Potato Research, 63:241-251. This version of the article has been accepted for publication, after peer review but is not the Version of Record and does not reflect post-acceptance improvements, or any correction. The Version of Record is available online at https://doi.org/10.1007/s11540-019-09438-9.

Potential of the *Blattisocius mali* mite (Acari: Blattisociidae) as biological control agent of potato tubermoth (Lepidoptera: Gelechiidae) in stored potatoes

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ABSTRACT:

Potato tubermoth (PTM) *Phthorimaea operculella* (Lep.: Gelechiidae) is one of the pest species affecting Solanaceae worldwide. It can cause up to 80% of losses in potato open air crops as well as damage up to 100% of tubers during storage. *Blattisocius* (= *Typhlodromus*) *mali* (Acari: Blattisociidae), a predatory mite, was studied as a potential biological control agent of PTM. A prey acceptance bioassay of PTM eggs was carried out. Additionally, two bioassays have been conducted under microcosm conditions, which assess the densities of mite releases at two levels of PTM infestation. The results showed that *B. mali* female adults accept PTM eggs as prey, and they cause a mortality rate 89.63±2.47%, 48 hours later. In addition to this, under microcosm conditions with potato tubers, we found at low infestation level that the effectiveness of the predatory mite varied from 72.50±28.50% to 100%, twenty-eight days later, according to the release rate of mites. Under high infestation level, the effectiveness of biological control of the pest varied from 53.36±25.55% to 88.85±7.17%, also according to the release rate of the mites. The possible use of biological control with *B. mali* of PTM, in different types of potato storage, is analysed and discussed.

Keywords: Phthorimaea operculella, prey acceptance, non-refrigerated storage.

INTRODUCTION

Pests and diseases cause pronounced losses in potato crops (*Solanum tuberosum* L.); thus, they represent as an average: 40.3% for plant pathogens and viruses, 21.1% for animal pests, and 8.3% for weeds (Oerke 2006). In the second group, the main arthropod pests in this crop are Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae), potato tubermoth complex (Lep.: Gelechiidae) and the aphids, mainly *Myzus* (*Nectarosiphon*) *persicae* species Sulzer (Hem.: Aphididae) which is not just a pest but also an important virus vector in the crop (Radcliffe 1982; Oerke 2006). Among the potato tubermoth complex: the common potato tubermoth (PTM), *Phthorimaea operculella* (Zeller); the Andean potato tubermoth, *Symmetrischema tangolias* (Gyen); and the Guatemalan potato tubermoth, *Tecia solanivora* Povolný (Kroschel and Schaub 2013) are economically important pest species. Of these species, the PTM is a worldwide pest of Solanaceous crops and weeds, which is especially devastating to potatoes (Das and Raman 1994); causing up to 80% losses in field, and up to 100% losses during storage (Trivedi and Rajagopal 1992; Hanafi 2007; Rondon 2010; Aryal and Jung 2015). PTM is also the pest species that currently causes high economic losses in Spain, whether in open air crops or during tuber-storage. Recently, the introduction of Guatemalan potato tubermoth to Spain, has caused serious problems in the Canary Islands and North of Spain (Gallego et al. 2019).

To date, chemical insecticides have good results controlling PTM populations in field and in stores (Kroschel and Koch 1996; Hanafi 1999; Gao 2018). Nevertheless, there is resistance to insecticides (Dogramaci and Tingey 2008; Kuhar et al. 2013) together with increased restrictions (at least in EU countries) on permitted active substances (e.g., Kathage et al. 2018). Moreover, chemical insecticides create issues of chemical residues in potatoes for consumption (Narenderan and Meyyanathan 2019). Therefore, other options must be considered to control this pest (Douches et al. 2010; Aryal and Jung 2015), including the use of biological control agents (Gao 2018; Khorrami et al. 2018; Gallego et al. 2019).

A review of the morphology, biology and ecology, including parasitoid species used in biological control programmes (through the release of exotic natural enemies), is found for PTM in the works of Trivedi and Rajagopal (1992) and Kroschel and Schaub (2013). Likewise, Rondon (2010) and CABI (2018) list natural enemies (predators, parasitoids and enthomopathogens) of this pest species.

The use of predatory mites as natural enemies is increasing, particularly those that belong to the Phytoseiidae family, with excellent results in open field and greenhouses (Gerson et al. 2003). Furthermore, within biological controls of greenhouse crops, there is a present trend to replace the parasitoid species with species of predatory mites (Vila and Cabello 2014). Despite the high potential of biological pest control for stored products (Hansen 2007a, b), the use of biological control against pest arthropods in stored vegetal products is not very well developed (Credland 2010; Riudavets 2018). Nevertheless, there are some exceptions, such as the commercialization and use of parasitoid species, mainly *Trichogramma* spp. (Hym.: Trichogrammatidae) in stored products in central Europe (Schöller 2010). On the other hand, as PTM attacks both plants in field and tubers in the store, the effectiveness and methods of using biological control agents (predators, parasitoids and entomopathogens) have to maintain control in two different situations (e.g., Pokharkar and Jogi 2000; Rondon 2010).

It is known that many mite species of the old family Ascidae are free-living predators which live in the higher layers of soil, plants and stored products, where they feed on nematodes and small arthropods. They are often phoretic on flies and moth adult, whose eggs and small larvae serve as their food (Gerson et al. 2003). Recent studies have led to the separation of these species into three families, two of which Ascidae and Melicharidae were placed in the superfamily Ascoidea, and one (Blattisociidae) in the Phytoseioidea (Krantz and Walter 2009; Santos et al. 2018).

Within the Blattisociidae family, *Blattisocius* genus is a group of cosmopolitan species that appear in vegetal products stores. They feed on eggs and young larvae, as well as on different mite species (Gerson et al. 2003; Hagstrum et al. 2013). Some of the species belonging to this genus have been highlighted due to their potential as biological control agents of stored products (Nielsen 1999a; Hansen et al. 2001; Moraes et al. 2015; Athanassious and Rumbos 2018). However, the biology of most *Blattisocius* spp. remains poorly researched (Thomas et al. 2011). *Blattisocius* (= *Typhlodromus*) *mali* was described from specimens found in apple tree in Holland (Oudemans 1929). Its distribution area is China, Egypt, United Kingdom, Greece, India, Holland, Poland, Taiwan and Turkey and in facilities such as farm, flour mill and off-farm storage (Hagstrum et al. 2013).

Considering the above information, the aim of this work was to assess the potential of the *B. mali* mite as a biological control agent of PTM. To that end, an acceptance bioassay of PTM eggs as *B. mali* prey and two bioassays to evaluate the effectiveness of biological control under simulated conditions of storage ("microcosm") were carried out.

MATERIALS and METHODS

Biological materials and experimental conditions

B. mali mites were obtained and identified from a serendipitous infestation of PTM colony found in stored potatoes located at Tordesilla (Valladolid, Spain) in June 2018, using the keys of Nesbitt (1951) and Haines (1978). The specimens were reared at the Agricultural Entomology Laboratory of the University of Almería for 6 months, before the beginning of the experiments. *B. mali* mite colonies were kept in the laboratory in plastic containers (250 ml) filled with bran and the prey mite *Acarus gracilis* Hughes (Acaridae) (provided by Entomotech, Almeria, Spain); to do this, the methodological procedure of Gerson et al. (2003) was followed. The environmental conditions were 25±1°C and 80-90% relative humidity (RH).

The PTM population was reared in the same laboratory and it began with specimens provided by the Plant Protection Laboratory of Almeria (Andalusian Regional Government, Spain). To that end, the methodology described by Fenemore (1977) was used, and small potatoes (Variety: Marilyn, 1st category, size 28/45 mm) were used to feed the larvae. The environmental conditions of the offspring were 25±1°C, 60-80% RH and photoperiod of 16:8 (L:D) h.

Prey acceptance bioassay

A first "no-choice" bioassay test was carried out, in which the prey acceptance and mortality of PTM eggs by *B. mali* was assessed. To that end, female mites were placed individually in glass test tubes (7.0 cm x 1.0 cm of diameter). A piece of white cardboard (0.9 cm x 5.0 cm) was placed in each test tube ($1.0 \text{ cm} \times 7.0 \text{ cm}$) where five PTM eggs were stuck using a thin paintbrush (00) and water was then introduced together with a piece of sponge ($0.5 \times 0.5 \text{ cm}$) moistened in water. The test tubes were then sealed with cotton. During the next 48 hours, in the previously stated environmental conditions, females were left to prey on the eggs. In the check (control), the process was carried out as above but without introducing adult female mites into the test tubes.

The experimental design was univariate and completely randomised, with the only factor predatory mite compared with check. The number of repetitions was 39 for the mite and 20 for the check. At the end

of the bioassay, the eggs were examined under a binocular microscope and the number of eggs preyed on and/or partially consumed by mites was counted. Then, eggs were left developing over 7 days to allow for the possible emergence of PTM larvae.

The values corresponding to the number of PTM eggs that survived were analysed statistically through a generalised linear model (GLM) with the Poisson distribution and the log link function; likewise, the average values were compared by pairs through the Wald test at P = 0.05. To do this, IBM SPSS version 23 statistical software was used.

The effectiveness of the control of PTM eggs by the mite was assessed by the modified Abbot formula (Robertson and Preisler 1992):

$$EP = \left(\frac{M - M'}{100 - M'}\right) * 100$$

where, EP = effectiveness rate, M = mortality rate in the treatment (mite) and M' = mortality rate in the check.

Microcosm bioassay

Two bioassays were carried out under "microcosm" conditions, adapting the methodology described by Arthurs et al. (2008). To carry out the assessments, three tubers (Variety: Marilyn, 1st category, size 28/45 mm) were infested with different PTM egg densities (< aged 24 hours): 10 or 50 eggs/container, respectively in each bioassay. The eggs were stuck with a uniform distribution on the tuber buds (eyes) using a moistened thin paintbrush (00). The aim was to simulate oviposition of adult PTM females, which in the laboratory, lay eggs in groups of 2-20 eggs, usually close to the eyes (Al-Ali et al. 1975). Then, a vermiculite layer was arranged (150 ml, 14.6 g) on the bottom of a plastic container (height: 15.5 cm, diameter 10.5 cm and volume: 1 l) as pupal substrate. 10 ml of water was added to the vermiculite in the container to maintain high humidity to facilitate hatching. The three tubers infested with PTM eggs were carefully placed on the vermiculite layer. Then, different doses of non-sexed adult predatory mite *B. mali* were added by hand on the tuber surface using a moistened thin paintbrush (00). Finally, the container was closed with a round piece of filter paper stuck with vaseline.

In each bioassay, the experimental design was univariate and completely randomised, with the only factor predatory mite density at three levels (5, 10 and 20 mites released, one-time, per container); in addition to the check (control). The number of replications was 5 per bioassay and treatment. In both bioassays, the containers were kept at the previously stated environmental conditions, until the formation of pupae and/or emergence of adults, 28 days from the beginning of the bioassays.

The values corresponding to the number of surviving PTM (pupae or adults) 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. To do this, the IBM SPSS version 23 statistical software was used. The effectiveness of B. malis, at every release rate and bioassay, was also assessed by modified Abbot's formula, 28 days later.

Results

Prey acceptance

Figure 1 shows the number of surviving PTM eggs, as well as the mortality caused by the *B. mali* female adults, compared with the check. In the statistical analysis of the number of surviving eggs, a highly significant effect was found (Omnibus test: likelihood ratio $\chi^2 = 88.987$, df = 1, P < 0.0001). Therefore, the mortality rate 48 hours later was $89.63\pm2.47\%$ and the effectiveness of predation rate, compared with the check was $89.00\pm7.65\%$. Most of the eggs were preyed on and fully consumed compared with those that were partially consumed ($3.59\pm12.03\%$, in this last case).

Microscosm bioassay

Figures 2A and B show the number of survivors and the mortality rate in a PTM population under microcosm conditions exposed to different releasing rates of *B. mali* predatory mites and in the two bioassays carried out with two pest infestation levels.

In the statistical analysis of the number of survivors in bioassay 1 (Fig. 2A), with an initial infestation level of 10 PTM eggs per container, a highly significant effect was found; the Omnibus test showed that the model was highly significant to explain variance (likelihood ratio $\chi^2 = 66.096$, df = 3, P < 0.0001). Therefore, 28 days later, the effectiveness rate of the predatory mite was 72.50±28.50%, 94.17±8.12% and 100%, compared with the check, when 5, 10 and 20 mites/container were released, respectively.

Likewise, for bioassay 2 with an initial infestation of 50 PTM eggs per container, the statistical analysis of the number of survivors (Fig. 2B) was found with a highly significant effect (Omnibus test: likelihood ratio $\chi^2 = 209.117$, df = 3, P < 0.0001). Therefore, 28 days later, the effectiveness rate of the predatory mite was 53.36±25.55%, 88.85±7.17% and 88.85±7.17%, compared with the check, when 5, 10 or 20 mites/container were released, respectively.

DISCUSSION

Two species of *Blattisocius* genus, *B. keegani* Fox and *B. tarsalis* (Berlese), have been cited on vegetal origin products stored in Spain (Riudavets et al. 2002a; Pascual-Villalobos et al. 2006). Therefore, according to the literature consulted, this is the first time that *B. mali* is cited in a study for Spain and similar habitats, even though this species has a relatively important worldwide distribution (Hagstrum et al. 2013). Likewise, it is also the first time that PTM eggs are cited as prey of this mite species, according to the results obtained from the acceptance bioassay (Fig. 1). In this sense, it must be mentioned that other species of the same genus as *B. keegani* (Trivedi et al. 1994; CABI 2018), as well as species of other families, *Macrocheles muscaedomesticae* (Scopoli) (Acari: Macrochelidae) (Hassan et al. 2002), have been mentioned as predators of PTM eggs.

According to the values of surviving PTM eggs found (Fig. 1), the predation rate of *B. mali* female adult represents an average value of 2.24 killed eggs/female and day. This value is higher than those found for *B. tarsalis* in *Plodia interpunctella* (Hübner) eggs (Lep.: Pyralidae) by Darst and King (1969), *Ephestia kuehniella* eggs Zeller (Lep.: Pyralidae) by Nielsen (1999b), and *Amyelois transitella* eggs (Walker) (Lep.: Pyralidae) by Thomas et al. (2011). In addition to this, the values are similar to those found for *B. tarsalis*

in *E. cautella* eggs (Haines 1981; Nielsen 1999a) and in other insect eggs (Riudavet et al. 2002b). However, the values found are lower than those reported by Nielsen (2003), also in *E. kuehniella* eggs, for *B. tarsalis*.

In relation to the consumption of prey eggs, the rate of prey eggs partially consumed was low (3.59±12.03%); most of the eggs were totally consumed by *B. mali* female adult. This finding contrasts with Nielsen's reports (1999b) on *B. tarsalis* in *E. kuehniella* eggs, which found that eggs were partially consumed, thus confirming a general theory at the time that predatory mites leave the most part of the nutritious content of prey egg. Later, however, Riudavets et al. (2002b) found, in *E. kuehniella* eggs, that for this species of predatory mite the proportion of eggs partially consumed depended on egg densities; whereby at low densities, partial consumption rates are relatively low. This is also corroborated with the results of our acceptance bioassay.

As it was pointed out in the Introduction, most of *Blattisocius* spp. remains poorly researched (Thomas et al. 2011). In fact, the review carried out by Moraes et al. (2015) only includes information on life cycles and prey species for three species of the genus: *B. dentriticus* (Berlese), *B. keegani* and *B. tarsalis*. For this reason, this work contributes further knowledge about the biology of this group of species.

As it was stated in the Introduction, in the case of PTM, the possibility of use of biological control must consider two different situations, outdoors crops as well as tubers in stores. In this sense, it is known that in warm areas, the species of the old Ascidae family colonise artificial habitats such as food stores, mushroom-growing facilities and greenhouses, but rarely colonise plants; in contrast with this, in hot and wet conditions, they usually live in plants (Gerson et al. 2003). For this reason, the use of *B. mali* for the biological control of PTM in conditions of potato storage should be considered.

In support of the previous information, it is known that some *Blattisocius* species have been cited as potential biological control agents against pests of stored products (Nielsen 1999a; Hansen et al. 2001; Moraes et al. 2015; Athanassious and Rumbos 2018). Furthermore, the only bioassay published that was carried out under conditions of a flour silo for the control of *E. kuehniella* in Denmark, obtained good pest control, compared with the chemical control taken in previous years, through regular flooding releases of *B. tarsalis* (Schöller 2004).

However, in the biological control of pests in flour the capacity to introduce the *B. tarsalis* mite into the material presents problems. *B. tarsalis* is only capable of regulating *E. kuehniella* flour moth populations when the depth of the material (flour) does not exceed 8.0 mm (Flanders and Badgley 1963), and in some cases less: 5.3 mm (White and Huffaker 1969) or 1.0 mm (Nielsen 1998). This problem cannot be foreseen in the case of potato tubers piled up in stores (Bethke 2014) because there are enough spaces among tubers to allow the dispersion and action of the predatory mite, as was found in the bioassay under microcosm conditions (Fig. 2).

The use of *B. mali* under conditions of tuber storage must take into account both the proportion of the harvest that is stored and also the conditions of such storage. In the former, it is difficult to evaluate the amount of potatoes that are stored although estimates show that approximately 25% of potatoes used for human consumption are stored for some time, but with much variability between different countries and climate zones (Bethke 2014). In the latter, the conditions of such storage influence the development of PTM populations and subsequent damage to tubers (Hanafi 1999; Andreadis et al. 2016). These conditions also affect the potential to use predatory mites. In developing countries, tubers are stored without cooling (Rondon 2010) and also in developed countries in the case of autochthonous cultivars and very localised productions (e.g. Rios 2012). Likewise, the control of the storage temperature is essential for effective long-term storage, especially for some specific uses of the tubers. Potatoes must be kept at a temperature above 10 °C (15 to 20 °C) and 85-95% RH for at least 2-3 weeks before lowering to the desired temperature for long-term storage (1-2 °C) (Dean 1994; Alonso-Arce 2011). Under these conditions, PTM should not be a problem (Andreadis et al. 2016) nor are these the appropriate conditions to use the predatory mite.

Finally, it ought to be highlighted that the results found in this work should be validated through bioassays on a large scale under conditions of non-refrigerated stored potatoes. This is even more necessary if we take into account that bioassays have been carried out with parasitoid species which are effective in field conditions, such as *Copidosoma koehleri* Blanchard (Hym.: Encyrtidae) (Baggen and Gurr 1998; Pokharkar and Jogi 2000) or *Trichogramma* spp. (Hym.: Trichogrammatidae) (Urquijo 1944; Saour 2004); but, under conditions of stored potatoes, they showed low levels of PTM control (Keasar and Sadeh 2007; Saour 2009; Mandour et al. 2012).

Funding: This work has been funded within the project titled: Development of new methods for the integrated management of potato moths *Phthorimaea operculella* and *Tecia solanivora* (Ref.: RTA2015-00074-C02-00). It was funded within the programme of main research projects in 2015 and complementary actions within the framework of the state program R+D+I aimed at society challenges (safety and food quality challenge, sustainable and productive agrarian activity, sustainability of natural resources and sea and marine research). The National Institute for Agricultural and Food Research and Technology (INIA) and Ministry of Economy and Competitiveness, Spain.

Compliance with Ethical Standars

Conflict of Interest The authors declare that they have no conflict of interest.

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Figure 1: Number (\pm EE) of survivors and mortality rate (\pm EE) of *Phthorimaea operculella* eggs when exposed for 48 hours to adult female *Blattisocius mali* mite compared with the check, under laboratory conditions (25 \pm 1 °C, 80-60% RH and 16:8 h light/darkness) (values with different letters mean significant differences at P = 0.05)

Figure 2: Number (\pm EE) of survivors and mortality rate (\pm EE) of *Phthorimaea operculella*, 28 days later, in two bioassays carried out under microcosm conditions (with potato tubers and conditions 25 \pm 1 °C, and 16:8 h light/darkness), when the initial pest infestation was (A) 10 or (B) 50 eggs of *P. operculella*/container, respectively, and three doses of *Blattisocius mali* predatory mite releases (0, 5, 10 and 20 adult mites/container). (in each figure, values with different letters designate significant differences at P = 0.05)

Fig. 1





