Social Sciences (SPSS) version 26 statistical software was used. Later, the values of the three trials were analysed by using a nonparametric Kruskal–Wallis one-way analysis with a level of significance of 0.05 was performed, using the same statistics software.

The effectiveness of the PTM–egg control by the mite was evaluated using Abbott's equation correction for natural mortality [31]:

$$EP = [(M - M')/(100 - M')] \cdot 100, \quad (1)$$

where, EP = efficacy percentage, M = the percentage of mortality in the treatment (mite) and M' = the percentage of mortality in the control (check).

2.3. Efficacy Bioassays in Microcosm

With the aim of simulating the storage conditions, two bioassays were carried out under "microcosm" conditions, adapting the methodology described by Arthurs et al. [8] and Gallego et al. [18]. For the evaluations, three tubers (variety: Marilyn, category 1, size 28/45 mm) were infested with two densities of PTM eggs (< 24 h of age): 10 or 50 eggs/container, respectively, for each of the assays. The eggs were evenly distributed

on the tuber buds (eyes) using a fine brush (00) moistened with water. This was done to simulate the oviposition of adult PTM females, who are known to oviposit groups of 2–20 eggs in the laboratory, usually in the vicinity of the buds [32]. Next, a layer of vermiculite (150 mL, 14.6 g) was placed at the bottom of a plastic container (height 15.5 cm, diameter 10.5 cm, and volume 1 L) as a substrate for pupal formation. The three tubers infested with the PTM eggs were carefully placed on this layer. Subsequently, the different doses of adults (not sexed) of the predatory mite (*B. tarsalis*) were added manually to the surface of the tubers using a fine brush (00) moistened with water. Finally, the container was closed with a piece of circular filter paper glued with vaseline.

In each bioassay, the experimental design was completely randomized univariate, with a single factor: density of the predatory mite at three levels (5, 10 and 20 mites/container), in addition to the control (check). There were 5 replications per bioassay and treatment. In the two bioassays, the containers were kept at 25 ± 1 °C and 16:8 h L:D until pupation and/or adult emergence (28 days after the start of the bioassays).

The values corresponding to the number of PTM survivors (pupae or adults) were statistically analysed using a GZLM with the normal distribution and the identity link function; in turn, the mean values were compared in pairs using the Wald test at $p = 0.05$. To carry this out, IBM SPSS version 26 statistical software was used.

The efficacy of *B. tarsalis*, for each release rate and bioassay, was also evaluated using Abbott's equation correction for natural mortality (Equation (1)) at 28 days.

3. Results

3.1. Evaluation of the Predatory Activity at Different Temperatures

Table 1 shows the mean values found relative to the number of survivors, the mortality percentage, and the efficacy percentage, at 7 days, in the three bioassays carried out, when the PTM eggs were exposed to adult *B. tarsalis* females for 48 h at three temperatures, compared to the controls (checks).

Table 1. Mean values of the number of survivors (±SE), mortality percentage (±SE) and efficiency percentage, when *Phthorimaea operculella* eggs were exposed for 48 h to the adult female of the predatory mite *Blattisocius tarsalis*, in the three bioassays carried out, under laboratory conditions, at three temperatures (10, 20 and 30 ± 1 °C; 80–60% R.H. and 16: 8 h L:D).

Temperature (°C)	Survivors (Number) [1]		Mortality (%)		Efficacy Percentage (%)
	Treatment	Control	Treatment	Control	
10	2.28 ± 0.22 a	4.48 ± 0.11 b	55.86 ± 4.14	9.66 ± 1.89	49.66 ± 5.06
20	0.90 ± 0.20 a	4.33 ± 0.14 b	82.00 ± 3.99	14.00 ± 2.74	78.17 ± 4.77
30	1.03 ± 0.19 a	4.20 ± 0.14 b	79.38 ± 3.76	16.67 ± 2.63	75.57 ± 4.34

[1] Mean value followed by different letters and in the same row show significant differences at $p = 0.05$ using the Wald test.

In the statistical analyses of the number of surviving eggs in the three bioassays carried out (at 10, 20 and 30 °C), highly significant treatment effects were found (Omnibus tests: likelihood ratio Chi-squared test = 21.286, d.f. = 1, $p < 0.0001$; 73.521, d.f. = 1, $p < 0.0001$; and 64.189, d.f. = 1, $p < 0.0001$, at 10, 20 and 30 °C, respectively).

In all cases, the number of surviving eggs was significantly lower in the mite treatment than the corresponding control (check) (Table 1). This indicates that the mites had a predatory effect on the PTM eggs at the three temperatures tested. Additionally, when analyzing the values of surviving eggs at the three temperatures, the Kruskal–Wallis test results are statistically significant ($p < 0.0001$). The outputs also show that the null hypotheses were rejected. Therefore, there are significant differences in the values for 10 °C compared to 20 and 30 °C ($p < 0.0001$ and $p < 0.001$, respectively), but not between 20 and 30 °C (not significant).

However, it can also be observed (Table 1) that the temperature of the test influenced the mortality caused by the mite. Thus, at 10 °C, the mortality value was the lowest

(55.86 ± 4.14%); this was surpassed by the bioassays both at 20 and 30 °C (82.00 ± 3.99% and 79.38 ± 3.76%, respectively).

The mortality in the controls due to natural mortality and/or experimental mortality remained low at all three temperatures (9.66 ± 1.89%, 14.00 ± 2.74% and 16.67 ± 2.63%, respectively, at 10, 20 and 30 °C) and are adequate values.

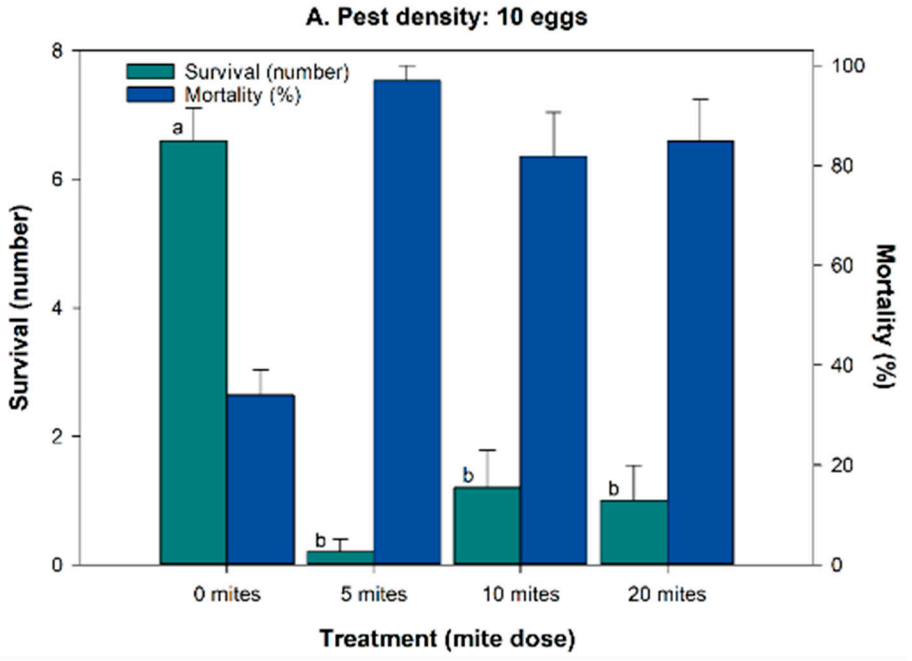
Correcting the mortalities obtained at each temperature in relation to the controls (Equation (1)), the efficacy percentage of PTM–egg control by the mites was maximal at 20 °C (78.17 ± 4.77%), followed closely by the value at 30 °C (75.57 ± 4.34%), both of which were higher than at a temperature of 10 °C (49.66 ± 5.06%).

3.2. Efficacy Bioassays in Microcosm

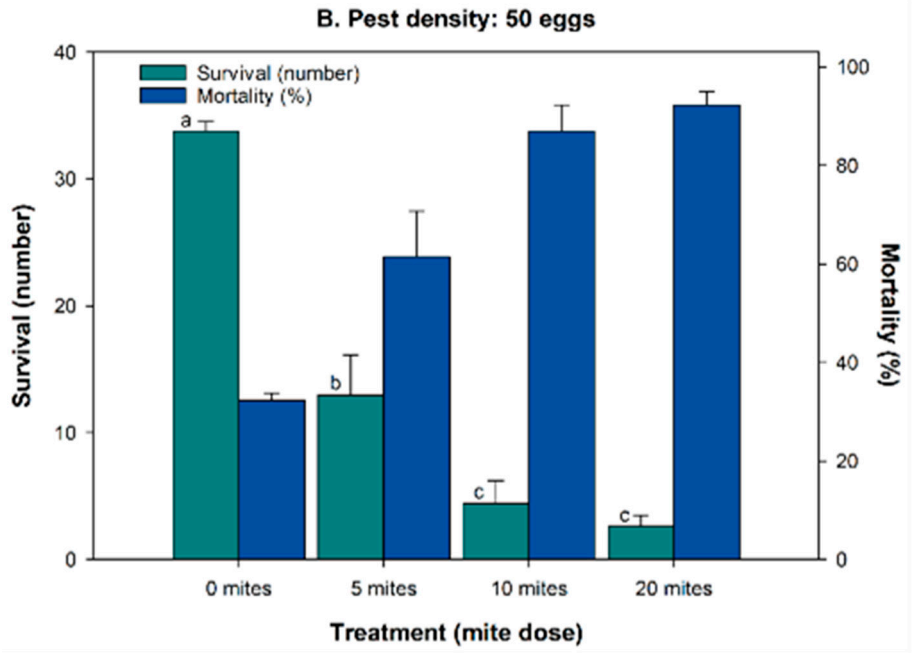
Figure 1 shows the number of survivors and the mortality percentage in a PTM population under microcosm conditions when exposed to different release doses of the *B. tarsalis* predatory mite in the two bioassays carried out at two infestation levels of the pest species.

A highly significant effect was found in the statistical analysis of the number of survivors in Bioassay 1 (Figure 1A), with an initial infestation level of 10 PTM eggs per container—the Omnibus test showed that the model was highly significant in explaining the variance (likelihood ratio Chi-squared test = 41.233, d.f. = 3, $p < 0.0001$). Thus, the efficacy percentage of the predatory mite at 28 days was 96.97 ± 3.03%, 81.82 ± 8.84%, and 84.85 ± 8.30%, with respect to the control (check), when 5, 10 or 20 mites/container were released, respectively.

Likewise, for Bioassay 2 (Figure 1B), with an initial infestation of 50 PTM eggs per container, the statistical analysis of the number of survivors found a highly significant effect—the Omnibus test showed that the model was highly significant in explaining the variance (likelihood ratio Chi-squared test = 49.172, d.f. = 3, $p < 0.0001$). Thus, the efficacy percentage of the predatory mite at 28 days was 61.54 ± 9.21%, 86.98 ± 5.34%, and 92.31 ± 2.74%, with respect to the control (check), when 5, 10 or 20 mites/container were released, respectively.



(**A**)



(**B**)

Figure 1. Number of survivors (±SE) and percentage of mortality (±SE) in *Phthorimaea operculella*, at 28 days, in two bioassays carried out under microcosm conditions (with potato tubers and 25 ± 1 °C, and 16:8 h L:D), when the initial infestation by the pest was 10 (**A**) or 50 (**B**) *Phthorimaea operculella* eggs/container, respectively, and three release rates of the predatory mite *Blattisocius tarsalis* were used (0, 5, 10 and 20 adult mites/container). (In each figure, values with different letters indicate significant differences at $p = 0.05$).

4. Discussion

The predatory mite *B. tarsalis* can be an important tool for the biological control of storehouse pests. In previous works, the potential for controlling two pyralid pests,

E. kuehniella and *C. cautella* [21,22,25] has been studied, and more recently in the control of PTM [19].

Of the environmental variables that affect the predatory capacity of arthropods, temperature is one of the most influential factors in predator–prey dynamics [33]. In this sense, it has been verified that *B. tarsalis* can maintain predatory activity on pest eggs in the 10 to 30 °C range. At a temperature of 10 °C, *B. tarsalis* predates 2.79 ± 0.21 *P. operculella* eggs after 48 h of exposure, which is equivalent to 1.4 eggs/day, values higher than those obtained by Nielsen [22] (less than 1.0 egg/day), at the same temperature and offering *E. kuehniella* eggs as the prey. This might be for several reasons. In the first place, the size of the prey egg can influence the number of eggs killed (the egg content is partially or completely consumed) so the greater the content, the earlier the mite would be satisfied [25]. In this case, the sizes of the eggs used were similar—*P. operculella* = 0.5 × 0.35 mm [34] and *E. kuehniella* = 0.57 × 0.30 mm [35]. Secondly, at high prey density, predatory mites tend to feed over shorter periods and leave the prey without completely consuming it [36,37]; this would explain the differences in the number of eggs partially consumed with respect to Nielsen [22], where the number offered was 10 compared to the 5 offered in this work. Furthermore, it can be corroborated that the partially consumed (collapsed) eggs are similar for the three temperatures tested at a constant prey density. A third factor that might explain the difference could be a different nutritional composition between the eggs of the two prey species [38].

At 20 °C, the number of PTM eggs killed (Table 1) is like that reported by Nielsen [22]. The data obtained show how the predatory activity of *B. tarsalis* is maintained within the range of temperatures tested (10, 20 and 30 °C). There are two reasons why this is of special interest for pest moth control. First, under refrigerated storage conditions, and depending on the subsequent use and cooling treatments of the tubers, as indicated in the introduction, there will be periods at which the temperatures will be between 8–16 °C. Furthermore, at temperatures below 11 °C, *P. operculella* development stops [11]. The results show (Table 1) that the mite's predatory activity continues in the 10–20 °C range, resulting in an efficiency percentage of between 49.66 ± 5.06% and 78.17 ± 17%, which can be useful for pest control in these situations.

Secondly, the mite also remains active in the 20–30 °C temperature range; this is a range of interest in non-refrigerated storage situations. Between these temperatures, the mite had an efficiency of 75.57 ± 4.34% at 30 °C and 78.17 ± 4.77% at 20 °C. In other works, it has been shown that the predator behaves more effectively against the pest at intermediate temperatures. For instance, at 25 °C, mortality values of 99% were recorded using the same prey eggs under identical conditions [19], and a maximum consumption, depending on the state of the female, of 1.4 *P. interpunctella* eggs per day [26]. Likewise, in the 20–27 °C range, the mite's egg-killing activity on *E. kuehniella* increased with temperature [22]. At 27 °C, it was able to destroy as many as 3.54 and 4.8 eggs per day of *C. cautella* and *E. kuehniella*, respectively [22,25].

Another factor that may affect the biological control result could be the interactions between the density of predators (the interactions between conspecifics) and the density of available prey (the predator–prey ratio). Thus, it is likely that co-existing predators in the same habitats that exploit and share the same resource interact through intraspecific competition (exploitative competition, interference competition and/or cannibalism). This could explain the results obtained in the microcosm test at the low pest density, where *B. tarsalis* obtained better efficacy results at the density of five individuals/container than at higher predator densities (10 and 20 individuals/container) (Figure 1B). These values do not agree with the ones obtained for *B. mali* under identical conditions and release rates, where the percentage of efficacy increased when the predator release rate increased (72.50 ± 28.50%, 94.17 ± 8.12% and 100%) [18] in comparison with the ones found in this work for *B. tarsalis*, where the percentage of efficacy decreased with the increase of the release rate. As for that, *B. tarsalis* exerts a prey regulation behaviour when the search area does not greatly exceed the area where the prey can be found, and when the density of

the eggs is such that the spatial protection given to them is not excessive [39]. This does not appear to be in line with our results since the spatial protection is the same for the different predator densities. Furthermore, Flanders and Badgley [39] also determined that the equilibrium positions in the control of *E. kuehniella* are determined by the intraspecific competition for its eggs and that the intensity of this competition decreases as the spatial protection offered to the prey by its distribution increases. This situation could be adapted to our results, since increasing the predator density decreases the spatial protection of the prey, thus increasing the probability of interaction between predators.

Conversely, cannibalism and intraspecific competition could also occur as the density of mites increases, thus affecting the number of eggs that survive. This is particularly true in the case of predatory mites of the Phytoseiidae family, a near relative of the Blattisociidae, where after their primary food sources are depleted, there is an increase in intraguild predation between the predator species [40]. However, in the case of the Blattisociidae family, the frequency and intensity of these interactions are not entirely clear. Thomas et al. [20] found no evidence of intraspecific predation in *B. keegani* Fox. It has also been observed that this species will only consume smaller, immature conspecifics just prior to starvation, and that its own eggs appear to be immune to attack from conspecific adults and nymphs [41]. This also seems to be reflected in the results obtained for *B. mali* in the same type of trial [18]. Therefore, further research is necessary to elucidate the role that intraspecific interactions play in the control of moth eggs by *B. tarsalis*.

Regarding the microcosm test at the high pest density, the efficacy percentage for the control of the pest eggs was dependent on the predator density. Efficacy values of between 61.54 ± 9.21% and 92.31 ± 2.74% were obtained (Figure 1B), slightly higher than the low predator dose (5 mites per container) reported in *B. mali* and slightly lower than the higher mite doses (10 or 20 mites/container) [18]. On the other hand, it has been reported that, at temperatures of 24–28 °C, *P. operculella* eggs hatch at between 3.9 ± 0.04 and 3.3 ± 0.05 days [42]; considering the natural mortality of the controls, this supposes an egg destruction rate of between 1.73 and 0.44 eggs/mite per day for the three predator ratios tested. These values indicate a good search capacity, especially at the lowest mite release ratio.

As stated by Moraes et al. [43] for Blatisociidae and other closes species, the specific aspects to be evaluated for each prospective species should include their response to different climatic conditions and the range of potential prey organisms in the habitat where the predator is proposed for release. Both aspects are evaluated in this work for *B. tarsalis* in relation to stored potato conditions.

5. Conclusions

In summary, under the conditions tested, *B. tarsalis* has shown great potential in controlling *P. operculella* at temperatures at which *P. operculella* can cause damage to stored tubers. At low temperature (10 °C), the mite's predation rate was 1.4 eggs/day, with no problems observed, and this rate increased in the 20 to 30 °C range. In addition, we evaluated what the most effective dose could be at 25 °C to achieve efficacy values higher than 81%—this was found to be when 10 mites were released, regardless of the pest density (low or high). In the absence of studies that look at other aspects, and at the large scale, *B. tarsalis* would seem to be a good control organism, providing an alternative to pesticide use in the control of *P. operculella* under non-refrigerated tuber storage conditions, and during certain periods of refrigerated storage.

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