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

ABSTRACT:

Potato tuber moth (PTM)*Phthorimaea operculella*(Lep.: Gelechiidae) is one of the pest species affecting Solanaceae worldwide. It can cause up to 80% of losses in potato cultivation in fieldas well asdamage up to 100% of tubersduring storage. *Blattisocius* (=*Typhlodromus*) *mali* (Acari: Ascidae),a predatory mite,was studied as a potential biological control agent of PTM. An acceptance assay of PTM eggs as prey was carried out. Additionally, two assays 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 that when the level of infestation of the pest was low, the effectiveness of the mite control varied from 72.50±28.50 to 100%, twenty-eight days later, according to the release rate of mites. Under high levels of infestation, 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 storages, are analysed and discussed.

INTRODUCTION

Pests and diseases cause pronounced losses in potato crops (*Solanum tuberosum* L.).Current reductions in the harvest are caused byapproximately:40.3% pathogens and viruses; 21.1% animal pests and 8.3% weeds (Oerke 2006).

The main arthropod pests in this crop are Colorado potato beetle*Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae), potato tuber moth complex (Lep.: Gelechiidae) and the aphids, mainly*Myzus* (*Nectarosiphon*) *persicae*species Sulzer (Hem.: Aphididae) which is not just a pest but also an important virosis vector in the crop (Radcliffe 1982; Oerke 2006).

Among these pest species, three of the Gelechiidae (Lepidoptera) familyform the knownpotato tuber moth complex: the common potato tuber moth (PTM), *Phthorimaea operculella* (Zeller); the Andean potato tuber moth, *Symmetrischematangolias* (Gyen); and the Guatemalan potato tuber moth, *Tecia solanivora* Povolný (Kroschel and Schaub 2013). In warm climates, the storage of potato tubersis affected by the PTM(Hanafi 2007). PTM is also the pest species that currently cause high economic losses in Spain, whether in

outdoors crops or during tubers storage.Recently, the introduction of Guatemalan potato tuber mothto Spain, has caused serious problems in the Canary Islands and North of Spain (Gallego et al. 2019).

The PTM is a worldwide pest of solanaceous crops and weeds, which is especially devastating to potatoes(Das and Raman 1994); causingup to 80% losses in field and up to 100%, losses during storage (Trivedi and Rajagopal 1992; Rondon 2010; Aryal and Jung 2015).

A review of the morphology, biology and ecology, including parasitoid species used in usual biological fight 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.

To date, chemical insecticides have good results controllingPTM 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).

As PTM attacksbothplants in field as well as tubers in the store, the effectiveness and methods of using biological control agents (predators, parasitoids and entomopathogens)has to maintain control in two different situations (e.g.,Pokharkar and Jogi 2000; Rondon 2010).

The use of predatory mitesas natural enemies is growing, particularlythose that belong to thePhytoseiidae family, with excellent results in open field and greenhouses (Gerson et al. 2003). Furthermore,withinbiological controlsof greenhouse crops,there is a currently trendtoreplace the parasitoid specieswith species of predatory mites (Vila and Cabello 2014).

Despite the high potential of biological pest control for stored products (Hansen, 2007 a,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 commercialisation and use of parasitoid species, mainly *Trichogramma* spp. (Hym.: Trichogrammatidae) in stored products in central Europe (Schöller 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 taken by flies and adult moths, 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 Ascoidea, and one (Blattisociidae) in Phytoseioidea (Krantz and Walter, 2009; Santos et al. 2018).

Within the Blattisociidae family, *Blattisocius* genusare cosmopolitan species that appear in vegetal products stores. They feed on eggs and young larvae, as well as on different mite species (Gerzon 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 as: farm, flour mill, off-farm storage (Hagstrum et al. 2013).

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

MATERIALS and METHODS

Biological material 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 bred in 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, Almería, Spain); to do this, the methodological procedure of Gerson et al. (2003) was followed. The environmental conditions were 25±1ºC and 80-90% of R.H.

The PTM population was bred in the same laboratory and it began with specimens provided by the Plant Health Laboratory of Almería (Andalusian Regional Government, Spain). To that end, the methodology described by Fenemore (1977) was used, and small potatoes (type side dish), were used to feed the larvae.Filter paper discs impregnated with moth eggs obtained from the mating chamber and oviposition of adults (approximately 20 pairs of adults wereconfined in 1000 ml plastic glasses sealed with a rubber band and a surgical gauze) were put in contactwith the tuberswhose surfacewas prepared with some holes to facilitate the introduction of neonate larvae.Additionally, a vermiculite layer was arranged to favour pupae formation. Once the larval

development was completed, the substrate was sieved to remove the pupae and place them again in mating chambers and oviposition until the emergence of adults.The environmental conditions of the offspring were 25±1°C, 60-80% of relative humidity (R.H.) and photoperiod of 16:8 (L:D).

Acceptance of prey assay test

A first “no-choice” bioassay test was carried out, in which the acceptance and predation 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). In each test tube a piece of a white cardboard (0.9 x 5.0 cm) impregnated with 5 PTMeggs was then introduced together witha piece of sponge (0.5 x 0.5 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 leftto prey onthe eggs. In thecheck, the process was carried out as above but without introducing adult female mites into the test tubes.

The experimental design was univariate and fully 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 assay, 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 over7 days to allow for the possible emergence of PTM larvae. The environmental conditions were 25±1ºC and 80-90% of R. H. and 16:8 h of Light/Darkness.

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.

Effective bioassays in microcosm

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 PTMegg densities (<aged 24 hours): 10 or 50 eggs/container,

respectively in each assay. The eggs were stuck withuniformdistributionin theeyes of thetubers 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 pupa 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 carefullyplaced on the vermiculite layer. Then, different dosesof 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 fully randomised, with the only factor: predatory mite density at three levels (5, 10 and20 mites/container); in addition to the check. The number of replications was 5 per bioassay and treatment.In both bioassays, the containers were kept at 25±1ºC and 16:8 light:darkness hours, 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; at the same time, the average values were compared in pairs through aWald test atP = 0.05. To do this, the IBM SPSS version 23 statistical software was used.

The effectiveness of *B. malis,* at every release dose and bioassay, was also assessed by a previously modified Abbot formula,and was indicated 28 days later.

Results

Acceptance of prey assay test

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 ratioχ^2 = 88.987, df = 1, P < 0.0001). Therefore, the mortality rate 48 hours later was 89.63±2.47 and the effectiveness of predation rate, compared with the check was 89.00±7.65%. Most of the eggs were preyed on and fullyconsumed compared with those that were partially consumed (3.59±12.03%, in this last case).

Bioassays of effectiveness in microcosm

Figures 2 A and B show the number of survivors and the mortality rate in a PTM population under microcosm conditions exposed to different release doses

of *B*. *mali* predatory mites and in the two bioassays carried out with two levels of infestation of the pest species.

In the statistical analysis of the number of survivors in bioassay 1 (**Figure 2 A**), with an initial infestation level of 10 PTM eggs per container, a highly significant effect was found, theOmnibus test showed that the model was highly significant to explain variance (likelihood ratioχ^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 or 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 (**Figure 2 B**) was found with a highly significant effect, theOmnibus testshowed that the model was highly significant to explain the variance (likelihood ratioχ^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 citedstudies on vegetal origin products stored in Spain (Riudavets et al., 2002 a; Pascual-Villalobos et al., 2006). Therefore, according to the literature consulted, this is the first time that *B*. *mali*is cited in a study forSpain 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 assay (**Figure 1**). In this sense, it must be mentioned that other species of *B*. *keegani*genus (Trivedi et al. 1994; CABI 2018), as well as species of other families: *Macrochelesmuscaedomesticae* (Scopoli) (Acari: Macrochelidae) (Hassan et al. 2002) have been mentioned as predators of PTM eggs and other PTM states.

According to the values of surviving PTM eggs found (**Figure 1**), the predation rate of *B*. *mali*female adult represents an average value of2.24 dead eggs/female and day. This value is higher than those found for *B*. *tarsalis* in*Plodiainterpunctella*eggs (Hübner) (Lep.: Pyralidae) by Darst and King (1969), in*Ephestiakuehniella*eggs Zeller (Lep.: Pyralidae) by Nielsen (1999b), or*Amyeloistransitella*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 1999 a) 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 tothe 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 findingcontrastswith Nielsen's reports on (1999b) *B*.

*tarsalis*in *E*. *kuehniella*eggs, which found that eggs were partially consumed, thus confirming ageneral theory at the time that predatory mites leavethe most part of the nutritious contentprey egg. Later, however, Riudavets et al. (2002b) found, in *P.interpunctella* eggs,that forthis 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 of prey assay test.

As it was pointed out in the Introduction, most of *Blattisocius* spp. remains poorly researched (Thomas et al., 2011). In fact, if we review the life cycles and preys consumed by *Blattisocius*species,Moraes et al. (2015) only include the species: *B*. *dentriticus*(Berlese), *B*. *keegani*and*B*. *tarsalis*. For this reason, this work contributes furtherknowledge 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 storageshouldbe 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 assay 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 thebiological control of pests in flourthe capacity to introducethe *B. tarsalis*mite into the material presents problems.The*B. tarsalis* is only capable of regulating the *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 tubersto allow the dispersion and action of the predatory mite, as was found in the assay under microcosm conditions (**Figure 2**).

The use of*B. mali*under conditions of tuber storage must take into account both the proportion of the harvest that isstored and also the conditions of such storage. In the former, it is difficult to value the amount of potatoes that are stored – although estimates show that approximately 25% of potatoes used for human consumption are stored for some time, there ismuch variability between different countries and climate zones (Bethke 2014). In the latter, the

conditions of such storageinfluence the development of PTMpopulations 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 ofautochthonous varieties and very localised productions (p.e.: Rios 2012). Likewise, the control of the storage temperature is essential for effectivelong-term storage, specially for some specific uses of the tubers. Potatoes must be kept at a temperature above 10ºC (15 to 20ºC) and 85-95% of R.H, for at least 2-3 weeks before lowering the temperature up to the desired temperature for long-term storage (1º-2ºC) (Dean 1994; Alonso-Arce 2011). Under these conditions, PTM shouldnot be a problem (Andreadis et al., 2016) nor are thesethe appropriate conditions to use the predatory mite.

Finally, it ought to be highlighted that the results found in this work shouldbe validated through assays on a large scale under conditions of non-refrigerated stored potatoes. This is even more necessary if we take into account that assays have been carried out with parasitoid species which are effective in field conditions, such as*Copidosomakoehleri*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).

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

Figure 2: Number (±EE) of survivors and mortality rate (±EE) of*Phthorimaea operculella*, 28 days later, in two bioassays carried out under microcosm conditions (with potato tubers and conditions 25±1ºC, and 16:8 h light/darkness), when the initial infestation by the pest was (A) of 10 or (B) 50 eggs of *P. operculella*/container, respectively, and three doses of

*B.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).