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Postharvest Biological Control of Guatemalan Potato Moth, *Tecia solanivora*, by *Trichogramma euproctidis* and *Blattisocius tarsalis*

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Abstract: *Tecia solanivora* (Berlese 1918), is a quarantine pest in Europe. Identified in Guatemala in the 1970s, it spread throughout Central and South America, reaching the Canary Islands in 1999 and mainland Spain in 2015. The pest has caused prohibitive economic losses both in the field and in storage, where losses can reach 100%. In the absence of approved chemical treatments, the use of an egg predatory mite, *Blattisocius tarsalis* (Berlese 1918), and the egg parasitoid, *Trichogramma euproctidis* Girault 1911, is being studied for use in storage. Previous laboratory studies have confirmed their potential for use in non-refrigerated stores, typically between 15 and 20 ºC. In the present work, we compared the efficacy of both natural enemies under semi-storage conditions. We observed that while both *T. euproctidis* and *B. tarsalis* reduced the *T. solanivora* populations (with an efficacy of 82.95 ± 7.32% and 49.06 ± 2.69%, respectively) and the number of mites per tuber, only *B. tarsalis* resulted in undamaged tubers (65%). For this reason, the mite was selected and tested in storage conditions, obtaining promising results in the protection of infested tubers, suggesting potential for further investigation, adaptation and standardization of its use in real conditions.

Keywords: storage; predator mite; egg parasitoid; *Solanaceae*; Lepidoptera; integrated pest management

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1. Introduction

Tecia solanivora (Povolný 1973) (Lepidoptera: *Gelechiidae*), commonly known as the Guatemalan potato moth, is native to Guatemala and was reported for the first time in 1956 [1]. Its spread has occurred mainly through the illegal trade and shipment of seed potatoes and tubers to several countries in Central and South America [2]. In 1999, the moth arrived in Tenerife (Spain) from Venezuela, and then spread to all the Canary Islands, from where is assumed it arrived in continental Spain, specifically in Galicia (2015) and Asturias (2017), i.e., the pest arrived in Europe [3,4].

Currently, the Guatemalan potato moth is the main pest on potato crops in the Canary Islands. It produces severe damages and high harvest losses, ≈50% in the field, and even 100% in storage facilities [5]. Due to its severity and to prevent its spread throughout Europe, *T. solanivora* was declared a quarantine pest in 2006 by the European Union. Eradication programs have been established in Galicia and Asturias (Spain) since 2015 and 2017, respectively, but because of the large implantation of this pest in the Canary archipelago at this moment, it was assumed to be not eradicable there [6].

In the field, unlike other moths, the *T. solanivora* larvae do not feed on the leaves or stems of the plant. The adult females lay their eggs in the irregularities and crevices of

the soil, near the stems. When the eggs hatch, the larvae migrate to the tubers and begin feeding on them, where they are protected from phytosanitary treatments [3,7]. During harvest, the eggs laid on the potatoes, as well as the first instar larvae, are difficult to distinguish from soil particles by the operator in charge of discarding infested tubers. Thus, frequently, these tubers bearing eggs/young larvae enter the warehouses and arrive at the market [8].

In warehouse facilities, substantial losses occur due to favorable environmental conditions for the proliferation of the Guatemalan potato moth. The low temperatures favor egg laying, which can reach 311 eggs along adult life at 15 °C, and the darkness favors the activity of the adult moths [3,9]. In addition, potatoes are stored unprotected. Unfortunately, during storage, options for chemical control of the Guatemalan potato moth are limited because most insecticides are not allowed to be applied just before potatoes are placed on the market as this would render them unsafe for human consumption. Presently, no pesticides are registered to control this moth in storage [8,10]. Therefore, research efforts are now focused on finding effective natural enemies of *T. solanivora* [11,12].

On this subject, Solà [13] reviewed papers focused on the use of natural enemies for biocontrol in storage facilities. She found the most studied organisms are parasitoids (51%), followed by entomopathogens (21%), and the smallest group is predators (18%). In all cases, most of the studies were performed exclusively under laboratory conditions. Concerning parasitoids, the most studied species are in the families *Pteromalidae* and *Braconidae*, followed by *Bethylidae*, *Ichneumonidae* and *Trichogrammatidae*.

Several entomopathogenic organisms have been tested against the Guatemalan potato moth, e.g., the fungi *Beauveria* spp., but these organisms showed very low potential in laboratory studies [14]; some strains of the bacterium *Bacillus thuringiensis* (Berliner, 1915) showed good result under laboratory conditions, but it has not been tested against the pest in warehouses [15]. For its part, the virus *Phthorimaea operculella*-granulovirus (PhopGV) has been tested in experimental conditions in warehouses with promising results [16], but unfortunately, the use of this natural enemy is not authorized in Spain.

In the case of predators, works mainly focused on mites, such as species in the genus *Blattisocius*, e.g., *B. dendriticus* (Berlese 1918), *B. keegani* (Fox 1947), and *B. tarsalis* Berlese (1918), and also on heteropterans, mainly anthocorids [13]. The use of predators and parasitoids on *T. solanivora* has received little attention and there are few papers available. However, several advantages of their application in storage facilities have been pointed out: once released in a storage facility, many biological control agents can maintain their population as long as there are available hosts, and they are often small and can feed on pests in the deep areas of stacked products. Furthermore, unlike chemicals, which must be carefully applied to fill the entire storage area, natural enemies can be released in a single, small area and are easily removed with normal cleaning routines. Nevertheless, the use of natural enemies requires more information about their biology and careful timing than traditional chemical insecticides [17]. On this matter, Osorio et al. [11], proposed the use of two generalist predators, the heteropterans *Lyctocoris campestris* Fabricius (1794) and *Buchananiella contigua* White (1880), based on laboratory experiments. Rubio et al. 2004 [18] tested *Trichogramma lopezandinensis* Sarmiento 1993 under semi-storage conditions, a *Trichogramma* species not present in the Canary Islands (Biota Database, https://www.biodiversidadcanarias.es/biota/ (accessed on 21 November 2023). Gavara et al. [19,20] tested the parasitoid *Trichogramma euproctidis* and the predator mite *Blattisocius tarsalis* in laboratory conditions. Predators and parasitoids are usually specialized in certain developmental stages of the target host or prey. In this case, both natural enemies are egg specialists [21–23]. This is an advantage because the larvae die before hatching, which is crucial as it prevents the larvae from feeding and protects the tubers [20]. The authors found that both *T. euproctidis* and *B. tarsalis* have a high potential to be tested against *T. solanivora* in the non-refrigerated conditions of smallholder potato storage (usually between 15 and 20 °C).

In the present work, therefore, *B. tarsalis* and *T. euproctidis* were tested in microcosms, semi-storage and storage conditions, following up the laboratory assays, with the aim to select and apply the most effective biocontrol agent against *T. solanivora*.

2. Materials and Methods

2.1. Insect Rearing

The primary individuals for the establishment of the *Tecia solanivora* culture were collected from local populations on the island of Tenerife. In the rearing process, 20 to 25 pupae were placed in a set of plastic glasses (d_1 = 11.5 cm, d_2 = 7.5 cm h = 15 cm; v = 1 L), the openings of which were covered with gauze tied with rubber bands and filter paper for oviposition. The gauzes were moistened with a solution of water and honey, mixed at 50%, using a paintbrush as a food source. The colonies were kept in climatic chambers under controlled conditions (20 °C, 70% RH, darkness). To ensure that eggs were not more than 24 h old, filter paper with fresh eggs was changed daily during the tests.

Trichogramma euproctidis was obtained as part of the natural enemy collection program in potato crops carried out by the Canarian Institute of Agricultural Research (Instituto Canario de Investigaciones Agrarias, ICIA). Rearing was performed with *Ephestia kuehniella* Zeller (1879) host eggs in Falcon™ tubes (v = 50 mL) with modified covers with stainless steel meshes. The culture was maintained at 20–25 ± 1 ºC, 60–65% RH, 16L:8D conditions. Parasitoids were fed on a thin line of honey placed on the walls of the tubes.

Tecia solanivora and *T. euproctidis* were reared by the company Agrobiologica S.L. (located at the Canarian Institute of Agricultural Research through a collaboration agreement), which regularly provided the insects needed for the experiments. The colonies were started at the beginning of 2021.

The original colony of *Blattisocius tarsalis* was provided by the Agricultural Entomology Laboratory at the University of Almeria, where the mite was collected and identified from a fortuitous infestation in a laboratory *Ephestia kuehniella* culture. Rearing was established in 2019, in the laboratory of the Plant Protection Unit of the Canarian Institute of Agricultural Research. In 15 × 20 cm plastic containers with a layer of vermiculite, the mite colony was maintained and propagated (25 ± 1 °C, 70% RH, darkness). The brood was fed three times a week with *T. solanivora* eggs supplied by BioAgroLogica SL.

2.2. Microcosm 15 °C

Following an adaptation of Gallego et al.'s [24] methodology (Figure 1), two bioassays were carried out, one at low infestation and another at high infestation level. In each of them, four treatments were applied that consisted of the following release densities: 0 (Control), 5 mites, 10 mites and 20 mites. The number of replicates per treatment was four.

First, 15 g of vermiculite (pupation substrate) was added to the bottom of 32 cylindrical containers (d_1 = 10.5 cm, h = 19 cm; v = 1 L), and 10 ml of distilled water was added to maintain humidity and favor egg hatching.

Next, 48 potatoes (cv. Slaney, size 60 × 75 mm) were infested in groups of three with *T. solanivora* eggs (not older than 24 h) to simulate two levels of infestation: a low level of 10 eggs/group (divided between the three potatoes) and a high level of 50 eggs/group (divided between the three potatoes). The eggs were spread in groups of 3–10 on the potato skin, on the irregularities or 'eyes' of the tubers, using a moistened brush. The groups of infested potatoes were then carefully placed in each container of their respective treatment.

Once the infested tubers were placed in the containers, *B. tarsalis* releases (unsexed adults) were carried out (5, 10 and 20 individuals), each in its respective treatment, and replicated at both infestation levels.

Finally, the plastic containers were sealed with pieces of muslin and rubber bands and placed in a climatic chamber (15 ± 1 °C, 70% RH) for 50 days. At the end of this period, the surviving larvae, pupae and adults of each treatment were counted and a second count was made 10 days later.

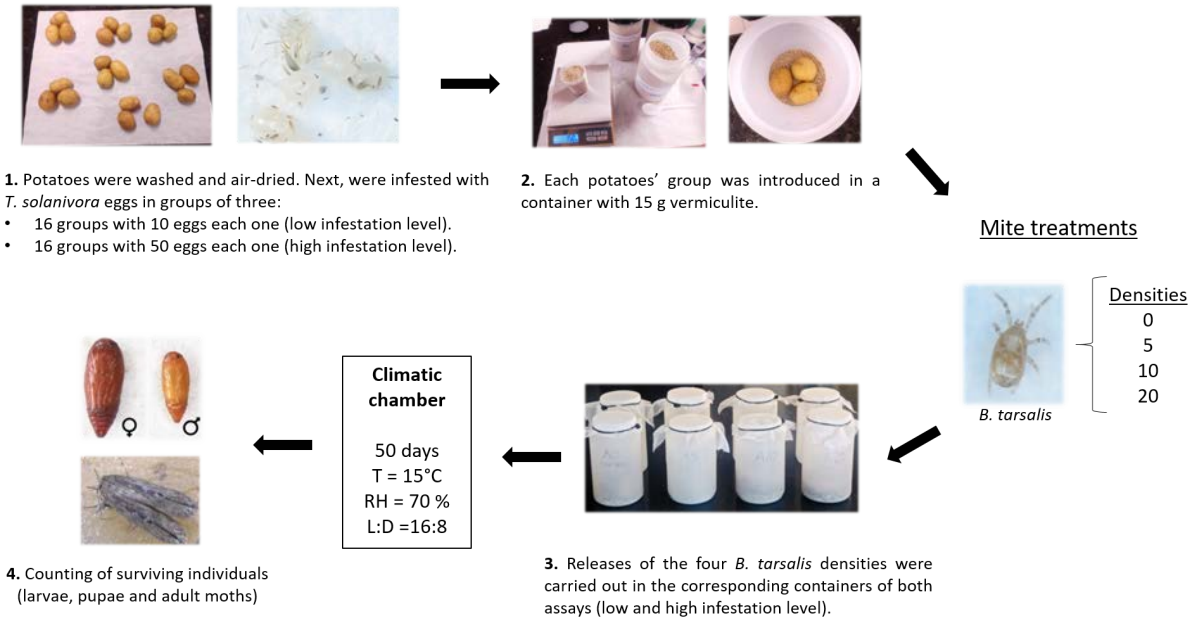


Figure 1. Summary steps of microcosm assay.

For statistical analysis, the data were tested for homogeneity of variance (F-test, Levene test) and normal distribution of residuals (Shapiro–Wilk test). For the high density (50 eggs), both parameters were confirmed and the means of each treatment were compared by ANOVA test using the GLM procedure Tukey's test at $p = 0.05$. In the case of low density (10 eggs), the data did not pass the normality test so were analyzed by a generalized linear model (GLM) using the Poisson distribution function and the log linear link function. In both cases, the analyses were carried out using IBM^{TM} SPSS^{TM} version 25 software. Finally, the efficacy was corrected with respect to the control with the modified Abbott equation [25]:

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

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

2.3. Semi-Storage

2.3.1. Experimental Procedure

In a cold chamber (17 °C, RH 60%, 0L:24D), 12 buckets ($d = 17$ cm, $d = 24$ cm, $v = 7$ L) with 20 potatoes tubers cv. Slaney (62 × 73.5 mm), previously washed, air-dried and numbered, were put over 1 cm of vermiculite as pupation subtract. Three treatments were carried out (control, *B. tarsalis* and *T. euproctidis*), each with four replicates (Figure 2).

Initially, 100 pupae (50 males and 50 females) of *T. solanivora* were sexed and when adults emerged, the most apparently healthy 40 males and females were selected. In all replicates of treatments, five couples (male and female) of moths were released and the buckets were sealed with a piece of fine muslin tied with a rubber band. After four days, 360 individuals (based on Gavara et al. [19] results) of *B. tarsalis* and 120 *T. euproctidis* (based on Gavara et al. [20] results) females were released in each replicate of their respective treatments.

After the release, the activity and development of moths and both natural enemies were allowed for 40 days. The muslin was then removed from the buckets and the infested potatoes were counted and carefully cut into thin slices to count surviving larvae, pupae and adults of *T. solanivora*. Healty-seeming tubers were returned to their respective buckets,

which were again sealed with muslin, and left in the cold chamber for 10 additional days. At the end of this period, the potatoes were inspected again as described above.

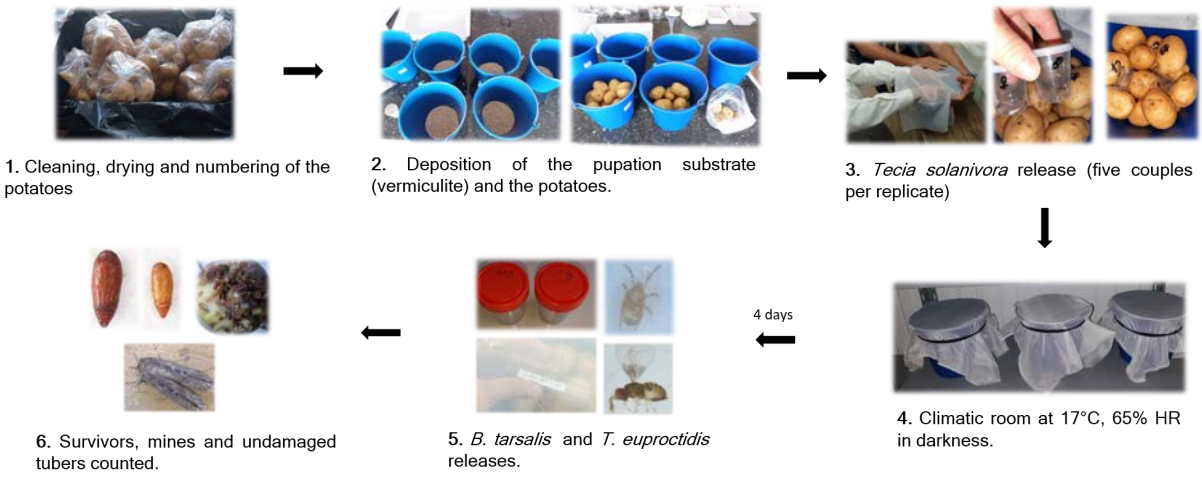


Figure 2. Summary steps of semi-storage assay.

2.3.2. Release Preparation of Natural Enemies

Based on the sex ratio of *T. euproctidis* obtained by Gavara et al. [20], the release procedure of this parasitoid consisted of cardboard with 150 *T. solanivora* parasitized eggs of 7–8 days of age (at 25 °C) per replicate of each treatment.

For *B. tarsalis* treatment, the mite-rearing container was shaken to homogenize the mite population within the vermiculite. Then, 20 samples (1 g each) were observed under a magnifying lens over a gridded petri dish, and mites were counted. The obtained average of mites per gram of vermiculite was used to obtain the weight of vermiculite needed to release 360 mites in each replicate of *B. tarsalis* treatment. The calculated average was 23.7 ± 1.2 *B. tarsalis* individuals g^{-1}, so 15.2 g of rearing vermiculite was applied in each replication of the treatment.

2.3.3. Statistical Analysis

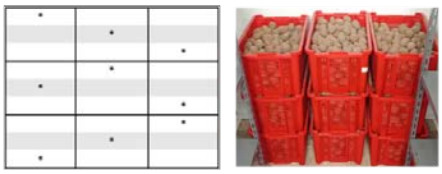
The experimental design was univariate and completely randomized, with only one factor—the presence of natural enemies—in three levels: Control (without natural enemy), *T. euproctidis* and *B. tarsalis*. The obtained mean values for the number of surviving *T. solanivora*, number of mites per tuber, and undamaged tubers percentage of the three treatments were compared using ANOVA test applying the GLM procedure by Tukey's test ($p = 0.05$), with previous confirmation of the homogeneity of variance (F test, Levene test) and the normal distribution of residuals (Shapiro–Wilk test). These analyses were performed with the statistical software IBM^{TM} SPSS^{TM} version 25.

2.4. Storage

The assay was carried out in two cold chambers with the same environmental conditions (16.58 ± 1 °C, 74.91 ± 5% RH, 0L:24D): one chamber for the control treatment and another one for the mite treatment. Each treatment consisted of nine stacked boxes (55 cm × 33 cm, h = 31 cm) containing 25 kg of potatoes (45 × 60 mm, cv Druid) with 10 numbered tubers. The numbered tubers were infested with 20 *T. solanivora* eggs (not older than 48 h) using a paintbrush moistened with distilled water. The infested tubers were placed in three different positions in the three boxes of each replicate: top, middle, and bottom as is shown in Figure 3.

1. Preparation of potatoes, numbering and infestation of 10 potatoes with 20 eggs of *T. solanivora:* 9 control and 9 treatment boxes.

2. Mite release (1315 individuals per crate) and potato storage was maintained for 30 days at 17 ºC.



3. Placement of infested potatoes among 25 kg of healthy potatoes at different heights in plastic boxes.

4. Collection and transfer of infested potatoes to mesh bags.

5. Counting of undamaged tubers and mines.

Figure 3. Experimental design of treatments in the storage assay: In 3, "*" indicates the location of the ten infested tubers in the fruit plastic boxes (up, middle or bottom).

Prior to stacking, 1315 individuals of *B. tarsalis* were released in each box of the Mite treatment. The mite sample was collected using the same method as described in the section "Preparation of natural enemies for release" of the semi-storage test: an average of 69.1 ± 4.1 (≈70) *B. tarsalis* individuals g^{-1} was obtained. Therefore, 19 g of mite-rearing vermiculite was added to each box to obtain 1315 mites.

After the release of *B. tarsalis*, the development of *T. solanivora* eggs and the predatory activity of the mites were allowed to occur for 30 days under the experimental conditions. Numbered potatoes from all replications of both treatments (Control and Mite) were then collected and placed in pre-labelled clothing bags. These bags were kept in a climate chamber at 25 °C, 70% RH and 0L:24D for one week to complete the development and emergence of the surviving larvae from the infested potatoes. Finally, the potatoes in each net bag were examined and the number of infested and healthy tubers and their number of mines was counted.

For the number of mites per tuber, the statistical analysis first checked normality and homogeneity using the Shapiro and Levene tests, respectively. Then, the ANOVA test with GML procedure with one factor at two levels (control and mite) was applied to compare the mean values with Tukey's test ($p = 0.05$). The analysis of the percentage of undamaged tubers was carried out using the Omnibus test ($p = 0.05$). The statistical analysis of the mite treatment position was supported by the Kruskal–Wallis test with one factor on three levels: Top, Center and Bottom.

3. Results

3.1. Microcosm 15 °C

Both at the low (10 eggs, Figure 4A) and high (50 eggs, Figure 4B) prey infestation levels, a significant effect of mite presence was observed comparing the number of surviving *T. solanivora* in Control vs. Mite treatments infestation (Omnibus test, $X^2 = 161.42$, df = 3, $p < 0.001$ at 10 eggs and $F_{3,2} = 13.38$; $p < 0.001$ at 50 eggs).

At both infestation levels no differences were found between *B. tarsalis* densities ($p > 0.05$ at all densities). The efficacies obtained for mites assayed densities (5, 10 and 20) for 10 eggs infestation were 92.85 ± 7.14%, 100.00 ± 0.00% and 100 ± 0.00%, respectively. The efficacies at high infestation levels were 50.28 ± 13.20% for 5 mites, 52.30 ± 7.94.53% for 10 mites and 71.02 ± 4.64% for 20 mites.

The number of surviving *T. solanivora* and their corresponding mortality rate at low and high infestation levels when exposed at the densities of 5, 10 and 20 *B. tarsalis* mites is shown in Figure 4.

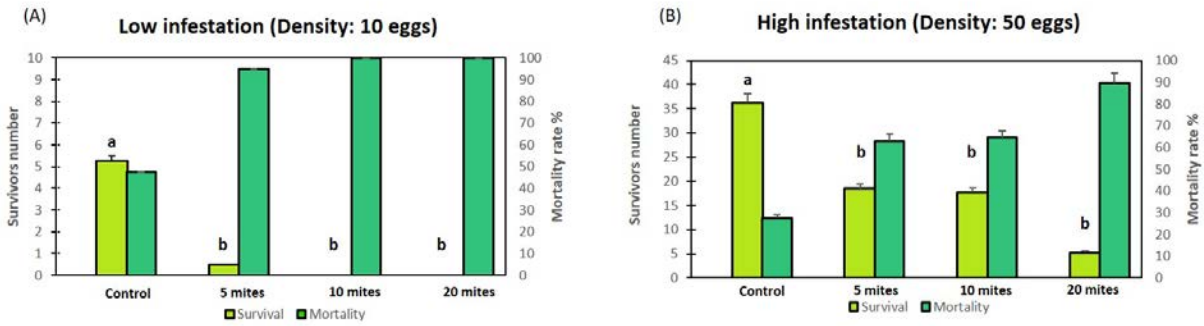


Figure 4. Mean values of surviving eggs (±SE) and mortality rate (±SE) at the infestation levels of 10 (**A**) and 50 (**B**) *T. solanivora* eggs, and doses of 0, 5, 10 and 20 mites. Values with different letters denote significant differences (Omnibus test, $p = 0.05$ for low density (**A**); one-way ANOVA, Tukey test, $p = 0.05$ for high density (**B**)).

3.2. Semi-Storage

3.2.1. Survival of *T. solanivora*

The statistical analysis showed highly significant differences for both natural enemies' treatments, *B. tarsalis* and *T. euproctidis*, with respect to Control in the number of surviving *T. solanivora* ($F_{2,9} = 177.64$, $p < 0.001$). The mite (*B. tarsalis*) treatment showed a significantly lower mean value of surviving *T. solanivora* compared to *T. euproctidis* treatment ($F_{2,9} = 177.64$; $p < 0.001$), with efficacies of 82.95 ± 7.32% and 49.06 ± 2.69%, respectively (Figure 5).

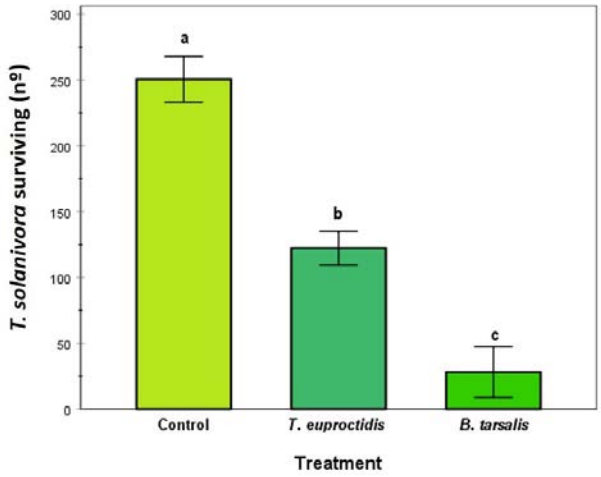


Figure 5. Mean number of surviving *T. solanivora* (±SE). Different letters indicate significant differences (one-way ANOVA, Tukey test, $p = 0.05$).

3.2.2. Tuber Damage

The number of mites per tuber showed the same pattern as the number of surviving *T. solanivora*. Both natural enemies, *B. tarsalis* and *T. euproctidis*, showed significant differ-

ences compared to the Control treatment ($F_{2,9}$ = 27.29; $p < 0.05$), with a lower significantly mean value for *B. tarsalis* with respect to *T. euproctidis* ($F_{2,9}$ = 27.29; $p < 0.05$) (Figure 6A).

The analysis of undamaged tubers (Figure 6B) showed that only *B. tarsalis* treatment obtained significant differences respect to the Control ($F_{2,9}$ = 14.80; p = 0.001), while *T. euproctidis* did not ($F_{2,9}$ = 14.80; p = 0.39), but it showed significant differences respect to *B. tarsalis* ($F_{2,9}$ = 5.21; p = 0.30).

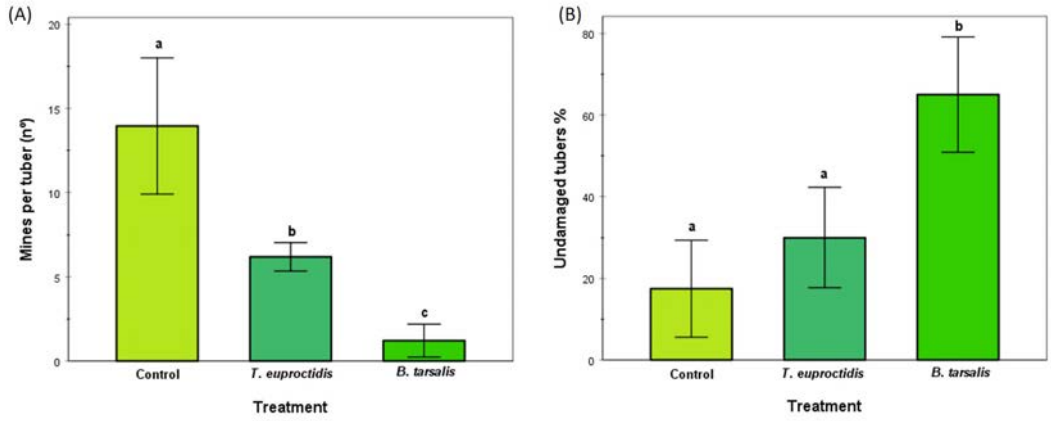


Figure 6. Mean number of mites per tuber (±SE) (**A**) and mean percentage of undamaged tubers (±SE) (**B**). Different letters indicate significant differences (one-way ANOVA, Tukey test, p = 0.05).

3.3. Storage

Statistical analyses showed that the mean number of mites per tuber (Figure 7A) and the percentage of undamaged tubers (Figure 7B) were significantly different in the *B. tarsalis* treatment compared to the Control ($F_{1,14}$ = 46.31; $p < 0.001$, and Omnibus test, X^2 = 40.34; df = 1; $p < 0.001$, respectively). On the other hand, the analysis of the effect of the position (Top, Center and Bottom) on tuber damage in the *B. tarsalis* treatment revealed that position did not affect mite efficacy ($F_{2,6}$ = 1.22; $p > 0.05$).

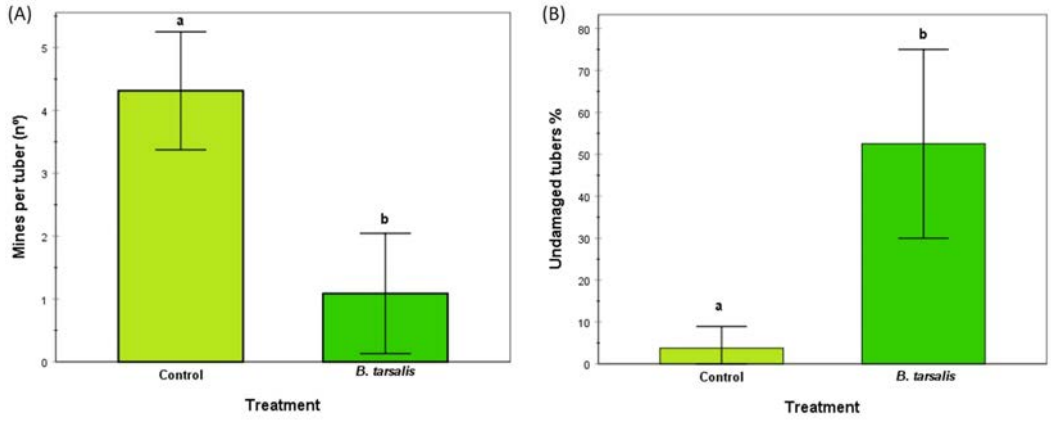


Figure 7. Mean number of mites per tuber (±SE) (**A**) and mean percentage of undamaged tubers (±SE) (**B**) of Control and Mite (*B. tarsalis*) treatment at storage conditions. Different letters denote significant differences (one-way ANOVA, Turkey-test, p = 0.05 and Omnibus test, p = 0.05; respectively).

4. Discussion

In the present work, the predator *B. tarsalis* and the parasitoid *T. euproctidis* were evaluated under microcosm, semi-storage and storage conditions to determine which of the two natural enemies was most effective in reducing populations and postharvest losses caused by the Guatemalan potato moth.

The potential of the predator mite was analyzed previously, under laboratory conditions, in the work of Gavara et al. 2022 [19]. This author pointed out that *B. tarsalis* would be unable to provide a successful biological control of the pest in big refrigerated store facilities (5–12 °C) due to the low predation efficacy obtained at 10 °C. Moreover it is in agreement with the very low activity and development showed at low temperatures in previous works by other authors [26,27]. Nevertheless, it could be used in non-refrigerated stored conditions, where the temperature range is usually between 15 and 20 °C, in the Canary Islands.

Concerning *T. euproctidis*, it was considered a good candidate as a biological control agent for *T. solanivora* under storage conditions by Gavara et al. [20], who confirmed its parasitizing capacity in darkness conditions and the constant parasitism activity in the range of 15–27 °C, with the maximum parasitism at 20 °C. Therefore, *T. euproctidis* would find suitable conditions for parasitism at the usual parameters of non-refrigerated warehouses. Our semi-storage assay showed that *T. euproctidis* reduced surviving *T. solanivora* by around 50%. However, the parasitoid did not avoid tuber damage, while *B. tarsalis* reduced the Guatemalan potato moth population by 80% and protected 65% of potatoes. These results suggested that the predator mite is the best bio-agent for pest control of *T. solanivora*. Solano-Rojas et al. [28] applied the same experimental method to compare the efficacy of *Trichogramma cacoeciae* (Marchal 1927) and *Blattisocius mali* (Oudemans 1929) against the tuber moth *Phthorimaea operculella* (Zeller 1873) and also obtained higher moth mortality with the mite than with the parasitoid.

We observed that in all treatments, especially in the control and *T. euproctidis* treatments, most of the infested tubers were located in the middle and bottom layers, those in contact with the walls and ground of the buckets. This is in agreement with the observations by Torres [29] who indicated that, under storage conditions, *T. solanivora* prefers to lay its eggs underneath the tubers, in the areas in contact with the floor or other surfaces, where it is more difficult for natural enemies to gain access. Searching efficiency has already been identified as a key criterion for the success of biological control, likely more relevant than the rate of intrinsic rise in natural enemies [21,30]. Our results suggest that *T. euproctidis* would have a low foraging ability to detect *T. solanivora* eggs and go inside gaps between tubers to descend to the layers below. Gavara et al. [20] also found no differences in the number of undamaged tubers for *T. euproctidis* compared to the control under semi-field conditions. It is known that the scales that adult hosts leave on eggs induce kairomonal activity in several parasitoid species, such as *Trichogramma* species [31]. As a possible explanation, they pointed to the *T. euproctidis*' poor ability to detect *T. solanivora* egg kairomones. It could be hypothesized that this may also occur under stored conditions, but further studies would be required in this regard. The low searching efficiency showed by the parasitoid entails increasing the number of individuals in the release density would not significantly improve the results. For this reason, *T. euproctidis* was discarded and only *B. tarsalis* was evaluated under storage conditions.

Based on non-refrigerated facility's temperature range, a first assay to test the mite activity was carried out at 15 °C under microcosm conditions. In this case, *B. tarsalis* reached its maximum mortality rate at the minimum release of five mites at both infestation levels (10 and 50 eggs, Figure 7). The efficacy of *B. tarsalis* against *T. solanivora* under microcosm conditions has already been tested at 25 °C in previous work of Gavara et al. [18], where similar results of efficacy were obtained at all mite densities at the low infestation level (10 eggs, ≈100%). However, at the high infestation level (50 eggs), compared to 25 °C, the efficacy of *B. tarsalis* in our experiments was lower: 50.28 ± 13.20%, 52.30 ± 7.94% and 85.77 ± 4.64% compared to 67.02 ± 13.53%, 82.29 ± 5.77%, and 98.52 ± 1.48% (for the densities of 5, 10 and 20 mites, respectively). These results denote the increase in the predator activity of *B. tarsalis* with temperature. This could be an advantage in non-refrigerated storages, subjected to changing temperatures, in which the control of the pest would be enhanced.

Although temperature is known to be one of the most important factors in predator–prey relationships, the coexistence of several predators in the same habitat, exploiting and sharing the same resources, can lead to intraspecific competition, which can also be detrimental to the biological control of a pest [32–34]. In this regard, our results did not show any negative effect on efficacy with the increase in the number of released mites.

In the semi-storage test, *B. tarsalis* showed good efficacy at high levels of infestation. This result was expected, as the trial was conducted under controlled conditions where the mites were confined to containers and had no choice but to move and feed on the moth eggs. However, in real commercial conditions, visual inspections are carried out during harvest prior to storage, resulting in the detection and removal of a proportion of infested potatoes. Consequently, the presence of infested potatoes should be relatively low, making it more difficult for the mites to find moth eggs.

Remarkably, in the storage test, *B. tarsalis* showed satisfactory efficacy even at very low infestation levels. It effectively reduced tuber damage (number of mites) by 50% (as shown in Figure 6), and this level of protection was consistently achieved throughout the volume of the plastic boxes. This underlines the ability of the mite to move between tuber stacks, efficiently finding *T. solanivora* eggs. Consequently, its efficacy can be increased with higher release densities. However, it is important to remember that actual infestation levels are typically higher than those tested, and as the semi-storage test results showed, *B. tarsalis* tends to perform even better under higher infestation conditions. Based on our results, a future application of *B. tarsalis* mites for biological control of the pest would require the study of different mite densities under real conditions. The need for additional mite releases, especially in long-term storage, should also be evaluated. Another critical aspect to consider is the timing of the release [35]. In this case, *B. tarsalis* should be applied as soon as possible after harvest to prevent hatching of the eggs.

The use of *B. tarsalis* should also consider the safety measures to be taken by the farmer or operator during the mite release process to avoid the occurrence of allergies and diseases associated with high concentrations of mites in enclosed spaces, particularly warehouses [36].

The choice of the storage system plays a crucial role in the use of natural enemies [21]. Although *B. tarsalis* was studied in storage boxes in this study, it is worth noting that potatoes are often stored in burlap sacks. In this scenario, despite the presence of holes that facilitate the passage of mites, it may be necessary to adapt the application method to the specific characteristics of the sack-based storage system.

It is important to emphasize that the use of a biocontrol agent such as the *B. tarsalis* mite is only a tool within an integrated pest management approach. In this context, it is important to recognize that *B. tarsalis* primarily targets the eggs and cannot reach the inside part of the potato where the larvae develop. Therefore, it remains imperative to maintain cultural practices such as routine inspection and removal of infested tubers from the crop.

5. Conclusions

Evaluation of the parasitoid *T. euproctidis* and the predatory mite *B. tarsalis* at semi-storage conditions showed that the parasitoid was ineffective, failing to produce intact tubers even when the *T. solanivora* population was low. In contrast, *B. tarsalis* showed promising results under storage conditions, suggesting potential for further investigation. Adaptation and standardization of its use in real conditions should be prioritized. In addition, the performance of predatory mites in alternative storage systems warrants further investigation. Finally, the economic viability of mass rearing and mite application should be analysed.

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