Evaluation and selection of biological control agents for the integrated management of the Guatemalan potato moth, *Tecia solanivora*

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

ICIA
INSTITUTO CANARIO DE INVESTIGACIONES AGRARIAS

Gobierno de Canarias

Jorge Gavara Vidal
April 2024

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Programa de Doctorat en Recursos i Tecnologies Agrícoles

Instituto Canario de Investigaciones Agrarias

DOCTORAL THESIS

"Evaluation and selection of biological control agents for the integrated management of the Guatemalan potato moth, *Tecia solanivora*."

Jorge Gavara Vidal

Directed by:

Ana Piedra-Buena Díaz

Estrella Marina Hernández Suárez

València, April de 2024

AGRAÏMENTS / AGRADECIEMEINTOS

En primer lugar, agradecer a mis directoras, Ana Piedra-Buena y Estrella Hernández por darme la oportunidad de haber vivido esta etapa de mi vida y haberme sacado de mi área de confort en "la Terreta". He encontrado en Tenerife un segundo hogar y estilo de vida que echaré siempre de menos. Ana, por acogerme y estar pendiente de mí todos estos años, además de directora, también una buena amiga y compañera provisional de despacho a la que chinchar de vez en cuando, y por aguantarme en el día a día y a deshoras. Gracias Estrella por asegurarse siempre de que no me falta de nada y sacar tiempo para atenderme a pesar de tu siempre abultada agenda. Y a Mery Jaizme, jefa de departamento primero, directora científica después, pero siempre acogiéndonos con afecto y velando por todos.

También agradecer a las compañeras del laboratorio, Laura, Rosita y Carina por su ayuda y guía cuando andaba perdido en temas cuotidianos mientras me instalaba en la isla y en el ICIA, y por los ratos divertidos haciéndonos la puñeta. José Ramón compañero y amigo, y Pepe por esas comidas entre semana que ya estoy echando de menos. A María, Nancy, Begoña, Carina, Nayra, Cari y Marta por sus ratos de charlas y apoyo y hacer que el laboratorio no sea aburrido. A Mihaela y Alberto el equipazo de Tecia por su ayuda y lo a gusto que se trabaja con ellos. A Saskia compañera de congresos, y de batallas varias de predoc en el día a día. A Vanesa y Moneyba por su ayuda durante los ensayos en la primera etapa de la tesis y en especial a Tere, la supertécnico de laboratorio por su indispensable ayuda a lo largo de toda la tesis, sus consejos tanto para la tesis como para la vida y su gran estima.

A los supervivientes de la tarde, Federico y Felipe por sus consejos y ayuda, y los "nuevos doctorandos" David y Marta, dejándose marear y molestar cuando me aburría e iba a darles charla.

Agradecer también a los compañeros y colegas de BioAgroLógica, Tomás, Tamara, Arnoldo y Alejandro, por su ayuda y el gran esfuerzo al atenderme con la provisión de insectos, aunque se los pidiera tarde y mal. Y por esos desayunos, con charlas, risas y cotilleos cuando iba a mendigarles café.

A las compañeras de Producción Vegetal en Zonas Tropicales y Subtropicales, Gloria Lobo y Goretti Díaz, por su apoyo, ayuda y prestarnos sus instalaciones para el desarrollo de la etapa de almacén.

A los compañeros del Servicio Técnico de Agricultura y Desarrollo Rural del Cabildo de Tenerife, Eugenia Trujillo y Santiago Perera, por su ayuda e interés constante y estar siempre disponible para resolvernos las dudas.

Gracias a los que me han acogido en estancia, Raquel Campos en el departamento de Viticultura del Instituto de las Ciencias de la Vid y del Vino de La Rioja, por iniciarme en el manejo de nematodos, y a su equipo de doctorandos.

Agradeço a David Horta, do CE3C da Universidade dos Açores, por me ter recebido e ensinado sobre a gestão de pragas-chave e pela oportunidade de ver uma ilha tão bonita.

Thanks to Nina Fatouros from the Biosystematics Group (Wageningen University and Research) and especially to Liana Greenberg for hosting me, helping me and teaching me new essays during my stay.

Un especial agradecimiento a Tomás Cabello y Juan Ramón Gallego del CIAMBITAL de la Universidad de Almería, por su ayuda y soporte ininterrumpido durante todo el desarrollo de la tesis, aún con las dificultades y molestias de la distancia. Sin duda ha sido una colaboración muy fructífera sin la cual la presente tesis no sería la misma.

A la meua família pel suport i per les seues visites vacacionals per a gaudir de l'illa i els moments divertits en companyia, especial agraïment al meu germà pel seu ajut en el tram final de la tesi. També als meus amics, tant els que em van visitar, com els que estaven esperant-me en la Terreta amb els braços oberts per aprofitar el temps i no perdre el contacte i els plans de sempre.

Finalmente, a los amigos peninsulares de Tenerife con los cuales ha sido un placer compartir estos años conociendo la isla, disfrutando el tiempo y haciendo planes imposibles en nuestras tierras de origen.

Abstract

The Guatemalan potato moth, *Tecia solanivora*, is an invasive species. It is native to Guatemala, from where it spread to Central and South America, arriving in the Canary Islands in 1999 and reaching mainland Spain in 2015. Although it is in the process of being eradicated from the Iberian Peninsula, it is established in the Canary Islands and is not considered eradicable.

The management of *T. solanivora* is very complex. Almost its entire biological cycle takes place under the soil and inside the tuber, which protects the pest, and only the adult stage is exposed. If no management strategies are applied, crop losses can reach around 50% in the field and 100% in storage. Cultural measures aim to reduce the pest population in the field during the growing, are combined with pheromone traps to prevent crop losses. However, pest damage often results in unacceptable economic losses. In addition, there is currently a lack of enough effective phytosanitary treatments that can be used in the field or approved for storage. In this situation, research into biological control agents has become particularly important. In this context, the study of oophagous natural enemies such as *T. achaeae*, *T. euproctidis* and *B. tarsalis* is of particular interest for the biological control of the Guatemalan potato moth, as they prevent the eggs from hatching. Once the eggs hatch, the larvae migrate to the soil, making it difficult to remove them before they enter the tubers where they are protected. The use of oophagous biocontrol agents therefore removes the pest before the potatoes are damaged.

Experimental methodology was applied to evaluate both *Trichogramma* species to select the best in three biocontrol contexts, laboratory, semi-field and semi-storage, and the predatory mite *B. tarsalis* under laboratory, semi-field and storage conditions. Although *T. euproctidis* showed good preliminary results in laboratory studies for both field and storage conditions, semi-field and semi-storage trials suggest that it is not a good biocontrol agent due to its poor ability to locate *T. solanivora* eggs. In contrast, *T. achaeae*, although less effective than *T. euproctidis* under laboratory conditions, proved to be a promising control agent under field conditions. These results highlight the importance of testing natural enemies under semi-field/semi-storage conditions prior to field or storage testing. Indeed, selecting biocontrol agents on the basis of positive laboratory test results alone could lead to wrong decisions and unnecessary expenditure of effort and resources. In the case of *B. tarsalis*, which has been shown to be a good biocontrol agent in

experimental storage, there is a need to improve mite releases (density of mites per release and number of releases) and evaluate their use in other storage systems. Finally, a study of their economic viability will be required, taking into account the cost of mass rearing.

It is important to note that the use of biological control agents, such as the parasitoid *T. achaeae* and the mite *B. tarsalis*, are only two of the tools available for integrated pest management. Under field conditions, cultural measures and trapping must be maintained to reduce adult populations and egg laying. In storage, visual inspection and screening of infested potatoes should also be maintained.

Resum

L'arna guatemalenca de la creïlla, *Tecia solanivora* és una plaga invasora originària de Guatemala des d'on es va disseminar al llarg de Centre i Sud-amèrica, arribant a les Illes Canàries el 1999 I alcançant l'Espanya peninsular el 2015. Tot i que en la península Ibèrica es troba en procés avançat d'erradicació, en l'arxipèlag canari ja s'ha establit i es considera que l'erradicació no és possible.

La gestió de *Tecia solanivora* es molt complexa degut a que pasa la major part del cicle vital davall del sòl, refugiada en l'interior del tubèrcle, només la fase adulta está completament exposta. Per al maneig de la plaga, habitualment s'apliquen mesures culturals durant tot el cicle vegetatiu, que es combinen amb l'ús de paranys de feromones, contribuint les esmentades mesures a reduir pèrdues en la collita. Tanmateix, estes continuen sent econòmicament inassumibles. A més ens trobem amb una carència de productes fitosanitaris per al camp prou eficaços i amb l'absència de productes autoritzats en l'àmbit dels magatzems. De portar-se a terme cap estratègia de gestió de la plaga, les pèrdues en la collita poden suposar al voltant del 50% al camp i del 100% als magatzems.

En este context, la recerca d'enemics naturals per a la gestió integrada de la plaga ha cobrat especial rellevància. Particularment, són interessants els organismes oòfags, com els estudiats en el present treball, els parasitoides *Trichogramma achaeae*, *Trichogramma euproctidis* i l'àcar depredador *Blattisocius tarsalis*, que permeten eliminar la plaga abans que es produïsca l'eclosió dels sous, fet que resulta transcendent, donat que una vegada es produeix l'emergència de la larva, esta migra cap a l'interior del sòl i penetra en els tubercles resultant molt difícil la seua eliminació. Per tant, estos organismes de biocontrol eliminen la plaga abans que les creïlles resulten danyades.

En el present treball es va aplicar una metodologia per realitzar un procés d'avaluació per ambdós espècies de *Trichogramma*, amb l'objectiu de seleccionar la millor, en els tres contextos, laboratori, semicamp i semimagatzem, I l'àcar depredador sota condicions de laboratori, semimagatzem i magatzem. Els resultats van revelar que si bé, *T. euproctidis*, mostrà molt bon potencial en els estudis preliminars de laboratori tant per a condicions de camp com de magatzem, els experiments de semicamp i semimagatzem van indicar que no es un bon agent de

control biològic per a *T. solanivora* donada la seua escassa capacitat de trobar els ous. En contraposició, *T. achaeae*, encara que va mostrar una eficàcia baixa en condicions de laboratori, es va mostrar prometedor en condicions de semicamp. Estos resultats il·lustren la importància de provar els agents de biocontrol en condicions de semicamp o semimagatzem abans de fer el pas a condicions de camp o magatzem, basar-se solament en resultats de laboratori podria suposar la presa de decisions incorrectes amb una pèrdua d'esforços i recursos. En el cas de *B. tarsalis*, s'obtingueren resultats òptims en totes les etapes de selecció, i per eixa raó va ser provat en magatzem experimental on es va mostrar com un bon agent de control de l'arna guatemalenca de la creïlla. En este sentit, s'han de continuar les investigacions amb l'objectiu de millorar els alliberaments (densitats i número), i avaluar el seu ús en altres sistemes d'emmagatzematge. Finalment, es requerirà l' estudi de la seua viabilitat econòmica tenint en compte el cost de la cria massiva.

És important assenyalar, que l'ús d'agents de control biològic, com el parasitoide *T. achaeae* i l'àcar *B. tarsalis* seran sols dos de les eines disponibles per al control integrat de la plaga. En condicions de camp, les mesures culturals i la instal·lació de parany deuen mantindre's per reduir les poblacions d'adults i les postes d'ous. Així mateix, en magatzem les inspeccions visuals i el rebuig de creïlles infestades deuen mantindre's.

Resumen

La polilla guatemalteca de la papa, *Tecia solanivora*, es una plaga invasora originaria de Guatemala desde donde se extendió a lo largo de Centro y Sudamérica, llegando a las Islas Canarias en 1999, y alcanzando la España peninsular en 2015. Si bien en la península Ibérica se encuentra en proceso avanzado de erradicación, en el archipiélago canario ya se ha establecido y se asume que su erradicación no es posible.

El manejo de *Tecia solanivora* es muy complejo debido a que pasa la mayor parte de su ciclo biológico bajo el suelo, protegida en el interior del tubérculo, quedando completamente expuesta solo la polilla adulta. Para la gestión de la plaga se aplican habitualmente medidas culturales durante el ciclo vegetativo, que se combinan con el uso de trampas de feromonas, contribuyendo estas medidas a reducir pérdidas en la cosecha, no obstante, estas siguen siendo económicamente inaceptables. Además, existe una carencia de productos fitosanitarios suficientemente eficaces en campo y la ausencia de productos autorizados para su uso en almacenes. De no ejecutar ninguna estrategia de gestión de la plaga, las pérdidas en las cosechas pueden alcanzar alrededor del 50% en campo y del 100% en almacén.

En este contexto, la búsqueda de enemigos naturales para la gestión integrada de la plaga ha cobrado especial relevancia. Particularmente, son interesantes los organismos oófagos, como los estudiados en el presente trabajo, los parasitoides de huevos, *Trichogramma achaeae*, *Trichogramma euproctidis* y el ácaro depredador *Blattisocius tarsalis*, que permiten eliminar la plaga antes que se produzca la eclosión de los huevos, hecho que resulta trascendental, dado que una vez se produce la emergencia de la larva, esta migra hacia el interior del suelo y penetra en los tubérculos resultando muy difícil su eliminación. Por lo tanto, estos agentes de biocontrol eliminan la plaga antes de que las patatas resulten dañadas.

En el presente trabajo se aplicó una metodología para realizar un proceso de evaluación para ambas especies de *Trichogramma* con el objetivo de seleccionar la mejor en tres contextos, laboratorio, semicampo y semialmacén y el ácaro depredador bajo condiciones de laboratorio, semialmacén y almacén. Los resultados revelaron que si bien, *T. euproctidis* mostró muy buen potencial en los estudios preliminares de laboratorio tanto para condiciones de campo como de almacén, los experimentos de semicampo y semialmacén indicaron que no es un

buen agente de control biológico para *Tecia solanivora* debido a su escasa capacidad para localizar sus huevos. En contraposición, *T. achaeae*, aunque mostró una eficacia más baja en condiciones de laboratorio, dio resultados prometedores en condiciones de semicampo. Estos resultados confirman la importancia de probar los agentes de biocontrol en condiciones de semicampo o semialmacén antes de dar el paso a condiciones de campo o almacén, dado que basarse solo en resultados positivos de laboratorio podría llevar decisiones incorrectas y una perdida innecesaria de esfuerzos y recursos. En el caso de *B. tarsalis*, se obtuvieron resultados óptimos en todas las etapas de selección, por lo que fue probado en almacén experimental donde se mostró como un buen agente de control de la polilla guatemalteca de la papa. En este sentido, se deberán continuar las investigaciones a fin de mejorar las liberaciones (densidades y número), y evaluar su uso en otros sistemas de almacenaje. Finalmente, se requerirá un estudio de su viabilidad económica teniendo en cuenta el coste de la cría masiva.

Es importante señalar que el uso de agentes de control biológico, como el parasitoide *T. achaeae* y el ácaro *B. tarsalis* serán solo dos de las herramientas disponibles para el control integrado de la plaga. En condiciones de campo, las medidas culturales y el trampeo deben mantenerse para reducir las poblaciones de adultos y las puestas de huevos. Así mismo, en almacén las inspecciones visuales y el cribado de patatas infestadas deben mantenerse.

Evaluation and selection of biological control agents for the integrated management of the Guatemalan potato moth, *Tecia solanivora*

I. Index

II. Figures index

General introduction

Chapter I

Chapter II A

Chapter II B

III. Table index

General introduction

Chapter I.

Chapter II A.

General introduction

1. General introduction

Every year, potato crops worldwide suffer significant economic losses due to pests of the moth complex, namely three species of the order Lepidoptera, within the genus Gelechiidae: *Phthorimaea operculella* (Zeller 1873), *Symmestrischema tangolias* (Gyen 1913) and *Tecia solanivora* (Povolný 1973) (Sánchez-Aguirre and Palacios 1995). While *P. operculella* has a cosmopolitan distribution, *T. solanivora* and *S. tangolias* have a more restricted distribution. In fact, the latter is not present in Europe (Carrillo and Torrado-Leon 2013).

The Guatemalan potato moth, *T. solanivora*, owes its common name to its origin, from which point it spread to the rest of Central America and later, to South America. From there it was introduced to the Canary Islands, before continuing to mainland Spain, initially Galicia, and then the Principality of Asturias.

In Spain, potatoes are grown in all the autonomous communities, and given the wide variety of production conditions, they can be planted in different areas at different times of the year, thus production is year-round (Vigil-Cando 2017). According to 2021 data, 2,141,350 tons of potatoes are grown every year, across a total surface area of approximately 66,000 hectares (MAPA 2024a), which means an average yield of 31.56 t/ha.

In the context of the Canary Islands, potato farming is important both economically and culturally, in particular, in mid-altitude zones, being a staple food for the local population (Carnero et al. 2008). In 2022, this crop was around 10% of the total agricultural production of the Canary Islands. It yielded 111,208 tons in 4.014 ha, which around 90% is located in Tenerife and Gran Canaria (ISTAC 2023) (**Fig. 1**).

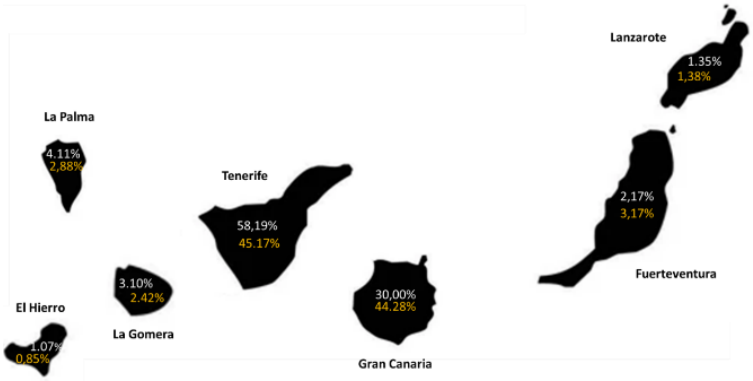

Figure 1. Percentage of cultivated potato area (white numbers) and production (orange numbers) per island respect to the whole Canary Islands. Source: own work, based on ISTAC (2024) data.

The cultivation of potatoes in the Canary Islands is characterised by the existence of numerous varieties that are not present in the Iberian Peninsula nor the rest of Europe. These potatoes are grouped together under the term "Papas Antiguas de Canarias" (Ancient Potatoes of the Canary Islands) (**Tab. 1**):

Table 1: Main varieties and species of Ancient Potatoes of Tenerife (Canary Islands).

Ancient potatoes of Tenerife
Solanum tuberosum* ssp. *tuberosum
¤ Moras or Brasileñas or Grasileñas
¤ Borrallas, Meloneras or Montañeras
Solanum tuberosum* ssp. *andigena
¤ Torrenta, Terrenta or Sietecueros
¤ Azucenas (Blancas, Negras)
¤ Bonitas (Llagadas, Ojo de Perdíz, Coloradas, Blancas, Negras)
¤ Coloradas, Coloradas de Baga or Londreras
Solanum chaucha
¤ Negras, Negras Yema de Huevo or Negras Herreñas.

* Note that this is only a selection of the most important Ancient Potatoes of Tenerife, there are other varieties in the Canary Islands. Source: Author's own work based on Ríos (2012a) data.

It is known that the potato originated in South America, specifically in the Andes, southern Peru and northern Bolivia, from where it was exported to the rest of the world. In the Canary Islands, the first reference of this tuber is from 1567 (Lobo-Cabrera, 1988). Many cultivars of Andean origin have been conserved *in situ* by local farmers over generations, and new cultivars may have emerged through selection in the different agricultural microregions (Ríos 2012b). The studies carried out indicate a great diversity, with cultivars belonging to *Solanum tuberosum* ssp. *andigena*, probably of Andean origin, others belonging to *Solanum tuberosum* ssp. *tuberosum*, probably from old European breeding programmes, or perhaps some material that could have originated in the archipelagos of Los Chosnos and Chiloe in Chile. Also, there are triploid cultivars that have so far been classified in the species *Solanum chaucha*. In addition, some cultivars with intermediate or distant characteristics from the above groups have been found, which could be hybrids obtained by different routes in the Canary Islands from potatoes originating in America (Ríos 2012b).

There is therefore a significant potential of *T. solanivora* to cause not only economic losses, which would be in addition to those already experienced in the Canary Islands, but also genetic erosion of potatoes cultivars present in the archipelago, or local varieties in the affected areas of mainland Spain. Besides, there is a high potential risk of the pest spreading to the rest of the Iberian Peninsula and mainland Europe.

Given the damage that it causes and the potential risks observed, *T. solanivora* is regulated by the European Union as a harmful quarantine organism. This was reflected in Spanish legislation through its inclusion in annex II, part A, section I, of Royal Decree 58/2005, of January 21 2005, adopting protective measures against the introduction and spread within the national territory and the European Community of organisms harmful to plants or plant products, as well as for export and transit to third countries.

Additionally, in 2017 the Ministry of Agriculture, Fishing and Food (MAPA) established a specific plan for this pest (Royal Decree 197/2017) in the form of the "National program for the control and eradication of *Tecia* (*Scrobipalpopsis*) *solanivora* (Povolný)". This plan obligates autonomous communities to monitor and detect the possible presence of the pest through their competent bodies. They must also notify the Ministry, declare infestation and buffer zones, and implement the measures outlined in the aforementioned Royal Decree.

2. Origin and distribution

Tecia solanivora was described by Povolný (1973) using moths and larvae collected in Costa Rica. To that country, it was likely introduced via a shipment of potato seeds from Guatemala, the country of origin, in 1970, specifically the region between the Isthmus of Tehuantepec in Mexico, northern Honduras and El Salvador (Puillandre et al. 2008, Vigil-Cando 2017).In the Americas, the first reports of the pest date back to 1971 in Costa Rica, and 1973 in Panama, Honduras and El Salvador (**Fig. 2**). In Nicaragua it was known to be present in the 1980s, but the moment of its arrival is unknown (Puillandre et al. 2008, Villanueva and 0Saldamando 2013).

Later, in 1983, it was introduced to Venezuela in the state of Táchira, through an import of infested potato tubers from Costa Rica, and then spread to the states of Mérida, Trujillo and Lara. Through the Venezuelan state of Táchira it passed to Colombia, where the moth was detected in 1985 in the state of Norte de Santander in potato crops in Chitagá, a municipality bordering Táchira (Arias et al. 1996, Puillandre et al. 2008, Vigil-Cando 2017). By 1990 the moth had already infested crops in the center of the country and had spread through Boyacá, Cundinamarca and Antioquia, being reported in Nariño, the southwestern department of Colombia bordering Ecuador, where its presence was officially declared in 1996 (Arias et al. 1996, Barragán et al. 2004, Carrillo and Torrado-León 2013, Puillandre et al. 2008).

Figure 2. Origin and spreading of *T. solanivora*. The area of origin of the Guatemalan potato moth was limited to Guatemala and the Isthmus of Tehuantepec. The arrows indicate where the pest spread and the year it was reported.

In 2010, *T. solanivora* was detected in El Porvenir, Mexico, a locality in the state of Chiapas near the border with Guatemala. This location is highly susceptible to the introduction of pests because there are many areas that lack phytosanitary controls; thus, it is thought that the moth was introduced to Mexico through the shipment of seed potatoes in this area (Roblero et al. 2011).

In Europe, its presence was first reported in 1999, when *T. solanivora* arrived in the Canary Islands through an illegal import of infested tubers, probably from South America. It was first detected in the northwest of Tenerife in the municipality of Icod de Los Vinos, and in 2000 its presence was confirmed (**Fig. 3**). In response, pheromone trapping was initiated that same year to determine the spread of the pest, and it was found to have reached the municipalities of La Guancha, San Juan de la Rambla and Los Realejos (Ríos 2012c). In March 2001, monitoring of the pest was carried out in potato fields and storage areas, and it was observed that in the north of the island the population of *T. solanivora* had increased dramatically, especially in the highest areas (> 750 m above sea level). The south, on the other hand, remained free of the pest (Carnero et al. 2008). Nevertheless, its advance could not be contained, and it eventually spread throughout the island, as well as to La Palma, Gran Canaria and El Hierro. It was also found in stores in La Gomera and Lanzarote. Nowadays it is established throughout the archipelago (Puillandre et al. 2008, Corral et al. 2017, Vigil-Cando 2017, MAPA 2024a).

Figure 3. Distribution of *T. solanivora* in the Canary Islands. Source: Ramos A. (Specialist Technician, ICIA).

Subsequently, in 2015 the moth reached the Iberian Peninsula, being detected in the autonomous community of Galicia, specifically in the municipalities of Ferrol, Narón and Neda (A Coruña province) (DOG no. 209 on November 3, 2015). The following year, the demarcations of infested zones were extended following the detection of new outbreaks in the province of Lugo (DOG no. 38 of 25 February, 2016). In 2017, the area of infestation was expanded once again (DOG no. 25 of 6 February, and no. 200 of 20 October, 2017), as the number of affected municipalities in the provinces of A Coruña and Lugo increased **(Fig. 4)**.

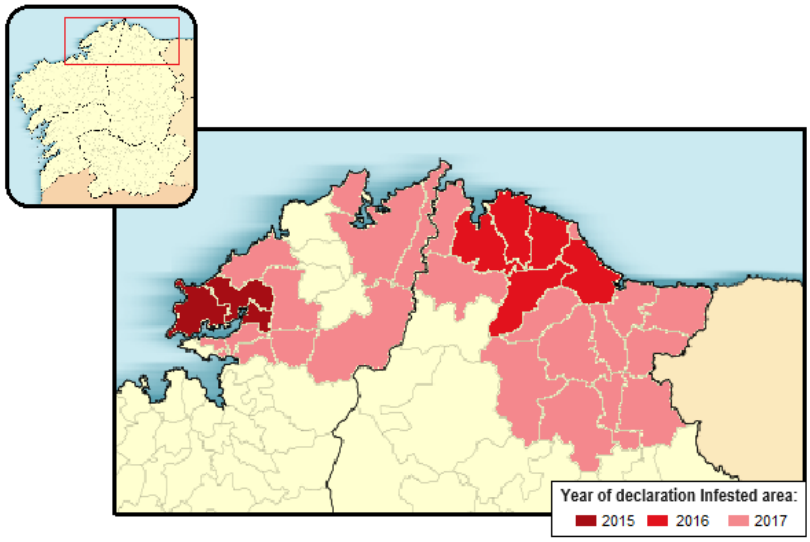

Figure 4. Distribution of *T. solanivora* in Galicia according to official declarations in the Diario Oficial de Galicia (DOG). Source: author's own work. DiarioGalicia (DOG). Source: author's own work.

From Galicia, the moth spread to the Principality of Asturias, where it was detected in 2017 **(Fig. 5).** The pest was declared present in the municipalities of Castropol, Cudillero, Navia, San Tirso de Abres, Taramundi, Valdés and Vegadeo (BOP**A** no. 33 of February, 2017). That same year, with the entry into force of Royal Decree 197/2017 of March 3, and

successive Official Gazettes of the Principality of Asturias (BOPA no. 69 of March 24, no. 126 of June 2, and no. 268 of November 20, 2017), 10 municipalities were declared as infested areas, 20 as buffer zones, and 5 as free zones subject to the declaration of planted crops (Vigil-Cando 2017) (**Fig. 5**).

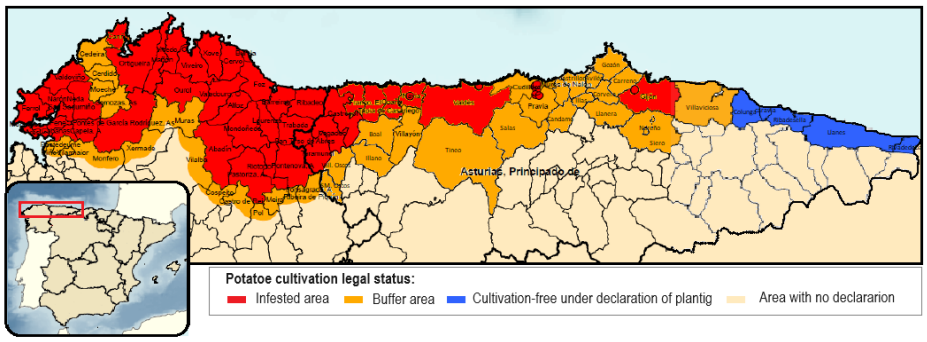

Figure 5. Distribution of *T. solanivora* in the Iberian Peninsula. Red indicates areas declarllllled as infested, orange indicates buffer zones, and blue indicates free areas subject to the declaration of planted crops. Source: author's own work, based on maps from the Ministry of Agriculture, Fisheries, and Food, 2019, and the Official Gazette of the Principality of Asturias, no. 268 of November 20, 2017.

The measures taken to control the pest in the mainland have proven to be very effective. In September 2019 a technical inspection was carried out in Galicia by , composed of two inspectors, a tuber expert, two interpreters and three officials from the MAPA. It resulted in a favorable report that found the measures being carried out to eradicate the pest to be resulting in a rapid and sustained reduction, which, if maintained over time, would lead to its eradication (Beramendi 2019). In 2020, given the improved situation, the municipalities of Ares, Cabanas, A Capela, Fene, Ferrol, Mugardos and As Pontes de García Rodríguez in the province of A Coruña, and Abadín and Ourol in the province of Lugo, were incorporated into the buffer zone. Likewise, bordering localities were no longer classed as buffer zones and became free zones (DOG no. 11, of January 17, 2020).

In the Principality of Asturias, these improvements allowed for the ban on planting potatoes to be lifted in the municipalities of Coaña, El Franco, Gijón, Muros de Nalón and Tapia de Casariego, converting these areas into buffer zones. Nevertheless, the municipalities bordering Galicia were established as no potato zones, to act as a phytosanitary barrier in case of a resurgence owing to unpredictable factors (BOPA no. 24 of Febrary 5, 2020). Finally, in 2023, after six years with no detection of *T. solanivora*, it was declared eradicated in Asturias and all municipalities except for San Tirso de Abres (bordering with Galicia), where potato production remains forbidden (BOPA no. 124 of Febrery 22, 2023).

3. Taxonomic framework and synonymous terms

The Guatemalan potato moth, *T. solanivora* (Povolný 1973), belongs to the superfamily Gelechioidea, according to phylogenetic studies (Urra 2016) and Tinedoidea, according to EPPO (Vigil-Cando 2017) within the order Lepidoptera, as shown below in **Table 2:**

Table 2. Taxonomic classification of *T. solanivora*.

Level	Name
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Suborder	Glossata
Superfamily	Gelechioidea
Family	Gelechiidae
Genus	Tecia
Species	***Tecia solanivora* (Povolný, 1973)**

Among the terms used to describe the species, there is its current name, *Tecia solanivora* (Povolný 1973), and the disused term, *Scrobipalpopsis solanivora* (Povolný 1973). In addition, this species is referred to using various common names in different countries and territories: polilla de la papa (Potato moth), polilla Guatemalteca (Guatemalan moth), polilla Centroamericana (Central American moth), polilla gigante de la papa (Giant potato moth), potato tuber moth, as well as others of very limited use (Ricón 2002). In the scientific literature it is referred to as the Guatemalan potato moth (the main name) and also as the Guatemalan tuber moth.

Identification

For morphological identification, egg and pupal stages are not reliable, therefore it is recommended to use larvae and adults. In the case of larvae, a binocular microscope should be used and the chaetotaxy should correspond to that illustrated in **Fig. 6**.

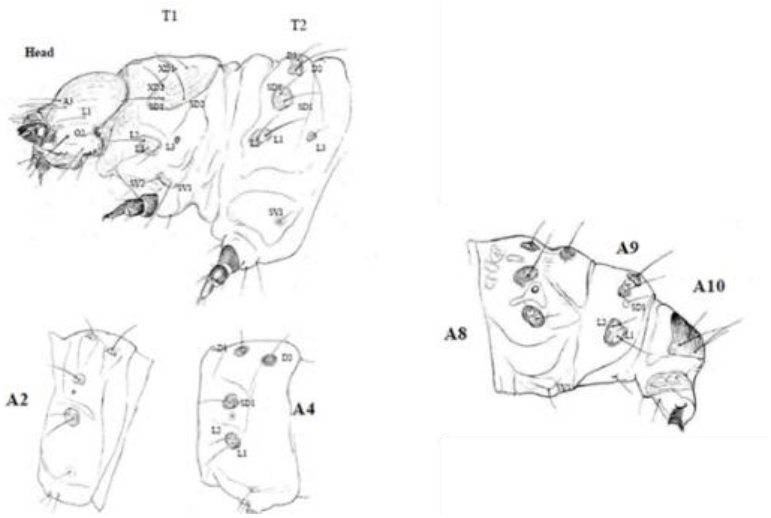

Figure 6. Chaetotaxy for identification of *T. solanivora* larvae. Source: Germain and Povolný 2006.

In the case of adults, although there are other differences, to ensure an unequivocal identificatin the genitalia should be observed under a microscope **(Fig. 7):**

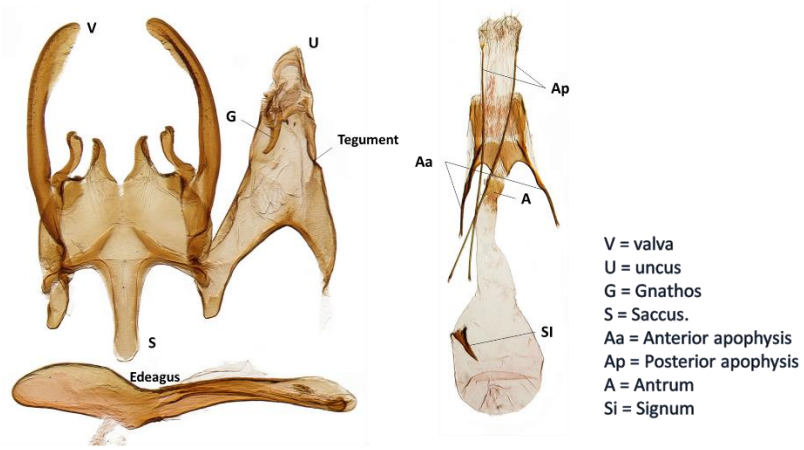

Figure 7. Genitalia of *T. solanivora*. Male genitalia (left). Female genitalia (right). Source: Vigil-Cando 2017.

- **Male genitalia:** differs from other species in that it has a narrowed uncus with a characteristically obtuse tip, with an elongated, hooked and well-developed

gnathos. Valvae with cylindrical base, elongating and curving regularly and acquiring the shape of spatulae at the tips. Aedeagus is long and strong, slightly shorter in length than the distance between the saccus and uncus (Germain and Povolný 2006, Hayden et al. 2013).

- **Female genitalia:** long apophyses, provided on the inner edge with a characteristic tubercle; the antrum is undeveloped and the signum has a strongly arched, spine shape (Germain and Povolný2006, Hayden et al. 2013).

4. Biology and life cycle

Tecia solanivora is a holometabolous insect with a life cycle made up of four stages, the duration of which varies greatly depending on temperature conditions: 42 days at a temperature of 25ºC and up to almost 200 days at 10ºC. Temperature also produces changes in the number of generations (Notz 1996). Differences in longevity have also been observed between sexes, with females always living a few days longer than males at the same temperature (Martinelli 2004).

The duration of the cycle and the number of generations are highly variable depending on environmental conditions, being strongly linked to temperature (Martinelli 2004, Rincón and López-Avila 2004, Notz 1996). These biological parameters have been studied by several authors with similar results (**Table 3**).

Table 3. Duration in days of each phase of the *T. solanivora* lifecycle and number of generations at 15, 20 and 25ºC. Table based on Martinelli et al. (2004) and own data. Authors: A) Nortz 1996. B) Torres et al. 1997, 1998. C) Carnero et al. 2008.

Stages of the cycle (Days)	Temperature									
	15ºC			20ºC			25ºC			
	A	B	C	A	B	C	A	B	C	ICIA 2020
Egg	22	15	15	10	7	7	6	5	5	6
Larvae	41	29	29	21	17	17	13	15	15	20
Pupa	57	31	31	22	14	24	18	12	12	11
Adult	-	36	20	-	34	18	-	19	10	-
Total cycle	122	75	95	53	56	56	37	42	42	37
no. Generations	3	4	-	7	7	-	10	9	-	-

The life cycle of *T. solanivora* (**Fig 1.**) begins when the adult female oviposits by depositing eggs both in groups and individually on surfaces that are rough or contain depressions, and

may choose different areas: the soil surface, clods and crevices near the stem of the plant, or on the tuber itself if these are exposed, and more rarely on the plant itself on leaves and stems (Torres 1998, Carrillo and Torrado-León 2013)

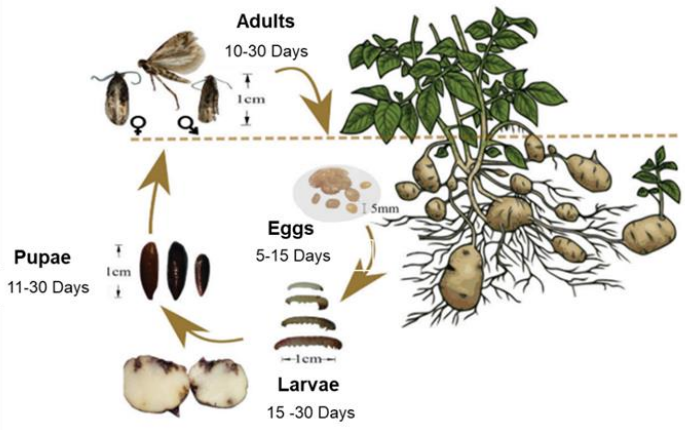

Figure 8. Diagram of the life cycle of *T. solanivora*. The minimum values for the duration of each stage are those corresponding to 25 ºC and the maximum values are those corresponding to 15 ºC. Source: Modified from Guerra-Luran and Acero-Godoy 2020.

In storage, eggs are deposited on or near the eyes of tubers, in the threads of bags and in hidden corners in the warehouse (Martinelli 2004, Vigil-Cando 2017). Each female lays an average of 225 eggs during her first 20 days of life (Ricón 2002).

The newly emerged larvae actively seek out the tuber, displaying strong positive geotaxic behaviour, burying themselves in the soil, without a chemical signal that determines their search (Camargo et al. 2010). Once on the tuber, they continue to move until they locate a point of penetration, entering through the eyes of the potato or the skin, leaving a totally imperceptible entry hole. Initially, the larvae produce horizontal galleries that they excavate through the external layers: cortex and vascular ring, until they choose a point through which they penetrate the most internal structures (pith), making galleries inside the tuber (Hilje 1994), until they complete their development. At this point, it stops feeding and leaves the tuber (fourth instar), via a visible exit hole, to pupate near the soil surface. Afterwards, the adult moth will emerge to copulate **(Fig. 9)** and initiate a new cycle.

Figure 9. Couple of *T. solanivora* adults copulating. Picture: Gavara. J. (ICIA)

5. *Tecia solanivora* development

Tecia solanivora is a holometabolous insect with a life cycle made up of four stages, the duration of which varies greatly depending on temperature conditions: 42 days at a temperature of 25ºC and up to almost 200 days at 10ºC. Temperature also produces changes in the number of generations (Notz 1996). The four stages are:

- **Egg:** ovoid, almost spherical in shape, measuring 0.46 - 0.6mm long and 0.39 - 0.43mm wide. They are pearly white when freshly laid **(Fig. 10A)**, turning a dark yellow before hatching **(Fig. 10B)** due to the darkening of the cephalic case of the larva, which is visible through the chorion at that time (EPPO 2006, Martinelli 2004, Torres 1998). This stage lasts between 5 and 25 days, depending on the environmental temperature (Notz 1996).

Figure 10. A) Freshly laid 24-hour eggs. B) Egg about to hatch, the arrow indicates the darkened cephalic case of the larva visible through the chorion. C) Larva in the process of emergence after breaking the chorion with the mandible. Pictures: Gavara J. (ICIA).

- **Larvae:** elongated with three pairs of thoracic legs, four pairs of abdominal pseudopods and one pair of anal pseudopods (Cuartas 2006). In addition, the larvae of *T. solanivora* have trapezoidal spots (pinnules) on the dorsum of each segment that allow them to be differentiated from those of *P. operculella* (Herrera 1998). To the naked eye, the larvae of these two moths in their advanced stages can be differentiated by the paler color of the body of *P. operculella* and the color of the cephalic case, which is brown in *T. solanivora* and black in *P. operculella* **(Fig. 11).**

Figure 11. Differences in color between larvae of *T. solanivora* (A and C) and *P. operculella* (B and D) at two different developmental stages. Note the paler pink color in *P. operculella* and the black cephalic case, versus the darker pink and brown cephalic case of *T. solanivora*. Pictures: Gavara J. (ICIA).

As regards their development, the larvae go through four stages, or instars, in which their size and color change:

- **First instar:** 1.2 - 1.4 mm in length with a white-transparent body, a dark brown head and prothoracic shield. The duration of this instar has been placed at around 8 days (Torres 1998, Espinel et al. 2009).
- **Second instar:** larger than the previous instar (3.6 mm). Cream color with darker brown spots. Lasts approximately 5 - 6 days (Torres 1998, Espinel et al. 2009).
- **Third instar:** measures between 3.6 and 15 mm approximately, with yellow-greenish coloration and more visible spots along the body and head, and a dark brown prothoracic shield. This instar can last about 8 - 9 days (Torres 1998, Espinel et al. 2009).
- **Fourth instar:** the larva reaches its maximum length at 16 mm and acquires a blue-greenish color with some transparency, and later a purple coloration in the dorsal part and green in the ventral part. The head is pale brown and the mouthparts are

chestnut brown. Each thoracic segment has a pair of true legs and black spots (Herrera 1998, EPPO 2006). For this last instar, the approximate duration is 6 - 8 days (Torres 1998; Espinel et al. 2009).

- **Pupa:** The pupa is obtect, fusiform and measures 7.3 - 9 mm. It is greenish when newly formed, turning light brown and then dark brown over time (Herrera 1998, Germain and Povolný 2006). During this phase, *T. solanivora* already presents differences between genera that allow for sexing of the species: on the one hand, there are differences in size, with pupae that will give rise to larger females of 8.5 mm compared to the 7.5 mm of males (**Fig. 12**). However, the 7 - 8.5 mm range may include both sexes so this criterion is unreliable (Ricón 2002Furthermore, these measurements can vary greatly depending on environmental conditions and feeding, so sexing based on size is not recommended.

Figure 12. Difference in size of *T. solanivora* pupae between sexes. Left: female, right: male. Note that the lengths do not correspond to those established by Ricón (2002), which denotes the variability of sizes depending on specific conditions. Pictures: Gavara J. (ICIA).

Another criterion for distinguishing sex is the location of the genital pore **(Fig. 13)**. In females, the genital opening is at the seventh abdominal segment, whereas in males it is located in the eighth. It may be easier to count from the segment where the sutures of the legs and antennae converge in the ventral part, in which case the genital pore is found in the fourth segment in females and the fifth segment in males (Ricón 2002, Rincón and López-Avila 2004). These criteria relating to the location of the genital pore are much more reliable than size in determining sex.

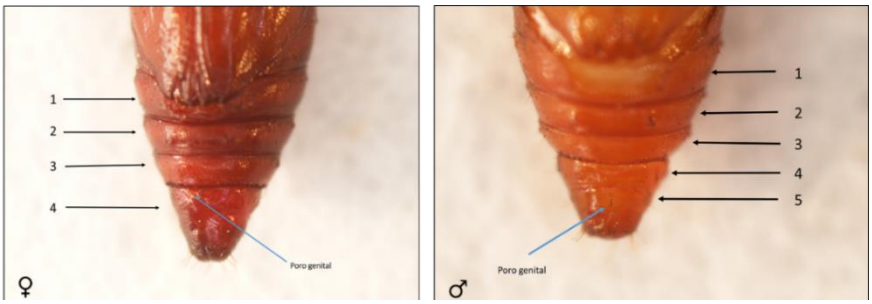

Figure 13. Genital pore position. Left: female pupa with pore in the fourth segment, right: male pupa with pore in the fifth segment. Pictures: Gavara J. (ICIA)

- **Adult:** a small grayish-brown moth with a body approximately 1 cm long and a wingspan of 12 - 15 mm **(Fig. 14A)**. At this stage, males and females show sexual dimorphism in size and coloration (Rincón and García 2007). Females are larger, with a longer and wider abdomen and three dark, rounded markings on each forewing, while males have only two (Povolný 1973, Carrillo and Torrado-León 2013). These rounded markings on the forewings, together with the presence of brown radial lines, allow for differentiation between *T. solanivora, P. operculella* and other moths **(Fig. 14)** (Cuartas 2006, Carrillo and Torrado-León 2013). Males and females of *T. solanivora* can be distinguished by observing the presence of genital spicules on the underside of the abdomen under magnification (**Fig 14c**).

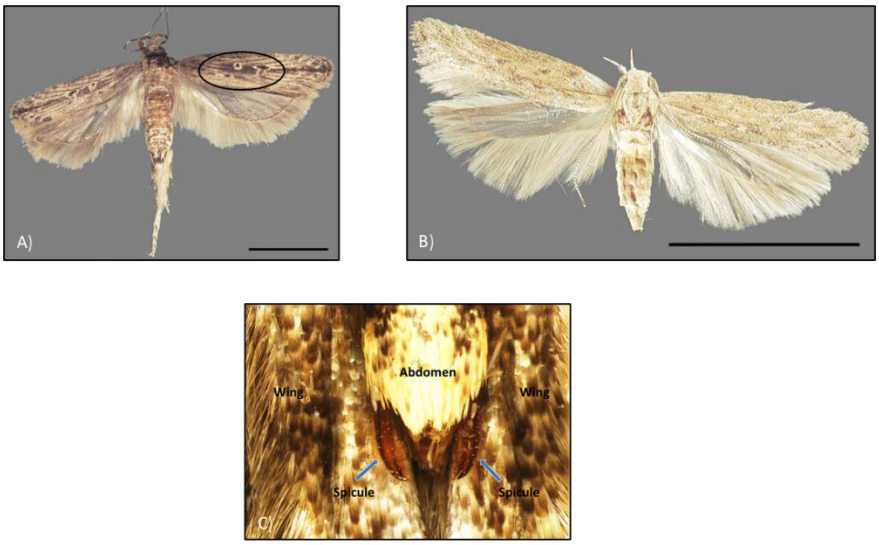

Figure 14. A) Female *T. solanivora* with the three characteristic rounded markings. B) Female *P. operculella.* C) Spicules of the adult female of *T. solanivora*. Source: A) and B), United States Department of Agriculture (USDA), C) Piedra-Buena A. (ICIA).

Although they are similar, it is possible to differentiate the adult moths of the potato complex by their different wing patterns **(Fig. 15)**: *T. solanivora*, has an elongated radial line, *P. operculella* with closed wings has a pattern reminiscent of an "X" in the center of the dorsum, and *S. tangolias* has rhomboid-shaped spots on the upper sides of the wings. Although *S. tangolias* is not present in Europe, it is important to be able to recognize it to warn of its presence.

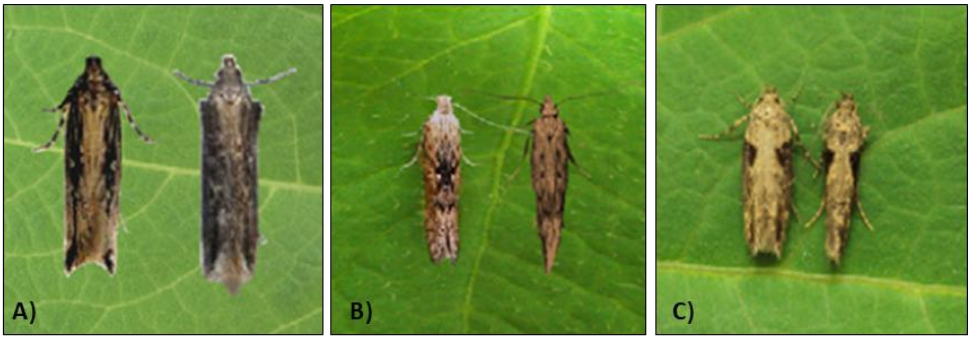

Figure 15. Different wing patterns of moths of the potato complex: A) *T. solanivora*. B) *P. operculella*. C) *S. tangolias*. Source: Kroschel and Schaub 2013 (modified).

6. Damage

Tecia solanivora is adapted to different mountain ecosystems distributed across a wide range of altitudes, having been found between 1600 and 3400 m in the northern states of the Andes (Torres 1998) and 1300 and 2300 m in Costa Rica. In the Canary Islands, Spain, the greatest damage is found at lower altitudes, between 500 and 600 m (EPPO 2006). Several studies have found a negative relationship between altitude and pest population density. However, management practices and climatic variables in the different areas invaded by the pest have been shown to be of greater influence (Povolný 1973, Barreto et al. 2004). Although the damage is similar to that caused by other potato moths, *T. solanivora* is considered one of the most harmful invasive pests in potato cultivation, which can have devastating effects in infested areas. The damage brought about by *T. solanivora* is caused only by the larval stages (Torres 1998, Carrillo and Torrado-León 2013, Karlsson et al. 2017). The larvae don't attack the aerial part of the plant, but only the tuber, where they feed and develop producing superficial galleries. This distinguishes it from the common moth, *P. operculella*, which feeds on leaves, shoots, stems and digs deep galleries. Moreover, it is also possible to differentiate attacks from both moths after the larvae have left the tuber,

given that the exit holes of *T. solanivora* are clean, whereas the edges of the holes of *P. opercuella* are dirty with traces of faeces (**Fig. 16 and 17**) (Trujillo and Perera 2017a, Tascón and Perera 2021). Damage can be classified as follows:

- **Direct damage (Fig. 16)**: Deterioration of tuber quality due to the presence of galleries that change and affect its general appearance (Trujillo and Perera 2017b).
- **Indirect damage:** These external openings, excavated galleries and excreta facilitate the entry of microorganisms, thus leading to rotting and total ruin of the tubers. The tubers cannot be marketed, nor are they suitable for human, industrial or animal consumption, or for seed. This causes considerable economic damage (Vigil-Cando 2017).

Damage can occur not only in potato fields but also post-harvest, during which time huge losses may be experienced. This is because storage conditions are ideal for the reproduction of the Guatemalan potato moth, since the tubers have no protection and the darkness that usually exists in warehouses favors the activity of the adults (Herrera 1998).

Figure 16. Infested potatoes of the Guatemalan potato moth: A) short galleries B) Clean exit holes. C) Damage in cross-section near the surface. Pictures: Estévez J.R. (Specialist Technician, ICIA).

Figure 17. Potato infested by *P. operculella* with soiled exit holes with traces of faeces. Picture: Perera González S. (Cabildo de Tenerife).

7. Monitoring and decision-making

As will be detailed in the section entitled "Biotechnical control measures", monitoring of *T. solanivora* is carried out through the combined use of pheromones and traps. Trujillo and Perera (2011) analyzed data obtained since March 2009 from the potato crop trapping network and concluded that *T. solanivora* follows a regular pattern of behavior each year. They observed that the pest appears at the beginning of the crop in January (winter), albeit in small numbers, and increases progressively in number until the beginning of May (spring), when there is a notable increase in captures. The population reaches its highest point following the harvest. These captures remain high until autumn, decreasing with the onset of the first rains **(Fig. 18)**.

Figure 18. Average annual population trend of *T. solanivora*. Source: Graph modified from Trujillo and Perera (2011).

Currently, there are no specific scientific studies available on the action threshold for *T. solanivora*, and the literature consulted shows significant disparity in terms of recommended values. According to Ricón (2022), the threshold is around 50 male captures per week (7.14 males per trap, per day). However, according to the guidelines of the MAPA (2015), measures against the pest should be taken when 70 moths per week (10 moths per trap, per day) if there are mature potatoes in the field. However, in the case of the latter, it should be clarified that this is not a specific threshold for *T. solanivora*, but a threshold also used for the common moth, *P. operculella*.

For biological control, none of these thresholds apply. The release or inoculation of natural enemies should be carried out when the first adult moth is trapped or at the beginning of tuberisation, or even earlier in the case of organisms that require an establishment period. However, each biocontrol agent should be the subject of a specific study to determine its optimum time of release/inoculation.

8. Control measures

Although control measures cannot eliminate *T. solanivora*, they can significantly reduce its population, if applied together and during all stages of farming:

8.1 Farming control measures

▪ Planting

First of all, one month (or at least 15 days) before planting, the soil should be thoroughly tilled. This exposes the eggs and pupae to drying environmental conditions and to the action of natural enemies. In addition, unharvested tubers from the previous plantation (volunteer potatoes) should be removed (Trujillo and Perera 2017a).

For planting, it is advisable to plant at a depth of 10 - 15 cm to prevent the moth from ovipositing near or on the tubers (Ricón 2002). If using a hoe, soil should be piled high on both sides, as this increases the distance between the newly emerged larvae and the potatoes. The larvae will have to travel further through the soil, making it more difficult for them to reach the tuber (Martinelli 2004, Trujillo and Perera 2017a).

The moment of planting and the potato variety are also important factors to be considered in pest management. The process of formation and development of the tubers should be timed to coincide with the rainy season, thus avoiding dry and hot periods (unless there is a sprinkler irrigation system), to avoid cracks in the soil appearing, where moth eggs may be laid (Ricón 2002).

In terms of potato variety, for “Papas blancas” (white potatoes) it is recommended to use certified seed, while in the case of “Papas de color” (colored potatoes) or second multiplication potatoes, seed should not be purchased from affected areas. Furthermore, it is important to avoid moving used sacks, big boxes or crates from infested areas to areas where the pest is absent, as they could contain moth larvae or pupae.

- ## Growing

Light irrigation must take place frequently in order to avoid cracks in the soil appearing, even towards the end of the crop. In the case of sprinkler irrigation, it is advisable to irrigate at dawn, between 5 and 8 a.m., as this is the time of day when adult moths are most active, so the mechanical action of the water droplets makes flying and copulating difficult (Trujillo and Perera 2017a).

In addition, where available, regular cleaning of walls, slopes and other places that can serve as refuges for moths, as well as crop rotation, are other measures that contribute to reducing pest populations (Trujillo and Perera 2017a).

- ## Harvesting

Harvesting should take place when potato skin set is complete, usually one week after cutting the foliage. It is important to avoid delays as this increases the exposure time of the tubers to possible moth attacks (Ricón 2002). In addition, it is important to remove all potatoes from the field (green, split and infested) to avoid the moths multiplying (Trujillo and Perera 2017a).

In the case of Tenerife and Gran Canaria, removed infested potatoes must be taken to specific containers provided by the agriculture services of the respective Cabildos (autonomous island governments of the Canary Islands) **(Fig. 19)**.

Figure 19. Container for infested potatoes. Picture: Perera González S. (Cabildo de Tenerife).

8.2 Biotechnical control measures

8.2.1 Pheromones

Biotechnical control measures include the use of sex pheromones, which are the most widely used monitoring tool for *T. solanivora* (Bosa et al. 2005, Carrillo and Torrado-León 2013). Sex pheromones are used to attract males and capture them in traps. This technique is used to detect if the pest is present in a new area, to learn about its distribution and to determine the population fluctuation during the crop cycle and throughout the year. The application of pheromones in the control of male moth populations is advantageous because it is cheap, requires little investment in labor, does no damage to the biological balance of the environment, and does not disrupt the pest's natural enemies (Vigil-Cando 2017).

Once the pest is established, these pheromones have been used to obtain information on the population density. Based on this information, decisions can be made on applying control measures, especially regarding the use of chemical insecticides (Trujillo and Perera 2011).

In addition to detection and monitoring, pheromones have other applications in integrated pest management programs using attractant traps and their combination with sterilants or insecticides (Bosa et al. 2005). It is also possible to use pheromones when applying the copulation interruption technique. This involves saturating the field environment with high concentrations of pheromones using dispensers or by spraying. The antennae receptors, as well as the nervous system of the males, are blocked, making it impossible for them to locate females ready to copulate. This interruption of copulation leads to a reduction or even the elimination of damage to the crop, as a result of the population decline in the next generation Although this technique has been tested experimentally by Bosa et al. (2005, 2008), at the time of writing there is no evidence of its application in real conditions on *T. solanivora*.

- **Types of traps**

Two types of traps are used to catch adult *T. solanivora* using pheromones: water traps and funnel traps. **The water trap (Fig.20a)** consists of a plastic carafe with two openings on the upper sides. The pheromone capsule is placed inside the carafe and held in place with a wire, which must remain dry without touching the soap or detergent solution used to fill the bottom of the carafe. **The funnel trap (Fig. 20b)** consists of a funnel with a lid and a base attached to it. An approved insecticide tablet is placed inside the base to kill the insects, and the pheromone diffuser is placed in a small cage located in the center of the lid (Trujillo and Perera 2011, 2017a).

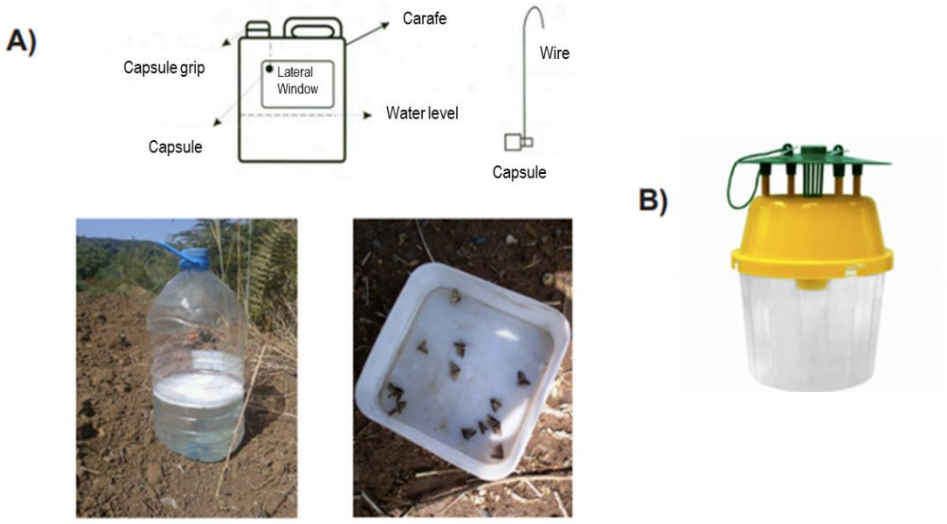

Figure 20. A) Top: water trap schematic. Bottom: water trap and adult *T. solanivora* collected from the trap. B) Funnel trap. Source: Trujillo and Perera 2017b.

Trujillo and Perera (2011) made a comparative study of the use of the two types of traps in different potato plots in Tenerife and found no significant differences in the total number of captures between water traps and funnel traps. However, it was observed that in the first examination after placement, catch numbers in the water traps were higher than the funnel traps, although over time, there was little difference between the two. Taking these data into account, water traps are recommended when detecting pest presence in a previously non-infested area.

- **Trap installation**

The traps should be placed at the edge, and if possible, outside of the plots. One trap should be placed for every two sowing bags (one bag serves approximately 200 m^2), at 25 m between them (Trujillo and Perera 2011).

In the case of water traps, the pheromone must not get wet and the trap must never run out of water. Otherwise, the adults will be attracted but not eliminated. The trap should remain in the field even after harvest, as the highest number of catches occurs in this period. The pheromone lasts between 40 and 60 days (depending on the supplier), and when it is replenished, the previous capsule should not be left lying in the field (Trujillo and Perera 2011).

8.2.2 Plant extracts

Currently, the application of plant extracts for pest control forms part of integrated pest management programs, especially for soft-bodied insects such as lepidopteran larvae, but also for other orders. Commercial products of this type are available (Salazar and Betancourth 2009, Cabrera et al. 2019).

There are several studies of the use of aromatic plants and their products as repellents or pesticides for different insects and other arthropods, with a wide variety of effects: antifeedant, toxic, ovicidal, as well as sub-lethal effects or as interferences in development and reproduction (Cabrera et al. 2019, Ramírez et al. 2010).

In the case of potato moths, most studies have been conducted on *P. operculella.* These management techniques have subsequently been tested on *T. solanivora* because of their similarity and proximity in biology and behavior. Ramírez et al. (2010) tested the effect of different essential oils from a group of Laminaceae on tubers and soil for the management of *T. solanivora.* Application to the tubers resulted in a significant reduction in the proportion of hatched eggs in treatments with extracts of patchouli (*Pogostenon cablin*, Linnaeus) and thyme (*Thymus vulgaris*, Linnaeus). These two plants also showed an ovicidal effect in the soil, as well as with the treatments with marjoram (*Origanum majorana*, Linnaeus), peppermint (*Mentha piperita*, Linnaeus 1753), rosemary (*Rosmarinus officinalis*, Linnaeus 1753) and basil (*Ocimum basilicum*, Linnaeus 1753).

However, despite the observed effect on egg hatching, no differences were found in the number of adults emerging from the treated tubers or in their longevity. The authors associate these results with the low persistence of the oils due to their high volatility, pointing out the need for further studies and evaluation of the doses, and the search for new formulations to increase their effectiveness.

Salazar and Betancourth (2009) tested the use of extracts of eucalyptus (*Eucalyptus globulus*, Labill 1800), rue (*Ruta graveolens* Linnaeus) and the commercial product alisin (chilli and garlic extract) under field conditions in two locations in Nariño (Colombia). These authors obtained a significant reduction in potato damage, but also pointed out the need to look for new formulations, or to increase the frequency of application. In the Canary Islands, Cabrera et al. (2019) tested the repellency of plants endemic to the Canary Island laurel forest, finding repellency values of 73% for *Picconia excelsa* (Aiton 1844), which has potential to be studied for potato moth control in the field.

These studies show that the use of plant extracts in the integrated management of *T. solanivora*is is an interesting and promising technique, albeit in need of adjustments in the formulations.

8.2.3 Varietal resistance

Breeding pest-resistant host plants is one component of integrated pest management that is gaining increasing attention due to its low environmental impact, long-lasting action, relatively low cost and easy implementation by growers (Cely-Pardo et al. 2022, Tai and Vickruck 2022).

Furthermore, it has been found that when a resistant variety reduces the population of a pest by up to 50% per generation, there is a drastic reduction in just a few generations in the pest insect, the effect being cumulative and persistent in contrast to the immediate and time-declining effect of most insecticides (NAS 1978).

In the context of the Guatemalan potato moth, the use of varietal resistance has been studied at the Colombian Agricultural Research Corporation - AGROSAVIA. By pre-selecting from the existing genetic resources of the Colombian Central Potato Collection, chosen for their resistance to *T. solanivora* and their agronomic characteristics, as well as existing commercial varieties, they have obtained promising candidates for varieties resistant to the Guatemalan potato moth or as possible breeding parents. Materials selection has also been used to study pest control through genetic transformation strategies, involving the characterization of Cry proteins of *Bacillus thuringiensis* strains active against *T. solanivora* (Section 5.3.1 Entomopathogenic organisms). This also resulted in varieties that have shown positive results in field trials and have good prospects for further study of their use (Villanueva et al. 2014, Sánchez-León et al. 2021, Cely-Pardo et al. 2022).

Nowadays, there is no research being carried out ; Spain on varietal resistance of potato crops to the Guatemalan potato moth. This could be of interest in the case of the Canary Islands, given that there is a great diversity of potato varieties that could be analyzed in the search for varietal resistance, as has been done in Colombia.

8.2.4 Attract and kill

This technique consists of the combined use of attractant pheromones and chemical substances. Unlike mass trapping, individuals are not captured but are exposed to an active insecticide (Blanco 2013). In this case, its use in the field would consist of applying drops of a pheromone and insecticide formulation on the surface of the leaves of the plant.

The attract and kill technique has already been developed and trialed against the common moth, *P. operculella*, and the Andean moth, *Symmetrischema tangolias*, under experimental conditions, and has delivered some promising results (Kroschel and Zegarra 2010, Kroschel and Schaub 2013). In the Canary Islands, the ICIA, in collaboration with the Agriculture and Rural Development Service of the Cabildo of Tenerife and the CIP

(International Potato Centre), is fine-tuning the method with a product authorized in Spain for application in the management of *T. solanivora* (Press Release 2021).

8.2.5 Sunlight treatment

This technique involves greening potatoes by exposing them to the sun (Gallegos et al. 2005), leading to the production of solanine: a toxic substance with insecticidal properties. This technique can only be applied to seed potatoes since green potatoes are not suitable for human or animal consumption due to their toxicity.

Studies carried out to test this technique have shown that seven days of exposure to sunlight for potato greening is sufficient. No reduction in the number of damaged tubers was observed when the exposure was increased to 15 days. Furthermore, it was observed that lethality is higher in early larval instars and that sunlight does not work on larvae that are already inside the potato, therefore it is advisable to place in the sun as soon as possible after harvesting (Chulde-La Fuente et al. 2003). This technique has shown to be effective, achieving reductions of up to 50% of the damage caused by *T. solanivora* in storage. Further improved results can be obtained by combining this with the use of baculoviruses (Section 5.3.1 Entomopathogenic organisms). In addition, it is a cost-effective and non-polluting method (Gallegos et al. 2005).

8.2.6 Modified atmosphere

Modified atmospheres are routinely applied post-harvest, and are very useful in the case of quarantine pests such as *T. solanivora.* They allow for the obtaining of healthy seed potatoes, and export is made possible by being able to ensure pest-free material. Although there are currently no technical impediments to the application of these treatments in the present case, there are legal limitations, as explained below.

Sanitation with a modified atmosphere involves generating an enveloping atmosphere over the product with high concentrations of CO_2. This technique is based on the effect that high concentrations of this gas have on the physiological, metabolic and ethological characteristics of insects, producing lethal effects on their growth, development and reproduction (Perera et al. 2011, Lobo et al. 2021).

The application of this technique for *T. solanivora* was first analyzed in 2005 through a collaboration between the Cabildo of Tenerife and the UDI of Phytopathology (Dept. of Plant Biology- ULL), and continued in 2006 with the application of gases in the Post-Harvest and Food Technology Laboratory (Dept. of Tropical Fruit Growing) of the Canarian Institute of Agricultural Research. During this period, atmospheres with different percentages of CO_2, O_2 and N_2 were tested, with promising results (Perera et al. 2011). Consequently, these working groups continued the assays to adjust the technique, testing different exposure

times and evaluating their effects both on pest control and the maintenance of the organoleptic properties and germination ability of the exposed potatoes, until they were able to meet the necessary efficacy and requirements to be considered a quarantine treatment. It proved to be optimal when applied at concentrations of 30% CO_2, 20% O_2 and 50% N_2, with an exposure time of ten days, achieving 100% efficiency in the control of *T. solanivora*, without producing adverse effects of any kind on the tuber (Lobo et al. 2021).

This technique was subsequently established by the MAPA as mandatory for the movement of potatoes from affected areas to areas free of pests within the national program for the control and eradication of *T. solanivora* (RD 197/2017). However, it is important to emphasize that this treatment is only effective under specific application conditions and with appropriate facilities. At present, the research group involved in the development of this technique is facing new assays to obtain the approval of the European Food Safety Authority (EFSA) as a quarantine treatment for potato exports.

8.3 Biological control measures

As an introduced pest*, T. solanivora* lacks specific native natural enemies. This, alongside favorable environmental conditions, and the absence of effective chemical control, have resulted in its rapid population growth and establishment. Given this situation, it is necessary to search for natural enemies that can serve as biological control against this pest (Ricón 2002, Espinel et al. 2009).

Natural enemy species for the eggs and larvae of *T. solanivora* have been documented **(Table 4)**, and include both parasitoids and predators, as well as parasitic and pathogenic nematodes (Vigil-Cando 2017). However, their degree of influence on pest populations is unknown and few of them have been found to play a significant role in pest management (Niño 2004, Carrillo and Torrado-León 2013).

To accelerate the search for biological control agents, given the similarities in biology and habits of both moths, some authors have suggested and tested the natural enemies of known efficacy against the common moth, *P. operculella*, on *T. solanivora* (Torres 1998, Carrillo and Torrado-León 2013). Among the biological control candidates evaluated in South America are the granulovirus PhopGV (Zeddam et al. 2003, Espinel et al. 2009) and the parasitoids *Copidosoma koehleri* (Torres 1998) and *Trichogramma lopezandinensis* (Rubio et al. 2004).

Table 4. Natural enemies associated with *T. solanivora*are documented in the different countries where it is present (Carrillo and Torrado-León 2013).

Nombre científico	Localización	Author
Egg parasitoids		
Chenolus phthorimarea	Guatemala	Navas et al., 1986
Copidosoma koehleri	Venezuela	Torres, 1998
Trichogramma lopezandinensis	Colombia	Rincón y López-Ávila, 1999
Trichogramma off. Pretiosum	Colombia	Osorio et al., 2001
Larvae parasitoids		
Apanteles spp.	Colombia	Osorio et al., 2001
Eggs and larvae predators		
Buchananiella contigua	Colombia	Osorio et al., 2003
Eggs predators		
Lyctocoris campestris	Colombia	Osorio et al., 2004
Parásitos (nemátodos)		
Sterneinema feltiae	Colombia, Venezuela	Sáenz, 2003; Torres, 1998
Patógenos		
Granulovirus PhopGV	Ecuador, Colombia	Zeddam et al., 2003
	Venezuela	Niño y Notz, 2000

8.3.1 Entomopathogenic organisms

- ***Bacillus thuringensis***

Bacillus thuringiensis (Bt) is a gram-positive, soil-dwelling bacterium. It is characterized by the production of paraspore crystals during sporulation, composed of Cry proteins. These proteins constitute the most important virulence factor of *Bt* since they have a specific toxic effect on insects of the orders Lepidoptera, Coleoptera and Diptera, among others (Rojas et al. 2013).

The infection process begins when a susceptible larva ingests the Cry crystals, resulting in the release of their protein subunits into the midgut. There, the crystals are activated by the action of host proteases and bind to specific receptors in the intestinal epithelium. This results in the formation of pores that alter the membrane potential, leading to cell lysis and tissue destruction, with the consequent death of the host **(Fig. 21)** (López-Pazos et al. 2010, Hernández-Fernández and López-Pazos 2011). Larvae affected by *Bt*

present a change in coloration from cream to brown, immobility, and body degradation due to the inability to eat (Martínez and Martínez 2009).

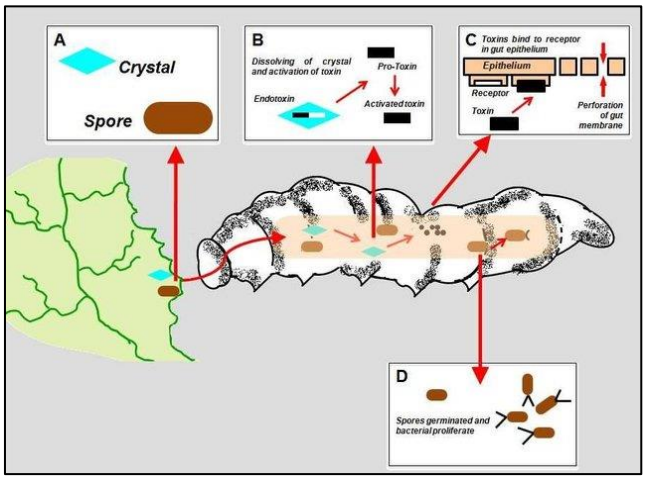

Figure 21. Infection process of *B. thurigiensis* in insect larvae. Source: Hernández-Fernández and López-Pazos (2011).

For potato moths, there is currently a commercial product in Spain based on *Bt*, registered by MAPA (2024b). However, elsewhere the use of native strains has been suggested as advantageous over commercial strains. Martínez and Martínez (2009) tested 30 strains native to Colombia against *T. solanivora,* under laboratory conditions, and found 15 strains that caused between 70 and 94% mortality. When compared with commercial products, in most cases the native strains showed similar efficacy, and some even showed higher efficacy. However, the native strains had been applied at lower concentrations than the commercial, suggesting that the efficacy of the native strains is higher than that observed, thus revealing great potential for field use in pest-affected areas.

- **Entomopathogenic fungi**

Research on the application of entomopathogenic fungi in the control of *T. solanivora* is scarce. However, studies conducted with fungi of the genera *Beauveria* and *Metarhizium* to evaluate their efficacy on eggs and larvae, both in the laboratory and in the field, have not obtained satisfactory results so as to recommend their use (Villanueva et al. 2014, Vigil-Cando 2017). Currently, no commercial products based on entomopathogenic fungi are available for the control of potato moths (MAPA 2024b).

▪ Baculovirus

Baculoviruses (Baculoviridae) are a group of pathogenic viruses consisting of two genera: Nucleopolyhedrovirus (nuclear polyhedrosis virus, NPV) and Granulovirus (GV). These are double-stranded circular DNA viruses whose virions are arranged in protein inclusion bodies (occlusion bodies, OBs), and infection of the host occurs by ingestion. Once ingested, due to the alkaline conditions in the midgut of the insects, the protein that makes up the OBs dissolves and the virions are released. These bind to the membranes of the epithelial cells of the mesentery, gaining access to their nucleus by fusing with the membranes, where viral DNA replication takes place. Thus, new virions are produced and spread throughout the tissues of the insect, producing large quantities of OBs which are released to initiate a new infective cycle, in which the larva dies due to tissue degradation (Caballero et al. 2001, Cuartas 2006).

Baculovirus-infected larvae are recognizable by a set of typical symptoms that manifest between one and several days after the onset of infection (Caballero et al. 2001, Cuartas 2006, Cuartas et al. 2009):

▪ Overlapping of instars due to delayed development, which increases the duration of larval stages.

▪ Color change: pale pink larvae, uniform milky white due to agglomerations of inclusion bodies, translucent colors allowing observation of internal organs **(Figs. 22 and 23).**

▪ Larvae with less mobility and greater flaccidity.

▪ Loss of appetite of larvae, with a tendency to move away from the food source.

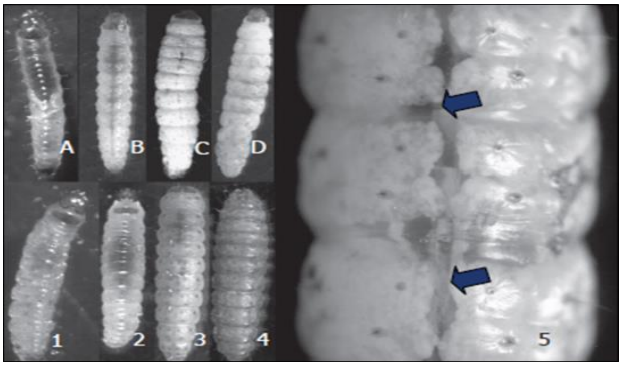

Figure 22. Signs of the development of granulovirus infection in the larval stage of *T. solanivora*. Diseased larvae: A) day 7, B) day 12, C) day 17, D) day 20, 5) detail of agglomerations of granulovirus inclusion bodies over the 5th, 6th and 7th abdominal segment. Healthy larvae: 1) day 7, 2) day 12, 3) day 17, 4) day 20. Source: Espinel et al. 2009.

One advantage of these viruses is that they have a very narrow host range, which, unlike chemical products makes them highly specific. For this reason, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), as well as the Oxford Institute of Virology (OVI), consider these viruses as the most promising tool in integrated pest management (CIP 2001, Cuartas 2006).

Of the two above-mentioned genera for the control of potato moths, granuloviruses are especially useful since they have only been isolated from Lepidoptera (Caballero et al. 2001). In different countries of the world (Perú, Colombia, Venezuela, Bolivia, Indonesia, Australia) the *Phthorimaea operculella* granulovirus, or PhopGV, has been reported to have naturally infected larvae of the potato moth*, P. operculella* (Cuartas et al. 2009, Gómez et al. 2009) both in field and in storage conditions. It is more easily found in places with high moth populations. Infected larvae, both live and dead, can be located on tubers, at the base of shoots, inside galleries or within their silk cocoons, although they can also be found in the aerial part of mined leaves (CIP 2000). Although the granulosis virus was isolated initially from *P. operculella*, it is able to infect the Guatemalan potato moth, and is therefore also used in the control of *T. solanivora*.

PhopGV, due to the high efficacy demonstrated by its different strains, is considered the central component of integrated management programs developed for the control of both *P. operculella* and *T. solanivora* in Central and South America (Zeddam et al. 2003, Gómez et al. 2009). However, its efficacy on the Guatemalan moth is lower, so native strains have been sought among the populations of this species to obtain genotypes of the virus that are better adapted, and possibly more effective on this host (Villamizar et al. 2006). Espinel et al. (2009) tested the use of native isolates from *T. solanivora* and an isolate from *P. opercullella* on *T. solanivora* larvae. They found that larvae infested with the native isolates had a greater and earlier overlap between instars. That is, these isolates were more efficient, while the *P. operculella* isolate showed greater difficulty in carrying out primary infection in the alternative host. The works of Villamizar et al. (2006) and Gómez et al. (2009) agree that native isolates of *T. solanivora* have greater efficacy than those from *P. operculella*, although they note that the effectiveness of the isolates can be enhanced or reduced when they are formulated.

One aspect to consider in virus multiplication was pointed out by Cuartas et al. (2009) when studying the production of inclusion bodies in native isolates from *T. solanivora* on *T. solanivora* and *P. operculella* larvae. The authors found that more inclusion bodies were produced in *P. operculella* larvae in all cases, suggesting the use of this species for mass production to achieve higher yields.

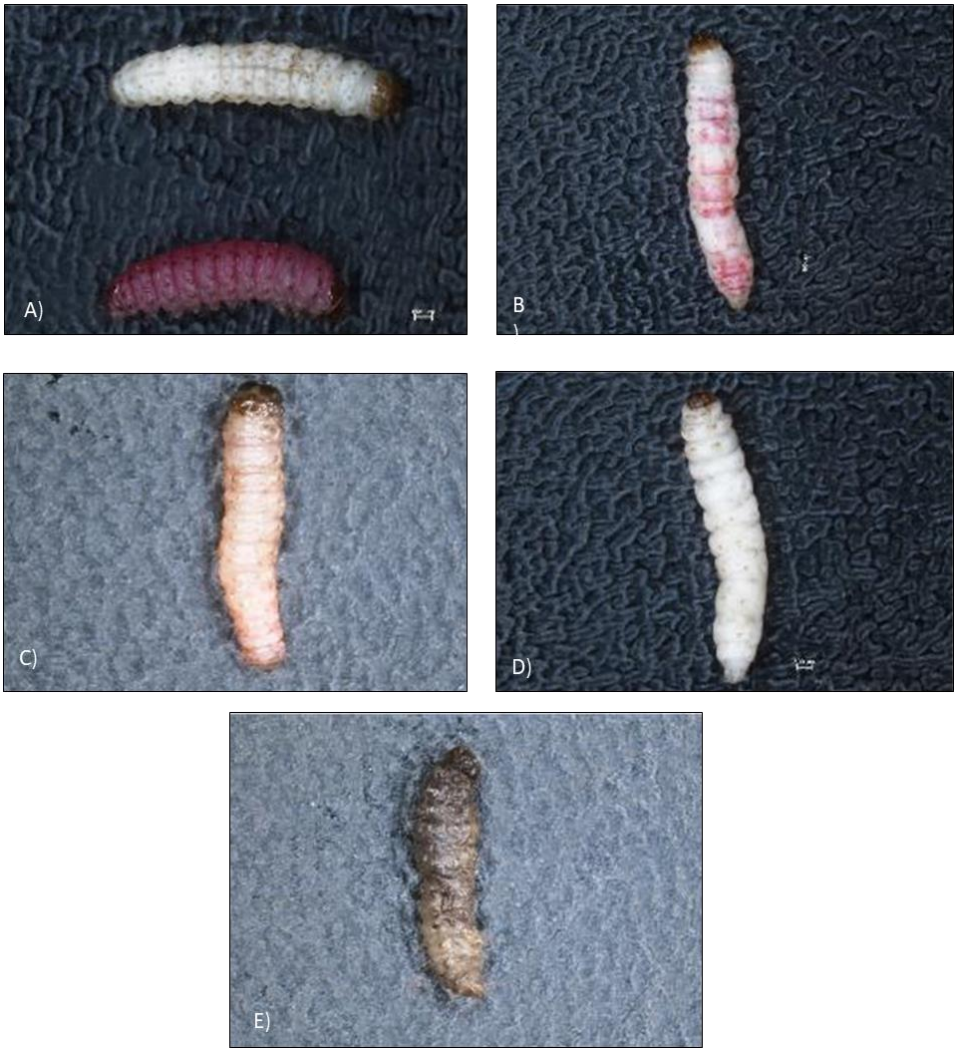

Figure 23. Symptomatology of baculovirus-infected larvae. A) Healthy larva with normal pink coloration, versus milky white larvae due to infection. B) Larva in an intermediate stage of infection with a pale pink color. C) and D) Larva in an intermediate stage of infection bleached with some flecks of natural pink on the abdomen and bleached in the dorsoventral area. E) Larva necrosed by infection. Pictures: Gavara J. (ICIA).

Regarding the application of baculoviruses in field conditions, it has been observed that solar UV radiation produces an inactivation effect on them, which has led researchers to devote great efforts to developing formulations that minimize the effect of this radiation. This would increase the efficacy of PhopGV. It is also essential to apply it at the right time since neonate larvae are at their most vulnerable stage. If the application is made against more developed larvae, concentrations up to a thousand times higher are needed to achieve median lethal concentrations (LC50). However, monitoring neonate larvae in the field is very complicated (Sporleder 2003).

Warehouse and field trials with baculovirus formulations against *T. solanivora* have yielded good results. In Costa Rica, Gómez-Bonilla et al. (2013) compared the use of traditional insecticides and PhopGV in storage and found equal or superior efficacy in the use of the granulovirus formulation with respect to the use of insecticides alone or combined with viruses.

In Spain, to date, there are no baculovirus-based products registered for the control of potato moths (MAPA 2023d).

- **Entomopathogenic nematodes**

Entomopathogenic nematodes of the Steinernematidae and Heterorhabditidae families have been included in integrated management programs as biological control agents against pest insects harmful to soil-dwelling plants. The Guatemalan potato moth falls into this category since it spends most of its life cycle under the soil inside potato tubers (Fan et al. 2001, Perera et al. 2009).

In both nematode families, the third juvenile stage (J3) is the only free-living stage **(Fig. 24)** with infective capacity, due to its symbiotic association with bacteria of the genus *Xenorhabdus*. During this free stage it actively seeks out the host, penetrating it. The Steinernematidae family does this through the natural openings (mouth, anus and spiracles), while the Heterorhabditidae family can also enter via the epidermis. Once inside the insects, the nematodes pass through the wall of the gastrointestinal tract, reaching the general cavity of the insect. There, at the infective stage, it transforms into a parasite by releasing the symbiont bacteria it carries in its intestine, causing the death of the host by septicemia (Sáenz 2003, Perera et al. 2009).

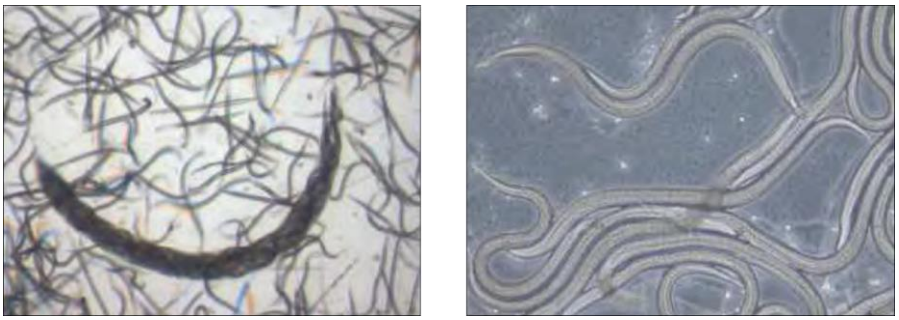

Figure 24. *Steinernema carpocapsae*. Source: Perera et al. (2009).

The basic symptoms to recognize in hosts affected by entomopathogenic nematodes are as follows:

▪ Larvae killed by J3 become brown and flaccid, with no foul odor **(Fig. 25A)**.

▪ After 10 days, white spots are observed on their cuticles due to the creation of new cuticles, due to the migration of new infective juveniles (**Fig. 25B**).

▪ On dissection, the tissues take on a rubbery appearance and appear to be completely disintegrated (**Fig.25C**).

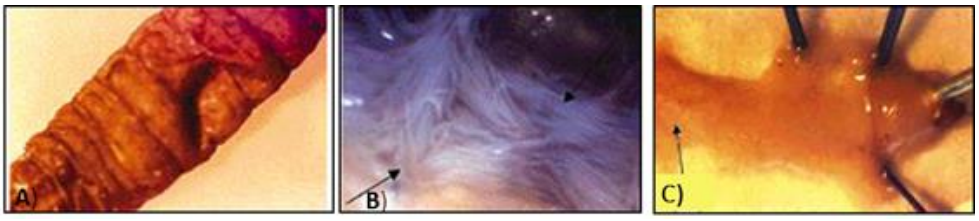

Figure 25. A) Last instar larva of *T. solanivora* affected by the entomopathogenic nematode *Steinernema feltiae*. B) Mass of infective juvenile *S. feltiae* in the intersegmental spaces of a final instar larva of *T. solanivora*. C) Rubbery appearance and disintegration of tissues under dissection of a final instar larva of *T. solanivora*. Source: modified from Sáenz (2003).

Several laboratory studies have shown that *T. solanivora* larvae are highly susceptible to infection by *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora*. These entomopathogenic nematodes cause 80 - 100% mortality of infected moth larvae in between 24 and 48 hours, in most cases (Fan et al. 2001, Sáenz 2003, Benítez and Duarte 2016). Sáenz (2003) pointed out the need to study the different restrictions that could be

encountered in the use of entomopathogenic nematodes in real field conditions. Perera et al. (2009) studied the differences in efficacy of two commercial products based on *Steinernema* on *T. solanivora* in potato crops under irrigated and rainfed field conditions. These authors found no significant differences with respect to the control treatment, attributing this to the fact that the trial coincided with rainfall. Benítez and Duarte (2016) pointed out that more laboratory, greenhouse and semi-field studies with these organisms are needed, since several previous experiences have prematurely discarded promising entomopathogens for biological control, when going directly from testing under laboratory conditions to real agricultural productions. From the above, it can be concluded that more research is still needed to better understand the optimal conditions for the application of entomopathogenic nematodes for potato moth control**.**

8.3.2 Parasitoids and predators

Various surveys, both in South America and in the Canary Islands, have identified several different parasitoids and predators of potato moths (Osorio et al. 2001, Martinelli 2004, Benítez and Duarte 2016, Piedra-Buena et al. 2019). Those that have obtained better results in different efficacy trials are detailed below.

- ***Copidosoma koehleri***

  - **Description and identification**

Copidosoma koehleri (Blanchard 1944), belongs to the superfamily Chalcidoidea, within the suborder Apocrita of the order Hymenoptera, as indicated in **Table 5**:

Table 5. Taxonomic classification of *Copidosoma koehleri*.

Level	Name
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera
Suborder	Apocrita
Superfamily	Chalcidoidea
Family	Encyrtidae
Genus	Copidosoma
Species	***Copidosoma Koheleri (Blanchard, 1940)***

As discussed above, proven parasitoids of the common potato moth, *P. operculella* are being tested for biological control of *T. solanivora,* including *C. koehleri,* which is a good candidate for this role (Ricón 2002).

The egg of *Copidosoma koehleri* is dumbbell-shaped, consisting of a bulb, a neck, and an elongated basal portion, approximately 0.17 mm length **(Fig. 26A)**. The adult body measures up to 2 mm long and is black with a metallic sheen **(Fig. 26D)**. It has a broad head and short antennae, which are lanceolate in males and truncated clavate in females. The second pair of legs has a pale-yellow coloration in its apical portion. In addition, these are developed, allowing it to jump, containing internal spines on the tibia. The forewings are hyaline, with a smoky spot in the region of the stigma. Females have an ovipositor that allows them to deposit the egg inside the host (Ricón 2002).

- **Life cycle**

Since it is a polyembryonic species, the fertilized egg of this encyrtid contains several embryos, with an egg of *C. koehleri* having up to 40. Each of these embryos divides, giving rise to two forms, one of which will result in the adult individual, and the other a soldier larva whose function is to eliminate other parasitoid species they find in the host (Cañedo et al. 2016). Regarding larval stages, observations and measurements of the width of the cephalic box (Dyar's law) point to the existence of four instars whose duration is linked to the development time of the host (Ricón 2002, Cañedo et al. 2016). L1 juvenile larvae, initially transparent and colorless, become creamy white at first and orange later as feeding progresses (**Fig. 26B**). They consume the internal tissue of the moth larva and its vital organs, preventing it from pupating. It then takes on a flaccid and unhealthy appearance, ceasing to feed and remaining almost immobile. Feeding continues until the host is empty and reduced to a carcass surrounding the parasitoid brood, the mummy (**Fig. 27A**). At this stage, the larval development *of C. koehleri* ends and it secretes a sheath and pupa. This pupa is initially slightly translucent white, but after 24 hours the eyes acquire a red pigmentation (**Fig. 26C**) and melanization of its structures begins, turning black. At this moment they are visible from the outside of the mummy, to which they give dark coloration (**Fig. 27B**). From this moment on, the appendages of the pupae begin to be released, and with their mandibles they open holes above the head of the parasitized larva, through which the adults emerge from the mummy (**Fig. 26D and 27C**). It can harbor between 25 and 75 new individuals (Sánchez-Aguirre and Palacios 1995, Ricón 2002, Cañedo et al. 2016).

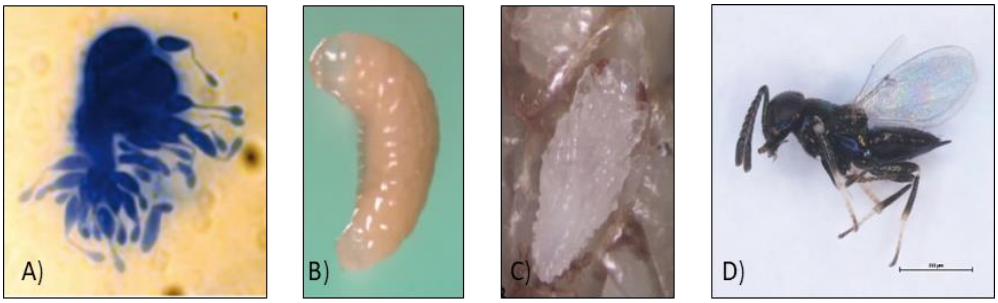

Figure 26. *Copidosoma koehleri* stages inside the mummy: A) Eggs. B) Larva.C) Pupa. D) Adult emerged. Source: A) Composition based on Santamaría et al. (2007), B) and C) Cañedo et al. (2016); D) Gavara J. (ICIA).

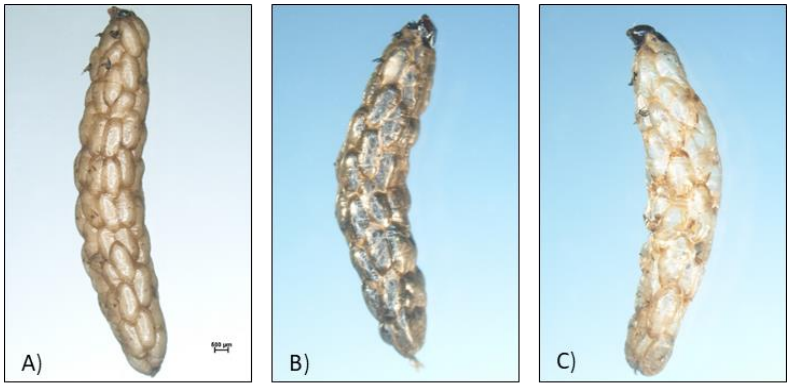

Figure 27. Mummy of *T. solanivora* produced by *C. koehleri:* A) newly formed pupae. B) pupae with black melanized structures. C) Empty mummy, when the adults have already emerged. Pictures: Gavara J. (ICIA).

This parasitoid was introduced in Venezuela in 1994 from Peru and Bolivia, where it showed low development and parasitism rates on *T. solanivora* under their conditions (Torres 1998). In the Canary Islands, laboratory rearing was carried out, obtaining variable parasitism rates (10-20%). In the field, parasitoid releases have been tested in potato crops in Tenerife, resulting in a reduction in the number of affected potatoes. However, this coincided with a rainy season, so the reduction occurred in all plots of the island and it was not possible to relate the reduction of infestations in the experimental farm with the parasitoidism of *C. koehleri* (Ricón 2002, Carnero et al. 2008). Subsequently, during the 2015, 2016 and 2017 harvests, studies were conducted comparing plots using an integrated

management strategy with the use of pheromones, authorized products (neem oil and *B. thuringiensis*) and releases of *C. koehleri,* with plots applying traditional management. Significantly lower percentages of damaged tubers were obtained in the plots under integrated management than in those under traditional management: 11% vs. 21%, respectively, in 2015, and 26% vs. 30% in 2017. In 2016, due to various logistical difficulties (destroyed traps, non-delivery of data by some farmers), the authors of the study were unable to establish reliable comparisons between farms under integrated management and those under traditional management (University of La Laguna, 2017).

▪ *Trichogramma* spp.

○ Description and identification

Trichogramma is a genus of the superfamily Chalcidoidea, within the suborder Apocrita of the order Hymenoptera:

Table 6. Taxonomic classification of *Trichogramma* spp.

Level	Name
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera
Suborder	Apocrita
Superfamily	Chalcidoidea
Family	Trichogrammatidae
Genus	Trichogramma
Species	***Trichogramma spp.***

These hymenoptera parasitize mainly moth and butterfly eggs. The vast majority of the species of this genus are polyphagous and parasitize a wide range of Lepidoptera, as well as insects of other orders. This preference for Lepidoptera has led to species of this genus being commonly used worldwide as biological control agents for pests of this order (del Pino et al. 2013a).

Identification of *Trichogramma* species has traditionally been based on morphological differences in the genitalia and male antennae (**Fig. 28**). However, this identification is very complex due to the small size of the individuals of this genus, which usually measure less than 1 mm, making it necessary to resort to experts in the genus. However, in recent years, molecular diagnostics have been developed to facilitate species identification (Polaszek et al. 2012, del Pino et al. 2013a).

Figure 28. Sexual dimorphism in antennae in *Trichogramma* sp. Left: female with smooth-looking antennae. Right: male with feathery antennae. Pictures: Gavara J. (ICIA).

Due to the great polyphagy of the species of this genus of egg parasitoids, their study for biological control has great potential. Of particular interest are the autochthonous species given that they are more adapted to local conditions (Hassan 1993). In Colombia, different species of *Trichogramma* have been found, identified and tested on *T. solanivora* (Osorio et al. 2001, Rubio et al. 2004). A total of six species of this genus have been found in the Canarian archipelago: *T. achaeae* (Nagaraja and Nagarkatti 1969), *T. bourarachae* (Pintureau and Babault 1968), *T. euproctidis* (Girault 1911), *T. evanescens* (Westwood 1833), close to *T. brassicae* (Bezdenko 1968) and *T. canariensis* (del Pino and Polaszek 2013), which have been found on other lepidopteran eggs and studied on other moths, such as the golden twin-spot moth (*Chrysodeixis chalcites*) and the tomato moth *Tuta absoluta* (Polaszek et al. 2012, del Pino et al. 2013a, 2013b).

In the present work, as explained in **Chapter 1**, *T. achae* and *T. euproctidis* were tested against *T. solanivora* on the basis of a previous prospection of eggs parasitoids in potato crops.

- **Life cycle**

The life cycle duration from egg to adult can vary from 8-17 days depending on temperature (15-30°C). Temperature on eggs, larvae and pupae also affects all aspects related to adult biology, such as emergence, sex ratio, longevity and fecundity (Vargas and Cabello 1989, Foerste and Foerste 2009, Firake and Khan 2014). In addition, it has been observed that light can influence the parasitism rate, being reduced under conditions of darkness and favored by light (Rubio et al. 2004).

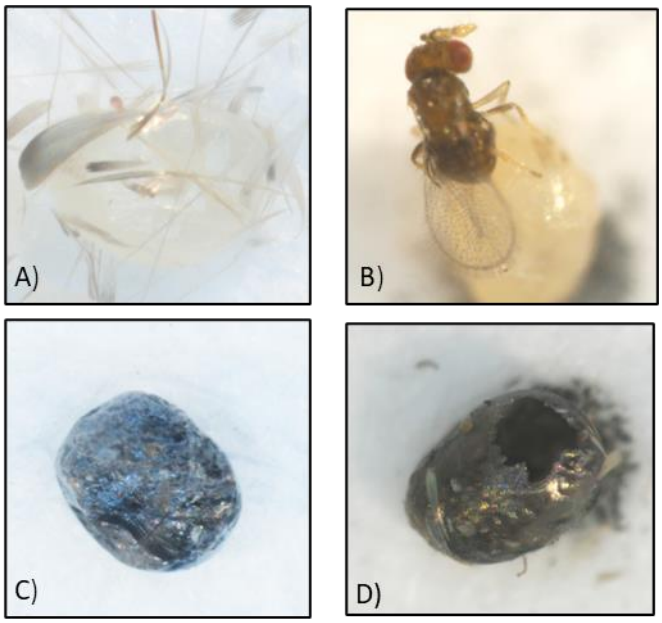

Figure 29. A) Healthy egg of *T. solanivora*. B) *Trichogramma* sp. ovipositing in a healthy egg. C) Parasitized egg with black coloration due to the growth of parasitoid larvae inside. D) Empty parasitized egg with exit hole due to the emergence of *Trichogramma* sp. Pictures: Gavara J. (ICIA).

The wasp finds the moth egg by capturing the kairomones from the scales that the female leaves near the egg during oviposition (**Fig. 29A**). Once a female finds a susceptible host egg, she examines it for a while by touhing it with her antennae, and if it is of the right quality and size, she drills a hole through the chorion (eggshell) and inserts two or three eggs inside **(Fig. 29B)** (Knutson 1998). When a female finds a group of host eggs for which she has a preference, she will generally remain on or near them for a long period until all or most of them are parasitized. Conversely, if less preferable host eggs are involved, the female will tend to reject them all or lay very few eggs before leaving the site to search for suitable eggs. Under laboratory conditions, a female parasitizes from one to 100 moth eggs during her lifetime. After the hatching of the parasitoid egg, the larval period lasts approximately 7 days, consisting of three instars, and then passes into the pupal period. During this period, parasitized eggs of *T. solanivora* can be distinguished from healthy ones as their color changes from white to black **(Fig. 29C)** (Knutson 1998). This pupal stage lasts about six days, after which the adult wasps emerge from the pupae and escape from the moth egg by chewing a circular hole in its shell. A black coating inside the chorion and the exit hole are evidence of parasitism.

Adults emerge from the host eggs early in the morning. First, the males, who remain near the eggs of the females to mate when they emerge. Reproduction is facultative, parthenogenetic and arrhenotocous. That is, females are diploid and come from fertilized eggs, while haploid males come from unfertilized eggs. Thus, mated females produce male and female offspring, and unmated females produce only male offspring. Females are capable of ovipositing as soon as they emerge, suggesting that at least a fraction of their eggs are already mature when they hatch (Knutson 1998, Ricón 2002).

▪ Ichneumonidae

In Martinelli's (2004) natural enemy survey research, an ichneumonid was found emerging from both the Guatemalan moth (*T. solanivora*) and common moth (*P. operculella*) pupae. Preliminary identification determined that it was a species of the genus *Pimpla* and provided the following description: wasp between 10 and 15 mm, males and females very similar with almost identical thorax, black abdomen, and reddish legs. The females are always larger and have an ovipositor approximately equal to the length of the abdomen (**Fig. 30**).

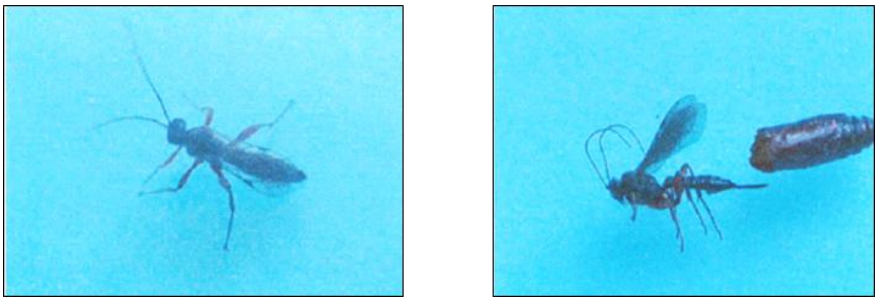

Figure 30. Ichneumonid found on *T. solanivora*. Left: male. Right: female just emerged from pupa. Source: Martinelli 2004.

Once the prepupa is exposed to the action of the parasitoid, the new fully developed adult takes only 15 - 19 days to emerge from the pupa. This emerging ichneumonid makes a characteristic cross-section at the end of the exuvia **(Fig. 31)**, which allows it to be differentiated from the cut of *T. solanivora*, which breaks it longitudinally (Martinelli 2004).

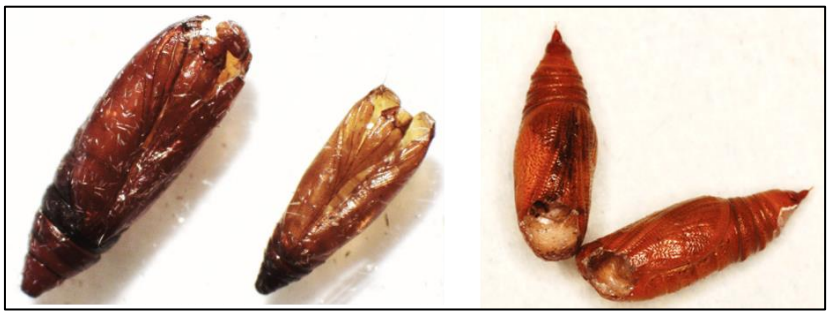

Figure 31. Left: longitudinal section of *T. solanivora*, right: typical cross-section of an ichneumonid. Source: left: Shaw et al. (2015), right: Gavara, J.

Laboratory tests did not show significant levels of parasitism of *T. solanivora* with this parasitoid; therefore, its identification was not pursued further (Cabrera, R., pers. comm.).

- **Predatory mites**

The Dept. of Biology and Geology of the University of Almeria (UAL) and the Plant Protection Unit of the ICIA are proceeding with the study of moth egg predatory mites of the genus *B. tarsalis* (**Fig.32**) for the control of potato moths in storage, including both the common moth, *P. operculella,* and the Guatemalan moth, *T. solanivora*, in order to provide alternatives to chemical control which is increasingly restricted in these areas, due to the scarcity of authorized products. In the first laboratory results against *P. operculella*, the mites under study, *Blattisocius tarsalis* and *Blattisocius mali* showed suitability for possible application in small, non-commercial farmers' storage (Gallego et al. 2020b; Gallego et al. 2020c; Gavara et al. 2021). In the present work the study of B.tarsalis on *T. solanivora* is aproached.

Figure 32. *Blattisocius tarsalis* preying on an *T. solanivora* egg. Picture: Gavara, J. (ICIA)

9. Chemical control

Although the application of phytosanitary products is useful for the management of the common moth, *P. operculella*, so far, products have shown little efficacy in the control of *T. solanivora*. Therefore, the use of insecticides is a complementary measure to be used alongside other practices (Trujillo and Perera 2017a). One of the main reasons for the lack of efficacy of phytosanitary products is due to larval development takes place inside the tuber, which limits the use of contact insecticides. Systemic insecticides are also restricted due to the resulting environmental and consumer risks (Niño 2004).

Objectives

The main objective of this work is to select new biological control agents for the management of the Guatemalan potato moth, *Tecia solanivora*, under field and storage conditions. This general objective is subdivided into two specific objectives, in accordance with the two chapters of this thesis:

1. Evaluation and selection of the egg parasitoids *Trichogramma achaeae* and *Trichogramma euproctidis* for field conditions (Chapter 1).

2. Evaluation of *Trichogramma euproctidis* and the predatory mite *B. tarsalis* for storage conditions (Chapter 2).

Chapter I.

"Evaluation and Selection for biological control in field and storage conditions of the *Trichogramma* species (Chalcidoidea: *Trichogramma*tidae), *T. achaeae* and *T. euproctidis*."

This chapter corresponds to the science article: "Evaluation and Selection of New *Trichogramma* spp. as Biological Control Agents of the Guatemalan Potato Moth (*Tecia solanivora*) in Europe" which was published in Insects journal of MPDI publisher on 1th August 2023 (DOI: 10.3390/insects14080679).

Evaluation and Selection of New *Trichogramma* spp. as Biological Control Agents of the Guatemalan Potato Moth (*Tecia solanivora*) in Europe.

Jorge Gavara [1-2], Tomás Cabello[3], Manuel Gámez[3], Saskia Bastin[1], Estrella Hernández-Suárez [1] and Ana Piedra-Buena[1]

1. Instituto Canario de Investigaciones Agrarias (ICIA), Finca Isamar, Ctra. de El Boquerón, s/n, 38201 San Cristóbal de la Laguna, Spain.
2. Department d'Ecosistemes Agroforestals, Universitat Politècnica de València, Camino de Vera, s/n, 46022 València, Spain.
3. Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAMBITAL), Universidad de Almería (UAL), Ctra. de Sacramento, s/n, 04120 Cañada de San Urbano (La), Spain.

Abstract

The Guatemalan potato moth (*Tecia solanivora*) is designated a quarantine pest by the European Union, causing severe production losses in potato crops. No effective chemical control alternatives are currently available, and cultural techniques are unable to reduce harvest losses to acceptable levels. With a focus on biological control, two egg parasitoids (*Trichogramma euproctidis* and *Trichogramma achaeae*) were selected and evaluated for use under field and storage conditions. Laboratory assays (choice and no-choice) indicated the preference of both parasitoids for *T. solanivora* vs. *Phthorimaea operculella*. *T. euproctidis* showed the highest parasitic activity for both moths. Analysis of functional response (at 15, 20, 25 and 27 °C) confirmed the high parasitic potential of *T. euproctidis*. Furthermore, in assays conducted under darkness conditions, *T. achaeae* was unable to parasitize eggs. However, in semi-field assays, *T. achaeae* was more efficient in searching for eggs in the soil than *T. euproctidis*. Based on these results, *T. achaeae* was selected to be tested under field conditions, and *T. euproctidis* was selected for testing under storage conditions.

Keywords: biological control; crop protection; egg parasitism; semi-field; host suitability; parasitoids; storage.

Resum

L'arna guatemalenca de la creïlla (*Tecia solanivora*), declarada plaga de quarantena per la Unió Europea ocasiona grans pèrdues en el cultiu de la creïlla. Actualment, no hi ha alternatives al control químic, que s'ha mostrat insatisfactori i l'aplicació de tècniques culturals tampoc aconsegueix reduir les pèrdues a nivells acceptables. En el present treball es centra en l'ús del control biològic, concretament es van avaluar i seleccionar dos parasitoides d'ous, *Trichogramma achaeae* i *Trichogramma euproctidis*, per a la seua aplicació en condicions de camp i magatzem. Els assajos de laboratori (Elecció i No-elecció) van mostrar la preferència d'ambdós enemics naturals per *T. solanivora* front *Phthorimaea operculella*. *T. euproctidis* va presentar major potencial parasític per les dos arnes, confirmant-se el mateix en l'anàlisi de la resposta funcional (15,20,25 y 27 °C). Per la seua banda, els assajos d'il·luminació van mostrar la incapacitat de *T. achaeae* per parasitar en condicions d'obscuritat. Tanmateix, l'assaig de semicamp va mostrar una major eficiència de *T. achaeae* trobant els ous de la plaga front *T. euproctidis*. D'acord amb els resultats obtinguts, es va determinar la selecció de *T. achaeae* per a ser testada en camp i *T. euproctidis* per a condicions d'emmagatzematge.

Resumen

La Polilla Guatemalteca de la papa (*Tecia solanivora*), declara plaga de cuarentena por la Unión Europea, causa pérdidas severas en el cultivo de la patata. El control químico se ha mostrado deficiente y no existen alternativas eficientes. Por su parte, tampoco las las medidas culturales logran reducir las perdidas en vineles aceptables. En el presente trabajo, se centra la atención en el control biológico, habiéndose realizado un evaluación y selección de dos parasitoides de huevos*, Trichogramma achaeae* y *Trichogramma euproctidis*, para su aplicación en condiciones de campo y almacén. Los ensayos de laboratorio (Elección y No-elección) mostraron la preferencia de ambos parasitoides por *T. solanivora* frente a *Phthorimaea operculella*, mostrando *T. euproctidis* un mayor potencial de parasitismo sobre a las dos polillas, confirmandose el mismo durante el análisis de la respuesta funcional (15,20,25 y 27 °C). Por su parte, los ensayos de iluminación mostraron la incapacidad de *T. achaeae* para parasitar en ausencia de luz. Sin embargo, en los ensayos de semicampo, *T. achaeae* se mostró más eficiente encontrando huevos que *T. euproctidis*. Atendiendo a estos resultados, *T. achaeae* fue seleccionada para ser testada en condiciones de campo y *T. euproctidis* para condiciones de almacén.

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 (Villanueva and Saldamando 2013). This pest spread to several countries of South America both through trade and the illegal transport of seeds and tubers. In 1999, the Guatemalan potato moth reached Tenerife (Canary Islands, Spain) from Venezuela, then presumably spread from the Canary Islands to mainland Spain: Galicia (2015) and Asturias (2017) (Vigil-Cando 2017, Gavara et al. 2023a). The Guatemalan potato moth pest causes important harvest losses in all countries where it is present. If not managed, 50% or more of the harvest may be lost in the field, and up to 100% may be lost in storage (Gallegos et al. 2005, Trujillo and Perera 2011). The European Union declared the Guatemalan potato moth a quarantine pest owing to its severity, and the import of potato tubers from countries or internal regions with *T. solanivora* presence is forbidden (Villanueva and Saldamando 2013). Female Guatemalan potato moths lay their eggs in soil irregularities and cracks near the stems of plants. When the eggs hatch, the larvae move to the tubers and start to feed on them (Torres 1998) (RD 197/2017). They remain inside the tubers, feeding and growing until the last instar, when they move toward the soil surface to pupae. Only adult moths live outside of the soil to reproduce and start a new cycle (Gavara et al. 2023a). The process described above necessitates a complex pest management regime. Chemical control is ineffective because only adult moths and the most exposed eggs are removed, whereas most eggs and larvae are protected under the soil. Consequently, current management is based on cultural techniques throughout the growing cycle and on pest population monitoring using pheromone traps (Trujillo and Perera 2011 and 2017). However, these techniques are not effective enough to reduce harvest losses to acceptable economic levels for growers either in the field or under storage conditions, as no authorized chemical treatments are currently available, and infested potatoes usually evade phytosanitary visual control due to their undamaged appearance during the first stage of infestation (Lobo et al. 2021).

The ineffectiveness of current phytosanitary and cultural techniques to control the Guatemalan potato moth has resulted in an increase in efforts to search for and evaluate potential candidates to improve integrated management of the pest (Villamil and Martínez 2014, Piedra-Buena et al. 2019, Keasar and Sadeh 2007, Osorio et al. 2001, Gavara et al. 2023a). In this sense, the study of egg predators or parasitoids is especially interesting with respect to the biological control of *T. solanivora*, as once the eggs hatch, the larvae migrate into the soil, making it difficult to remove them before they enter the tubers. In particular, this type of biocontrol agent is interesting for storage conditions, where the eggs are laid on the surface of potatoes. Few eggs parasitoids are found on *T. solanivora*; one example is the braconid, *Apanteles* sp., although it does not protect tubers, as this parasitoid does not

prevent the growth and feeding of larvae but may reduce the population of the pest in the next generation (Villamil and Martínez 2014, Cañedo et al.2016, Weber et al. 2022). The encyrtid, *Copidosoma koehleri* (Blanchard 1940), presents the same limitation, despite sometime proving successful under field conditions on common tuber moth, *Phthorimaea operculella* (Zeller 1873). Therefore, *C. koehleri* is considered to be among the main natural enemies of potato moths in tuber complexes (*Symmetrischema tangolias* (Gyen 1913), *P. operculella* and *T. solanivora*) (Sánchez-Aguirre and Palacios 1995). Several authors have tested this parasitoid against Guatemalan potato moth under laboratory conditions and found that it does not achieve satisfactory acceptance and preference results (Ricon, 2002, Báez and Gallegos 2011). Ichneumonidae (*Pimpla* sp.) were also found on *T. solanivora* but were discarded because the establishment of rearing was not possible owing to a considerable lack of female offspring (Martinelli 2004). A Colombian Trichogrammatid strain, *Trichogramma lopezandinensis* (Sarmiento 1993), has shown promising results as a biological control agent under semi-storage conditions (Rubio et al. 2004); however, this species has a restricted distribution and is not present in the Canary Islands nor in mainland Spain (CABI 2019). The species of the genus *Trichogramma* (Hymenoptera: *Trichogramma*tidae) would be ideal biological control agents for the Guatemalan potato moth. They have been extensively studied and are usually used as biocontrol agents against lepidopteran pests with successful results. They can also be used along with other control methods, and their mass rearing with alternative hosts is easy (Cabello et al. 1996, Smith 1996, Cherif et al. 2021). Furthermore, this genus has been tested and used against other Gelechiids as *Tuta absoluta* (Meyrick 1917) and *P. operculella*, which are present in potato crops, with promising results (Hassan 1993, Gallego et al. 2020a, Schäfer and Herz 2020).

The efficacy of *Trichogramma* species for biological control of pests depends the suitability of the host target eggs. Therefore, correct election of species/strain has been shown to be essential for a successful release program (Hassan 1989, Myint et al. 2022). The appropriate selection of a biological control agent should be based on laboratory, semi-field and field experiments, starting with the testing of indigenous *Trichogramma* species/strains, because they are better adapted to the climate, habitat and host conditions than non-local strains (Hassan 1993, Cherif et al. 2021). The same approach should be applied under storage conditions. In the present work, one local strain of *T. achaeae* (Nagaraja & Nagarkatti 1969) and *T. euproctidis* (Girault 1911) from the Canary Islands were evaluated under laboratory conditions for the first time against *T. solanivora* to evaluate their potential use under field and storage conditions. The present work also represents the first time that acceptance of *T. euproctidis* has been evaluated on *P. operculella*, whereas *T. achaeae* was evaluated in a previous work (Smith 1996). Mixed infestations of the two potato moths present in the Canary Islands are common, and both parasitoids may be able to contribute to the control of these pests.

2. Materials and Methods

2.1. Insects Rearing

Both *Trichogramma* species, *T. achaeae* and *T. euproctidis*, were obtained from local mixed populations from a parasitoid collection program on potato crops carried out by the Canarian Institute of Agricultural Research (Instituto Canario de Investigaciones Agrarias, ICIA).

The mixed population of *T. achaeae* and *T. euproctidis* was separated using using molecular methods (see next section). Purebred rearing of each species was established on *Ephestia kuehniella* eggs at 20–25 ± 1 °C under 60-65% RH with a photoperiod of 16L:8D. Rearing was carried out in Falcon™ tubes (v = 50 mL) using modified covers with stainless-steel mesh. Parasitoids were fed a thin line of honey placed on the walls of the tubes.

The two moth species, *T. solanivora* and *P. operculella*, were obtained from local populations on Tenerife Island. During rearing, between 20 and 25 pupae of each species were placed in individual cylindrical plastic glasses (r_1 = 11.5 cm, r_2 = 7.5 cm, h = 5 cm; v = 1 L) covered on the upper side with a gauze held by a rubber band and filter paper for oviposition. As a food source, the gauze was moistened with a 50:50 solutions of water and honey using a paintbrush. The colonies were kept in climatic chambers at 20 °C under 70% RH in the dark. During the assays, filter papers with the new lays were changed daily to ensure that eggs were less than 24 h old.

Both moths and parasitoids were reared by Agrobiologica S.L. company (Located at the Canarian Institute of Agricultural Research through a collaboration agreement), which periodically provided the insects needed for assays. All insect colonies were started during the year 2021.

2.2. Molecular Identification

Two local *Trichogramma* species, *T. achaeae* and *T. euproctidis*, were used in this study. Owing to their small size (<1 mm) and the absence of obvious morphological distinctions between closely related species, morphological identification of *Trichogramma* is particularly difficult (del Pino et al. 2013). Therefore, molecular identification was carried out following the approach described by Polaszek et al. (2012), DNA was extracted from a whole individual specimen using the Chelex protocol (Casquet et al. 2012). Each specimen was placed in a 1.5 mL Eppendorf tube and ground in 5 µL of proteinase K (10 mg·mL−1−1) and 75 µL 10% Chelex-100. The tubes were then sealed and incubated overnight at 55 °C. The polymerase chain reaction and sequencing protocols described by Bastin et al. (2021) were followed (Piedra-Buena et al. 2019). The polymerase chain reaction primers for ITS2 are provided by Stouthamer et al. (1999). Sequences were checked, edited and assembled with CLUSTALW within MEGA software (version 7). The obtained sequences were then compared with the known *Trichogramma* spp. sequences published in the Genbank

database: http://blast.ncbi.nlm.nih.gov/Blast.cgi (Accessed on 12 March 2023) using BLAST.

At the end of the parasitism period in each bioassay, females were extracted and collected in Eppendorf tubes with alcohol (90%) for subsequent molecular identification using the described method. Every sample of choice and illumination assays were identified, whereas in the rest of the assays, only 5 random samples were identified for each treatment. In all cases, molecular analysis confirmed the correctness the species used in each treatment.

2.3. Host Acceptance (No-Choice)

Four "no-choice" (acceptance) bioassays (two with *T. achaeae* and another two with *T. euproctidis* on eggs of *T. solanivora* and *P. operculella*) were carried out following an adapted version of the methodology described by Gallego et al. (2020a) (**Fig. 1**). In each trial, 20 newly emerged, mated *Trichogramma* females (less than 24 h old) without previous parasite experience and that had previously been provided with a fine line of honey as a food source were individualized in test tubes (7 cm × 1 cm diameter). In order to ensure their complete sexual maturation and acclimatization, the individualized females were subjected to a 24 h period under assay conditions in a climatic chamber (25 °C, 70% RH, with a photoperiod of 16L:8D).

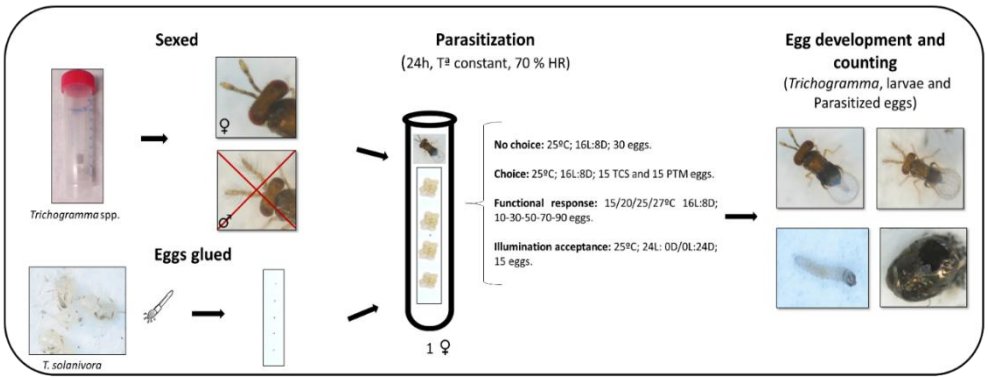

Figure 1. Summary of laboratory assay methods (no-choice, choice, functional response and illumination acceptance). TCS = *T. solanivora*; PTH = *P. operculella*.

After the acclimatization period, 30 fresh *T. solanivora* eggs (not sterilized, less than 24 h of age) were glued on white cardboard (0.9 cm × 5.0 cm) in groups of five using a distilled-water-moistened paintbrush. No special glue was used, as the moisture of the paintbrush was enough to keep the eggs fixed. Next, one cardboard section with eggs was

introduced to each *Trichogramma* sample. The test tubes were then closed with plastic film and returned to the climatic chamber, where parasitism was allowed for 24 h. At the end of the parasitism period, the females were removed, and development was allowed to continue for seven days in the climatic chamber. Finally, the numbers of parasitized, black (melanized) eggs and surviving larvae were counted. Removal of previously hatched larvae was not necessary because *T. solanivora* do not have a cannibalistic habit.

For statistical analysis, data were checked for homogeneity of variance (F test, Levene test) and the normal distribution of residuals (Shapiro-Wilk test). Next, the average values were compared by ANOVA test using the GLM procedure (Tukey's test, p = 0.05) in IBM™ SPSS™ Statistics Version 25. In each trial, the experimental design was random and univariate with a single factor at four levels: *T. euproctidis* against *T. solanivora*, *T. euproctidis* against *P. operculella*, *T. achaeae* against *T. solanivora* and *T. achaeae* against *P. operculella*, with 16 replicates.

2.4. Host Preference (Choice)

For the choice trials, the same methodology was as for the non-choice trials described above, with one difference: instead of 30 eggs, 15 *T. solanivora* eggs and 15 *P. operculella* eggs localized in opposite corners of the cardboard sections were offered at the same time to each isolated *Trichogramma* female (**Fig.1**). In these assays, removal of previously hatched larvae was not necessary because neither *T. solanivora* nor *P. operculella* showed cannibalistic or predation relations between them. *Trichogramma achaeae* and *T. euproctidis* host preferences were analyzed using the Manly preference index (β_2) (Manly 1972), with Chesson (1983) expression:

$$\beta_2 = \frac{\ln \frac{n_i - r_i}{n_i}}{\sum_2^1 \ln \frac{n_i - r_i}{n_i}} \qquad \textbf{(Equation 1)}$$

where β_2 is the Manly preference index (without replacement), r_i is the number of parasitized eggs, n_i is the number of exposed eggs and i = 1 or 2 represents the host eggs of each tested species. A preference index value of $\beta_2 > 0.5$ corresponds to preference, $\beta_2 = 0.5$ indicates indifference and $\beta_2 < 0.5$ represents rejection.

The number of parasitized eggs was analyzed by ANOVA test using the GLM procedure, and the average values were compared by Tukey's test (p = 0.05) and Manly preference index with one-sample t-test against a hypothetical mean of 0.5. Previously, the homogeneity of variance (F test and Levene test) and normal distribution of residuals (Shapiro–Wilk test) were checked. These analyses were carried out with IBM™ SPSS™ Statistics Version 25.

8.5. Functional Response and Parasitism

The functional responses of *T. achaeae* and *T. euproctidis* were determined at 15, 20, 25 and 27 ± 1 °C at densities of 10, 30, 50, 70 and 90 *T. solanivora* eggs under 70% RH with a photoperiod of 16L:8D. For these assays, the same procedures explained in the previous section were followed, but the data were collected upon the emergence of the *Trichogramma* adult. The following data were registered: parasitized eggs, emerged host larvae, collapsed eggs and number of emerged *Trichogramma* males and females. A total 10-15 replications were performed for each egg density in each *Trichogramma* species.

The data of parasitized eggs concerning to the functional response of both *Trichogramma* species at each tested temperature were subjected to two different statistical analyses. First, the homogeneity of variance and normality of the data were checked with the same procedure as in the previous assays; then, the density factor significance was analyzed by GLM analysis, and the mean values were compared using Tukey's test (p = 0.05) in IBM™ SPSS™ Statistics Version 25. Next, equations of functional response types I, II and III were adjusted for *T. achaeae* and *T. euproctidis* at all tested temperatures according to the following expressions (Cabello and Gamez 2007, García-Martín et al. 2008, Cabello et al. 2011):

- Type I:

$$N_a = N_t\left[1 - \exp\left(-a' \cdot T \cdot P_t\right)\right] \qquad \textbf{(Equation 2)}$$

- Type II:

(Equation 3)

$$N_a = N_t \cdot \left(1 - \exp\left[-\frac{a' \cdot T \cdot P_t}{1 + a' \cdot T_h \cdot N_t}\right]\right)$$

- Type III:

(Equation 4)

$$N_a = N_t \cdot \left(1 - \exp\left[-\frac{\alpha \cdot T \cdot N_t \cdot P_t}{1 + \alpha \cdot T_h \cdot N_t + \alpha \cdot T_h \cdot N_t^2}\right]\right)$$

where N_a is the number of parasitized hosts, N_t is the density of the host or prey, a' is the instantaneous search rate (equivalent to Nicholson-Bailey's "area of discovery": $a = a'T$, $days^{-1}$; T) on day^{-1}, T is the total available search time (days), P_t is the number of parasitoids, T_h is the host manipulation time and α' is parasitoid mortality potential. The cited adjustments were performed with Tablecurve 2D software, version 5.0.

The corrected Akaike information criterion (AIC_c) was applied to select the best adjustment model because it supposes better statistical precision for comparisons between models than the regression coefficient (r^2) (Motulsky and Christopoulos 2003. However, the latter was calculated to determine the goodness of each performed adjustment.

For parasitism and the sex ratio, the differences at the assayed temperatures (15, 20, 25 and 27 °C) in each species and between species, the number of parasitized eggs and the percentage of females were analyzed by two-way ANOVA and Tukey's test (p = 0.05) with the GML procedure.

2.5. Illumination Assays

Two illumination acceptance assays were conducted under light conditions, and two assays were conducted under dark conditions for *T. achaeae* and *T. euproctidis* following the same methodology as that applied in the no-choice assay, with the exception of the photoperiod, which was 24L:0D under light conditions and 0L:24D in dark assays.

In each trial, the experimental design was random and univariate with a single factor at two levels: parasitized eggs under light condition versus parasitized eggs under darkness conditions, with 20 replicates. Because the Shapiro-Wilk test confirmed the non-normality of the data, the numbers of parasitized *T. solanivora* eggs under illuminated and darkness conditions were compared using a non-parametric Kruskal–Wallis one-way analysis with a level of significance of p = 0.05. Both analyses were carried out with IBM™ SPSS™ version 25.

2.6. Search Ability under Semi-Field Conditions

The search ability assay (**Fig 2.**) was carried out under greenhouse conditions in insect cages (45 × 45 × 45 cm). Twenty potatoes (washed and numbered) were placed in 15 L trays and covered with a 5 cm layer of a mixed substrate of orchard soil and lapilli (2:1), previously disinfected with a steam machine (90 °C, 45 min). Three treatments were carried out (control, *T. achaeae* and *T. euproctidis*) with four replicates per treatment. Five couples (male and female) of *T. solanivora* were released in all treatments. The first release of 60 females of *T. achaeae* and 60 of *T. euproctidis* was carried out after a period of 48 h in each treatment. To obtain *Trichogramma* females, *T. solanivora* eggs developed for eight days at 25 °C were parasitized based on the sex ratio, as indicated in the "Results" section. This procedure yielded 80 and 76 parasitized eggs for *T. achaeae* and *T. euproctidis*, respectively. The second *Trichogramma* release was performed seven days later, with the same number of females of each species. No wasps were released in the control treatment. After a parasitism period of 20 days, the tubers were transferred to plastic boxes for 25 extra days to allow for the emergence of most of the larvae. The mean temperature during the greenhouse assay was 23.18 ± 5.30 °C, whereas the RH was 67.38 ± 16.06%. The evaluation of the assay consisted of counting, damaged tubers, number of mines per tuber and the survival of *T. solanivora* individuals (larvae, pupae and adults). Additionally, tubers were cut to search for any larvae remaining inside, which were also counted.

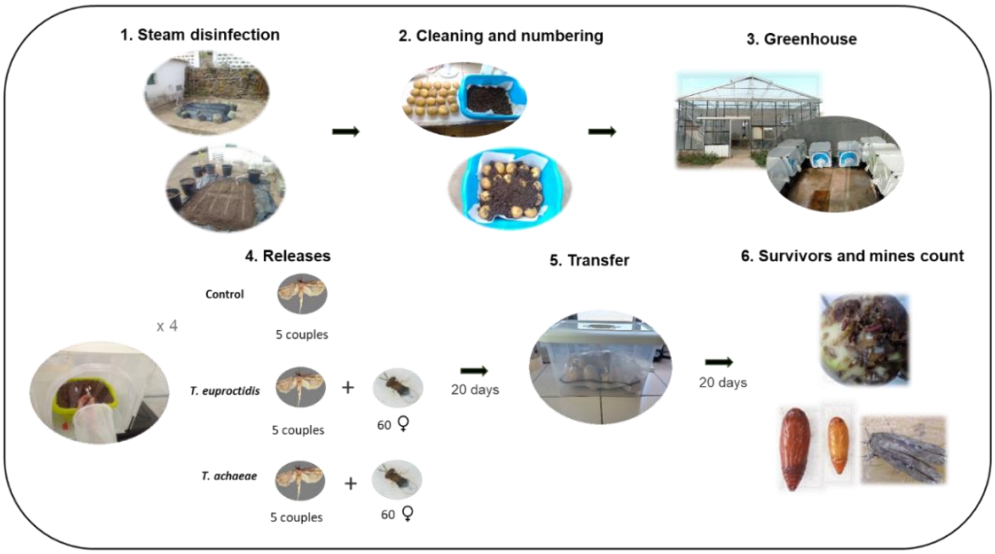

Figure 2. Summary of the searching semi-field assay

For statistical analyses, once the variance homogeneity and normality of data (Levene test and Shapiro-Wilk test, respectively) were checked, the mean number of survivors were compared by ANOVA test using the GLM procedure (Tukey's test, $p = 0.05$). In the case of the number of mines per potato and undamaged tubers, the data were analyzed by a generalized linear model (GZLM) using the Poisson distribution function and the log linear link function.

3. Results

3.1. Host Acceptance (No-Choice)

Statistical analysis showed the major parasitism capacity of *T. euproctidis* and *T. achaeae* against *P. operculella* relative to that of *T. solanivora*, with a higher number of parasitized *T. solanivora* eggs with *T. euproctidis* than with *T. achaeae* ($F_{2,3.59} = 50.72$; 59; $p < 0.05$) under no-choice conditions.

Figure 3 shows the parasitism level of *T. euproctidis* and *T. achaeae* against *T. Solanivora* and *P. operculella* eggs.

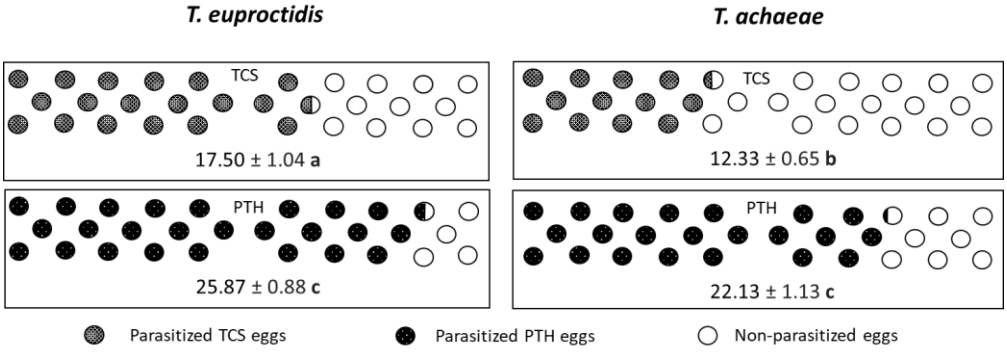

Figure 3. Mean number of parasitized *T. solanivora* (TCS) and *P. operculella* (PTH) eggs (±SE) when which are exposed to *T. euproctidis* and *T. achaeae* females at 25 °C under 70% RH with 16L:8D. Different letters denote significant differences in the number of parasitized eggs between treatments with different combinations of parasitoids (*T. euproctidis* or *T. achaeae*) and egg hosts ($p = 0.05$).

3.2. Host Preference (Choice)

Significant differences were found in mean values of the number of parasitized eggs in both species. *Trichogramma euproctidis* showed a higher number of parasitized eggs for *T. solanivora* than for *P. operculella*: 10.87 ± 0.58 vs. 4.81 ± 0.56 ($F_{1,1.29}$= 52.03; $p < 0.05$). The same pattern was observed for *T. achaeae*, with 6.35 ± 0.33 parasitized *T. solanivora* eggs vs. 3.88 ± 0.74 for *P. operculella* ($F_{1,1.32}$ = 5.27; $p < 0.05$). The Manly preference index (MI) showed the preference of *T. euproctidis* and *T. achaeae* for *T. solanivora* against *P. operculella* (t = 4.65; $p = 0.001$ and t = 2.03; $p = 0.001$, respectively).

Figure 4 shows the parasitism level of *T. euproctidis* and *T. achaeae* against *T. Solanivora* and *P. operculella* ggs exposed for 24 h under choice conditions.

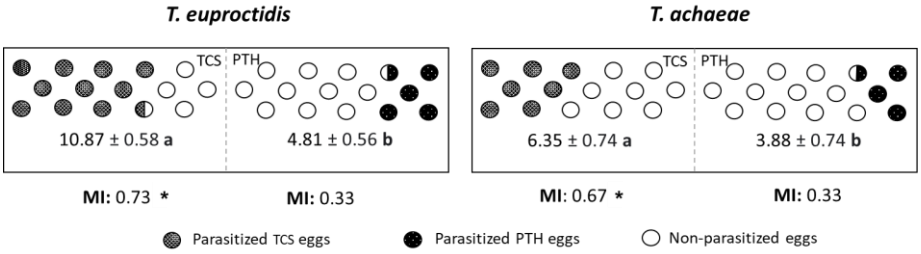

Figure 4. Number of parasitized *T. solanivora* (TCS) and *P. operculella* (PTH) eggs (±SE) and Manly Index (MI) when which are exposed to *T. euproctidis* and *T. achaeae* females the same time (25 °C, 70% RH, 16L:8D. Different lowercase letters represent significant differences (one-way ANOVA and Tukey's test; $p = 0.05$), and "*" indicates significant preference (*t*-test; $p < 0.05$) of each parasitic wasp

3.2. Functional Response

3.3.1. Trichogramma euproctidis

Analysis of functional response showed that at all the tested temperatures (15, 20, 25 and 27 °C), the parasitic behavior of adult females of *T. euproctidis* corresponded with type III. The adjustments showed the lowest values in the corrected Akaike indices (AIC_c). With respect to the adjustment parameters, the handling times (T_h) were similar; on the other hand, the mortality potential (α') showed similar values at the three highest temperatures (**Table 1**). The graphs in **Fig. 5** do not look like the typical "S " curves of functional response type III. It's because that the inflexion point of the type III curves,

from which the parameter "α" or parasitoid mortality potential occurs at very low densities of host eggs offered in the species *T. solanivora*.

Table 1. Parameters and statistical significance for the type I, II and III functional response equations when different densities of *T. solanivora* eggs were exposed to the adult female of *T.euproctids* for 24 h under laboratory conditions. "*" indicates the lowest AIC_c value at each temperature.

Temperature	Type	Fit Curve Parameters			Statistical Parameters		
		a'	a	T_h	d.f.	R^2	AIC_C
15 °C	I	0.5155	-	-	5	0.8052	7.30899
	II	5.9158	-	0.0466	4	0.8845	8.77405
	III	-	0.2569	0.0491	4	0.9053	6.96387 *
20 °C	I	0.7024	-	-	5	0.8681	7.33837
	II	1750.9	-	0.0372	4	0.9647	6.52153
	III	-	3.8×10^{16}	0.0372	4	0.9653	−0.6938 *
25 °C	I	0.6725	-	-	5	0.8232	7.78757
	II	4847.2	-	0.0436	4	0.9302	7.90383
	III	-	7.78×10^{11}	0.0394	4	0.9557	6.52805 *
27 °C	I	0.6218	-	-	5	0.7786	8.29285
	II	2.27×10^{15}	-	0.0412	4	0.9655	6.04313
	III	-	9.07×10^{13}	0.0418	4	0.9661	5.76405 *

Figure 5 shows the functional response curves of *T. euproctidis* against *T. solanivora* at four temperatures (15, 20, 25 and 27 °C).

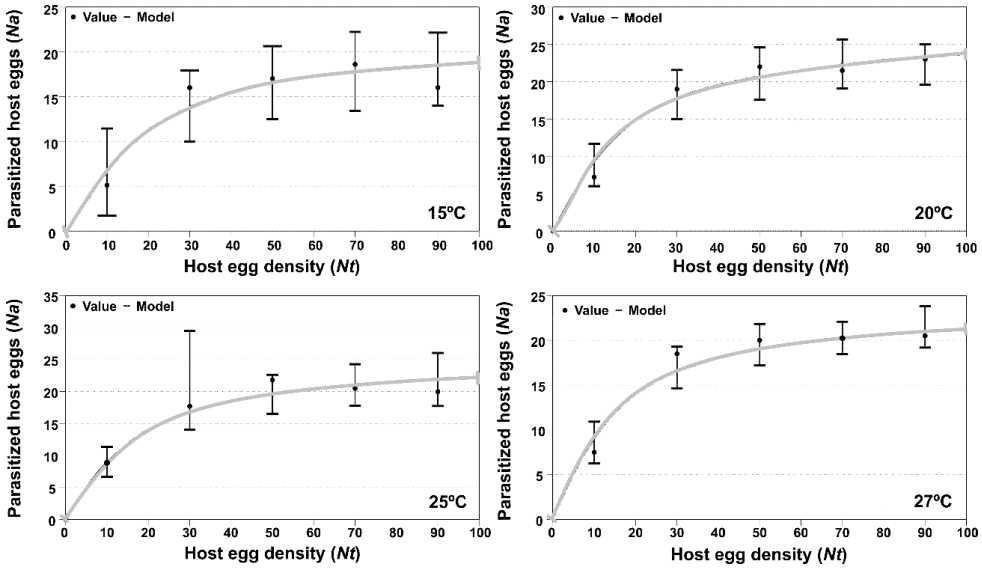

Figure 5. Functional response of *T. euproctidis* female parasitizing *T. solanivora* eggs at four temperature levels (15 °C, 20 °C, 25 °C and 27 °C) under laboratory conditions (70% RH and 16L:8D). Vertical bars indicate 95% confidence intervals. Note: For a correct interpretation of the figures it should be taken into account that the *y*-axis scales may be different.

Analysis of variance showed a significant effect of the host density on parasitism for all tested temperatures ($F_{4,45} = 26.47$; $p < 0.05$ at 15 °C; $F_{4,4.42} = 25.40$; $p < 0.05$ at 20 °C; $F_{4,4.50} = 31.78$; $p < 0.05$ at 25 °C and $F_{4,4.5} = 33.71$; $p < 0.05$ at 27 °C, respectively). At every temperature except 27 °C, *T. euproctidis* reached the maximum parasitism activity with 30 eggs.

3.3.2. Trichogramma achaeae

Trichogramma achaeae showed a type II functional response at the three lowest temperatures (15, 20 and 25 °C) that changed to type III at the highest temperature (27 °C). Thus, in relation to the adjustment parameters, in the present case, only the manipulation times (T_h) can be compared. As shown in **Table 2**, the values decreased with increased temperature. In **Fig. 6**, the graph at 27 °C, the typical sigmoidal shape of type III functional response cannot be seen for the same reason explained for *T. euproctidis* in the previous section.

Table 2. Parameters and statistical significance for the type I, II and III functional response equations when different densities of *T. solanivora* eggs were exposed to the adult female of *T. achaeae* for 24 h under laboratory conditions. "*" indicates the lowest AIC_c value at each temperature.

Temperature	Type	Fit Curve Parameters			Statistical Parameters		
		a'	α	T_h	d.f.	R^2	AIC_C
	I	0.2767	-	-	5	0.6347	5.49962
15 °C	II	3.4655	-	0.0845	4	0.9543	2.30479 *
	III	-	0.6533	0.0876	4	0.9515	2.48573
	I	0.3882	-	-	5	0.3079	9.34299
20 °C	II	2.23×10^{8}	-	0.0680	4	0.9660	2.93269 *
	III	-	1.48×10^{19}	0.0651	4	0.9608	2.99312
	I	0.3835	-	-	5	0.3843	8.68488
25 °C	II	19.4182	-	0.0585	4	0.9782	2.22521 *
	III	-	2.55×10^{17}	0.0588	4	0.9775	2.26747
	I	0.4465	-	-	5	0.6211	8.22257
27 °C	II	26.851	-	0.0547	4	0.9766	2.96402
	III	-	1.0972	0.0540	4	0.9783	2.73542 *

Figure 6 shows the functional response curves of *T. achaeae* against *T. solanivora* at the four tested temperatures (15, 20, 25 and 27 °C).

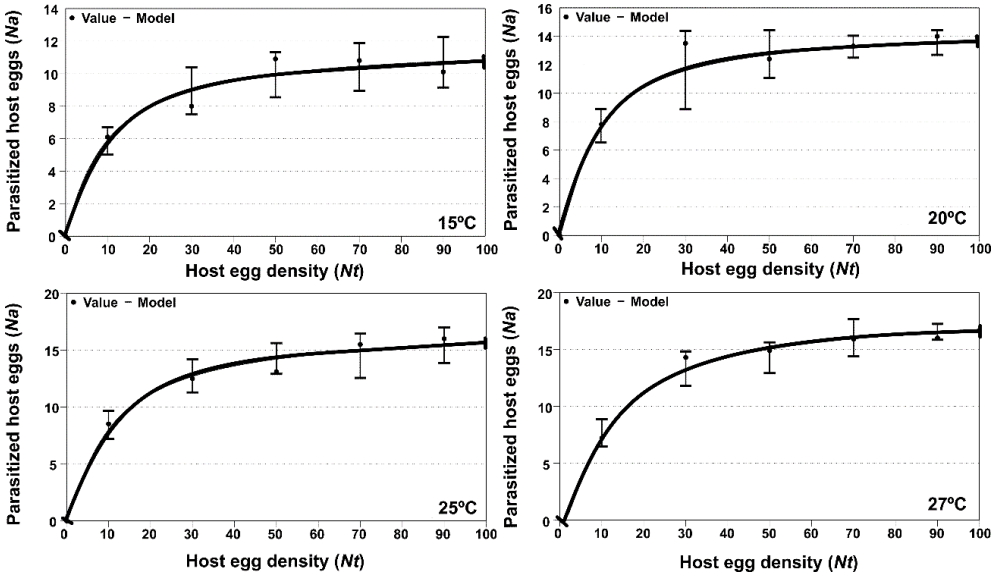

Figure 6. Functional response of *T. achaeae* female parasitizing *T. solanivora* eggs at four temperature levels (15 °C, 20 °C, 25 °C and 27 °C) under laboratory conditions (70% RH and 16L:8D). Vertical bars indicate 95% confidence intervals. Note: For a correct interpretation of the figures, it should be taken into account that the *y*-axis scales may be different.

With regard to host density, a significant influence on the number of eggs was observed at the four evaluated temperatures $F_{4,46} = 5.22$; $p < 0.05$ at 15 °C; $F_{4,46} = 11.70$; $p < 0.05$ at 20 °C; $F_{4,46} = 15.83$; $p < 0.05$ at 25 °C and $F_{4,46} = 9.65$; $p < 0.05$ at 27 °C). *T. achaeae* reached the maximum parasitism level at all tested temperatures at a density of 30 eggs.

3.4. Parasitism

Analysis of the number of parasitized eggs (**Fig. 7**) shows a significant effect of the species and the temperature ($F_{1,74}$ = 11.75; $p < 0.05$ and $F_{1,74}$ = 11.75; $p < 0.05$, respectively), with no effect of the interaction. *T. euproctidis* showed the highest parasitic activity between 20–27 °C, with significant differences relative to 15 °C ($F_{3,36}$ = 4.69; $p < 0.05$). In the case of *T. achaeae*, the highest level of parasitic activity was found at 20 and 25 °C ($F_{3, 36}$ = 6.37; $p > 0.05$). These temperatures only presented significant differences relative to 15 °C ($F_{3,36}$ = 6.37; $p < 0.05$). On the other hand, analysis of the number of eggs parasitized by *T. euproctidis* compared with *T. achaeae* at each temperature showed higher mean values for *T. euproctidis* at all tested temperatures: 15, 20, 25 and 27 °C ($F_{1,20}$ = 38.07; df = 1, 20; $p < 0.05$, $F_{1,18}$ = 13.73; $p < 0.05$, $F_{1,18}$ = 6.37; $p < 0.05$ and $F_{1,17}$ = 45.14;$p < 0.05$, respectively).

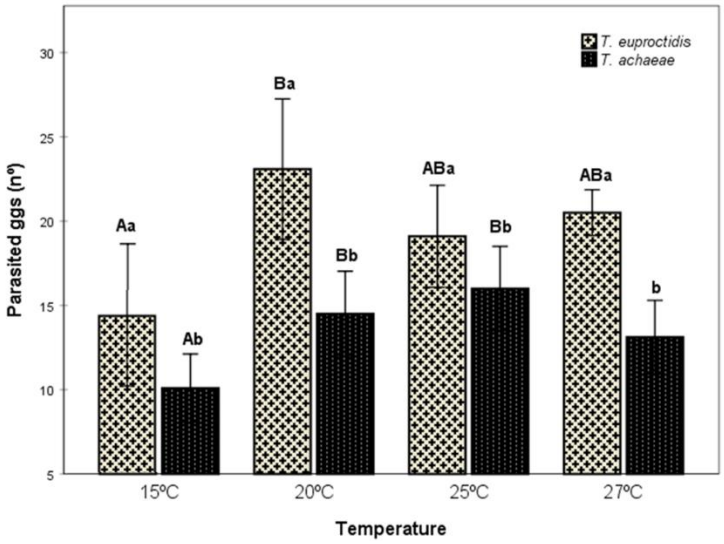

Figure 7. Mean number of eggs parasitized by *T. solanivora* (±SE) for *T. euproctidis* compared to *T. achaeae* at a host density of 90 eggs in the temperature range of 15–27 °C. Different uppercase letters represent differences in the number of parasitized eggs among the tested temperatures in each species. Different lowercase letters indicate significant differences between the two species (two-way ANOVA and Tukey's test, p = 0.05).

3.5. Sex Ratio and Fecundity

Statistical analysis of both *Trichogramma* progeny showed a significant influence of temperature and species ($F_{3,271} = 5.57$; $p < 0.05$ and $F_{3,271} = 5.57$; $p < 0.05$, respectively) with no effect of the interaction interaction ($F_{3,271} = 5.57$; $p > 0.05$) on the sex ratio. In *T. euproctidis* didn't find significant differences with temperature ($F_{3,137} = 1.29$; $p > 0.05$). In contrast, *T. achaeae* showed significant differences at 27 °C compared to 15 and 20 °C ($F_{3,134} = 8.08$; $p < 0.05$). However, a comparison of the two *Trichogramma* species only showed significant differences at the lower assayed temperatures (15 and 20 °C) with a higher number of *T. euproctidis* females ($F_{1,63} = 6.37$; $p < 0.05$ and $F_{1,63} = 12.27$; $p < 0.05$, respectively) (**Fig. 8**).

Fecundity results show the emergence of between one and two adults by each parasitized egg for both species at all the assayed temperatures (**Table 3**).

Table 3. Mean number (±SE) of emerged adults per parasitized egg obtained in the functional response assays at each temperature for *T. euproctidis* and *T. achaeae*.

Species	15 °C	20 °C	25 °C	27 °C
T. euproctidis	1.08 ± 0.3	1.20 ± 0.04	1.26 ± 0.04	1.27 ± 0.04
T. achaeae	1.41 ± 0.11	1.16 ± 0.04	1.13 ± 0.05	1.23 ± 0.04

Figure 8 shows the percentage of female *T. euproctidis* and *T. achaeae* at the tested temperatures in functional response assays.

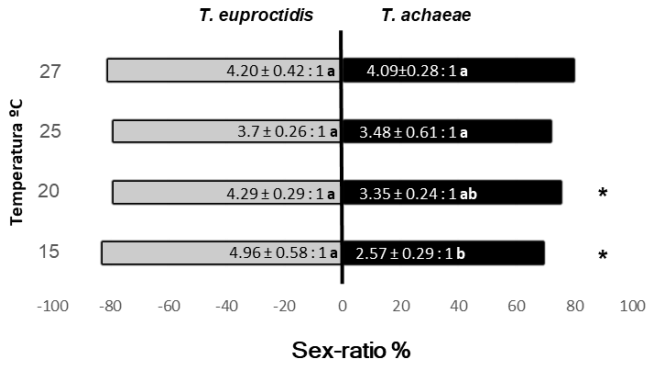

Figure 8. Mean sex ratio (F:M) (±SE) obtained for *T. achaeae* and *T. euproctidis* with temperature on *T. solanivora eggs*. Different lowercase letters indicate significant differences between temperatures (two-way ANOVA and Tukey's test, p = 0.05) for each species. "*" indicates significant differences between the two species at the same temperature (t-test, p = 0.05).

3.6. Illmination Acceptance Assays

The parasitism activity analysis result did not show significant differences between light and darkness conditions for *T. euproctidis* (Kruskal–Wallis test, X_2 = 125.00, $p > 0.05$). In contrast, *T. achaeae* was unable to parasitize the host eggs under darkness conditions (Kruskal–Wallis test, X_2= 210.00, $p < 0.05$) (**Fig. 9**).

Figure 9 shows the average number of parasitized *T. solanivora* eggs for *T. euproctidis* and *T. achaeae* under light and darkness conditions.

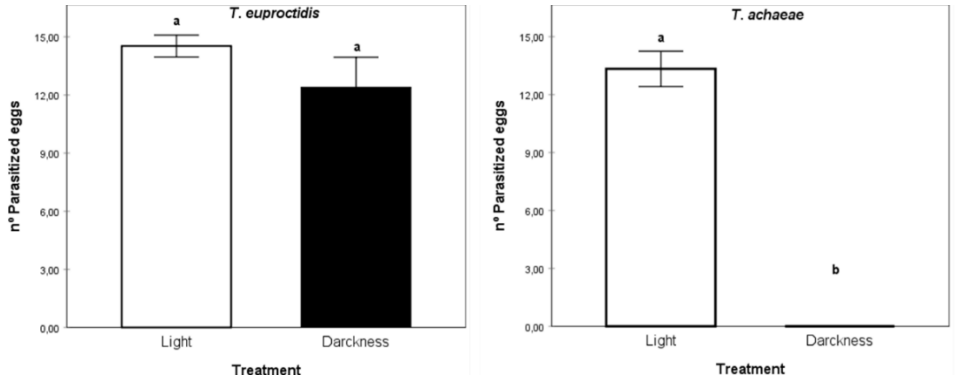

Figure 9. Mean number of *T. solanivora* eggs parasitized (±SE) by *T. euproctidis* and *T. achaeae* under light conditions compared with darkness conditions at 25 °C and 70% RH. Values with different letters indicated significant differences (Kruskal-Wallis test, $p = 0.05$).

3.7. Search Ability under Semi-Field Conditions

The statistical analysis results show significant differences in all the evaluated parameters between *T. achaeae* and control treatments: *T. solanivora* survival ($F_{2,9}$ = 6.25; $p < 0.05$, **Fig. 10**), number of mines per tuber (omnibus likelihood ratio X^2 = 7.459; df = 2; p = 0.24) and percentage of undamaged tubers (omnibus likelihood ratio X^2 =14.004; df = 2; p = 0.001) (**Fig.11**). *Trichogramma euproctidis* did not differ significantly in any parameter compared to the control (omnibus likelihood ratio X_2 = 7.459; df = 2; p = 0.62 for number of mines; omnibus likelihood ratio X^2 = 14.004; df = 2; p = 0.24 for undamaged tubers; and $F_{2,9}$ = 6.25; p = 0.137, for the number of survivors) and presented significant differences with respect to *T. achaeae* in the number of mines per tuber (omnibus likelihood ratio X^2 = 14.004; df = 2; p = 0.04) but not in the percentage of undamaged tubers (omnibus likelihood ratio X^2 = 14.004; df = 2; p = 0.056) (**Fig. 10** and **Fig. 11**).

Figure 10 shows the mean number of surviving *T. solanivora* obtained in the control, *T. euproctidis* and *T. achaeae* treatments under semi-field conditions.

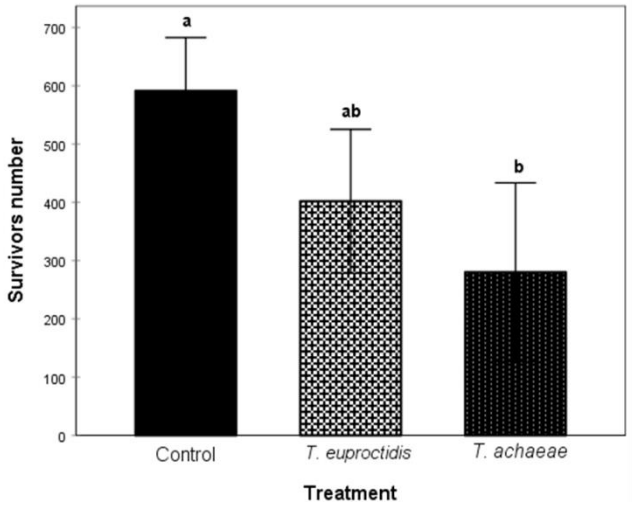

Figure 10. Mean (±SE) values of the number of surviving *T. solanivora*. Values with different letters indicated significant differences (one-way ANOVA and Tukey's test, $p = 0.05$).

Figure 11 shows the damages observed in the control, *T. euproctidis* and *T. achaeae* treatments, as well as the mean number of mines per tuber and the percentage of undamaged tubers.

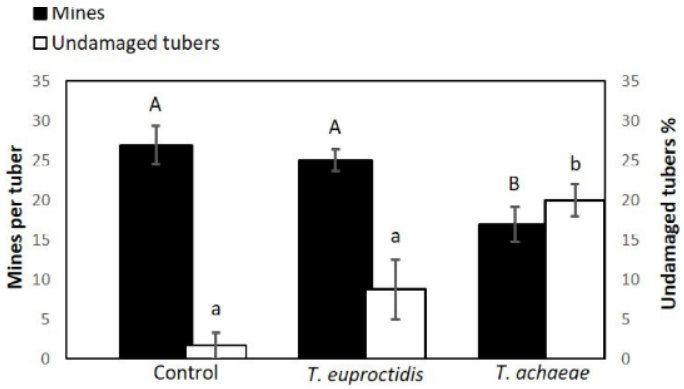

Figure 11. Mean values of the number of mines per tuber (±SE) and the percentage of undamaged tubers (±SE) obtained in each treatment. Different letters indicate significant differences (omnibus test, $p = 0.05$).

4. Discussion

In the present work, with the main objective of determining the potential application of *T. achaeae* and *T. euproctidis* as biological control agents against the Guatemalan potato moth (*T. solanivora*) in field and storage conditions, an evaluation and selection procedure were carried out. The applied methodology analyzed the four steeps defined for Vinson (1976) in host selection process of parasitoids: host habitat location, host location, host acceptance and host suitability. For the first steps, i.e., habitat location and host location, because the analyzed *Trichogramma* species were recollected on potato crops, it is reasonable to assume that the appropriate habitat corresponds to the field conditions rather than artificial storage conditions.

Host acceptance was analyzed using the non-choose assays. As mentioned in the Introduction, mixed infestations of *T. solanivora* and *P. operculella* are common. In such contexts, both hosts are available for parasitic activity of the analyzed *Trichogramma* spp. The presence of an alternative compatible host is known to result in a reduction in parasitism efficacy against the target pest and undesired ecological effects. Therefore, achieve maximum success in biological control programs, the selection of a candidate with a strong preference for the target pest is important (Babendreier et al. 2002, Mansfield and Mills 2004, Cherif et al. 2021). In the present work, we confirmed that *T. achaeae* and *T. euproctidis* have good acceptance for both hosts (**Fig. 2**). This is the first report of *T. solanivora* as a compatible host for the studied parasitoids, i.e., *T. achaeae* and *T. euproctidis*. Our study also confirmed the acetance of *P. operculella* by *T. achaeae*, which was previously reported by Gallego et al. (2020a). In all cases, both parasitoids showed significantly higher parasitism levels against *P. operculella* under no-choice conditions, and the major efficacy of *T. euproctidis* compared to *T. achaeae* was observed against both hosts. When the tested *Trichogramma* species were exposed to eggs of the two moths at the same time, both parasitic wasps showed a considerable number of parasitized eggs over Guatemalan potato moth, in agreement with the results of the Manly index, which indicated a clear preference for this host (**Fig. 4**). The obtained preference results are interesting because under field conditions *P. operculella*, eggs are present on the leaves and more accessible than *T. solanivora* eggs, which are laid in the soil (Tascón and Perera 2021, Gavara et al. 2023a). The major preference for Guatemalan potato moth eggs could facilitate wasps to find them in mixed infestations. Multiple factors have been pointed to with respect to the egg preference of *Trichogramma* species, such as egg age, chorion thickness and hardness, among other factors. However, previous works found that when *Trichogramma* are exposed to different host eggs, they usually select the largest eggs, most likely because a larger host size is preferred because parasitoids recognize that there are more nutrients available for their progeny (Mackauer and Sequeira 1993, Mansfield and Mills 2004, Samara et al. 2011, Myint et al. 2022). This phenomenon is consistent with our

results, i.e., that *T. solanivora* eggs are larger than *P. operculella* eggs (around 0.41 × 0.53 mm, compared to 0.50 × 0.35 mm (Samara et al. 2011, Kroschel and Schaub 2013, Martínez et al. 2019). In the evaluation of acceptance and preference, some authors have also counted the number of wasp-egg contacts; however, their results showing correspondence between high levels of parasitism and a high number of contacts were inconsistent (Hassan 1989 and 1993, Schäfer and Herz 2020). Therefore, wasp-egg contacts do not seem to be a clear criterion.

The last step, i.e., host suitability, is concerned with biological factors related to the development of the parasitoid in the potential host, i.e., survival, parasitism rate, sex-ratio of progeny and emergence, which are strongly influenced by temperature (Mackauer and Sequeira 1993, Samara et al. 2011). Another factor in the selection of a pest control agent is the functional response, i.e., the number of eggs parasitized in response to host densities (Berryman 2009, del Pino et al. 2020) which is a basic factor that allows contributes to the understanding of host interactions and is among the main influences on the stability of the system (Oaten and Murdoch, 1975). Functional response can be categorized into three forms: type I, linear increase in killed host/prey to a plateau; type II, a curvilinear rise to a plateau that then levels off under the influence of handling time or satiation; and type III, a sigmoidal increase in attacked hosts (Holling 1959, Hassell 2000, Mills and Lacan, 2004). In our study, the parasitoids showed different patterns in functional response, whereas *T. euproctidis* maintained type III (**Table 1**), and *T. achaeae* presented type II at the three lower temperatures and changed to type III at 27 °C (**Table 2**). The type II functional response, as shown by *T.achaeae* is the most common response found in *Trichogramma*tidae and has been suggested to be associated with suitable parasitoid hosts (Alphen and Jervis 1996, Rincón 1999, Fernández-Arhex and Corley 2003). This response result is in accordance to those reported by several authors at a temperature of 25 °C (Reay-Jones et al. 2006, del Pino et al. 2020, Manohar et al. 2020). However, our results contrast with the type I response reported in previous work for *T. achaeae* over *Ephestia kuehniella*, likely reflecting the effect of the different host (del Pino et al. 2020). In the same work, the author showed a change in functional response to type II at 25 °C, becoming type II to III at 27 °C, as reported by Wang and Ferro (1998) in *Trichogramma ostridina*. This case illustrates the influence of temperature on functional response. The constant type III response at all temperatures shown by *T. euproctidis* is a reflection of a major potential against *T. achaeae* because type III is considered the best kind of behavior to obtain a successful biological control of pests because it is theoretically the only response that can achieve direct density dependence at a low population host level and stabilize the host-parasitoid system (Rincón 1999). The potential of *T. euproctidis* in functional response is in accordance with parasitic activity, as this wasp showed a higher parasitism level than *T. achaeae* at all temperatures (**Fig. 7**). A possible justification for the parasitization behavior of *T. euproctidis* in relation to *T. achaeae*, under laboratory conditions is the different

manipulation times (T_h) (the sum of drumming, drilling and oviposition), as found in the functional response (**Table 1** and **2**); thus, the first species has a host manipulation time that ranges from 0.0372 to 0.0418 days compared to second species, with values ranging from 0.0540 to 0.0845 days, meaning that *T. euproctidis* spends less manipulation time saving within the day, leaving more time for searching and finding more host eggs. Handling time was previously identified as an important factor in parasitization by *Trichogramma* species (Pak et al. 1986).

observed that both parasitoids reach the maximum parasitism level at the density of 30 eggs, except *T. euproctidis* at 25 °C, which was at the density of 50 eggs, but only parasitized 21 eggs. Using different combinations of *Trichogramma* species and hosts, other authors also found that parasitoids could not parasitize all eggs within 24 h when 30 host eggs were exposed (Reay-Jones et al. 2006, Andrade et al. 2011, del Pino et al. 2020 Manohar et al. 2020), meaning that 30 eggs is the optimal offer for comparison of *Trichogramma* species.

Our results only reflect an influence of temperature on the sex ratio in *T. acaheae* at 27 °C, showing a higher number of females, whereas previous works obtained different conclusions about the effect of temperature on the number of female progeny (Bari et al. 2015, Andrade et al. 2011, del Pino et al. 2020, Tabebordbar et al. 2022), probably indicating a greater influence of parasitoid and host species than temperature. Comparing both species, we found a higher number of females on *T. euproctidis* compared to *T. achaeae* at 15 and 20 °C (**Fig. 8**). This is an interesting result because it is the usual range of small growers' warehouses, who usually store their harvest at room temperature. Under all evaluated temperature conditions, the parasitoids clearly exceeded the minimum of 50% female progeny recommended by the IOBC (Van Lenteren et al. 2003).

Regarding fecundity, our results did not show any influence of temperature. Between one and two emerged adults per parasitized egg was observed under all tested conditions for both species, indicating a high level of host suitability, over the 80% recommended by the IOBC, although considered a minor factor in inundative control strategies, which is the case for *Trichogramma* species (Van Lenteren et al. 2003, Schäfer and Herz 2020).

In spite of the better values shown by *T. euproctidis* for most of the analyzed biological parameters in the laboratory evaluations, in the semi-field assay, this species did not reduce *T. solanivora* populations or prevent damage in tubers. However, *T. achaeae* reduced the pest population, reaching an efficacy around 50%, and yielding 20% of undamaged tubers (**Fig. 9**). Taking into consideration the fact that the same number of individuals of both species was released and that the parasitic potential was higher at all tested temperatures for *T. euproctidis* under laboratory conditions, these results suggest a lower searching ability in soil of this wasp under field conditions. Therefore, even when it

can access the host, it is not an effective pest control agent. Although the importance of kairomones in host search and selection and their role in biological pest control are not mentioned in this paper, they have been addressed by other authors (Murali-Baskaran et al., 2018). In this regard, the superior effectiveness of *T. achaeae* in relation to *T. euproctidis* in the semi-field test may be due to the fact that the first species presents a greater attraction, as well as a greater stimulus in response to oviposition by the kairomones of the host species than the second species, as has been reported for other species and habitat conditions for this group of egg parasitoids (Murali-Baskaran et al. 2018). In contrast to semi-field conditions, the illumination assays showed the inability of *T. achaeae* to parasitize under darkness conditions compared to *T. euproctidis*, which kept its potential unchanged under both light and darkness conditions. These results indicate that *T. achaeae* is not suitable for storage conditions.

5. Conclusions

In the present study, a systematic methodology was applied to select and evaluate two *Trichogramma* species (*T. achaeae* and *T. euproctidis*) against the Guatemalan potato moth (*T. solanivora*).

In the laboratory assays (host acceptance, host preference and functional response), *T. euproctidis* showed better performance than *T. achaeae* in controlling the pest. However, in semi-field trials, *T. euproctidis* did not reduce *T. solanivora* populations or protect tubers from the pest, whereas *T. achaeae* behaved as an effective biocontrol agent under field conditions, suggesting a higher host searching ability or attraction to the host than that of *T. euproctidis*. Nonetheless, only *T. euproctidis* showed parasitic activity in the dark. Therefore, this species will be evaluated under semi-storage conditions in future studies.

The results obtained in this work highlight the relevance of performing assays with natural enemies under semi-field/semi-storage conditions before testing them in the field or in storage facilities. The selection of pest biocontrol agents based only on positive laboratory assay results could lead to incorrect decisions and unnecessary expense of effort and resources.

Chapter II.

“Evaluation and Selection for biological control in storage conditions of predator mite *Blattisocius tarsalis* (Berlese 1918) and egg parasitoid *Trichogramma euproctidis* (Girault 1911).”

This chapter corresponds to two science articles: “Evaluation of the Egg Predator *B. tarsalis* (Mesostigmata: Blattisociidae) for the Biological Control of the Potato Tuber Moth *Tecia solanivora* under Storage Conditions”, published in Agriculture journal of MPDI publisher on 24 June 2022 (DOI: 10.3390/agriculture12070920) and “Postharvest Biological Control of Guatemalan Potato Moth, *Tecia solanivora*, by *Trichogramma euproctidis* and *B. tarsalis*” which was published in Agriculture journal of MPDI publisher on 28 November 2023 (DOI: 10.3390/agronomy13122927).

Chapter II A

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

Jorge Gavara [1-2], Tomás Cabello[3], Manuel Gámez[3], Saskia Bastin[1], Estrella Hernández-Suárez [1] and Ana Piedra-Buena[1]

1. Instituto Canario de Investigaciones Agrarias (ICIA), Finca Isamar, Ctra. de El Boquerón, s/n, 38201 San Cristóbal de la Laguna, Spain.
2. Department d'Ecosistemes Agroforestals, Universitat Politècnica de València, Camino de Vera, s/n, 46022 València, Spain.
3. Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAMBITAL), Universidad de Almería (UAL), Ctra. de Sacramento, s/n, 04120 Cañada de San Urbano (La), Spain.

Abstract

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

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

Resum

Tecia solanivora és la principal plaga causant de danys en el cultiu de creïlla en camp a les Illes Canàries, tanmateix, les pèrdues són majors després de la collita en el període d'emmagatzematge. Actualment, no hi ha productes fitosanitaris autoritzats per a magatzems. L'anàlisi de l'àcar depredador *B. tarsalis* com a agent de control biològic es va portar a terme en el present treball. L'estudi de l'efecte de la temperatura va mostrar que *B. tarsalis* és capaç de mantindre la capacitat predatòria en el rang de 10 a 27 °C. En este sentit, Si bé es va observar que l'activitat depredadora s'incrementà amb la temperatura, no es van trobar diferències en les taxes de mortalitat entre els 10 i els 20 °C (amb un 33.52 ± 2.44 i 40,14 ± 3,54% d'eficàcia, respectivament), tampoc entre els 25 i 27 °C (59,26 ± 4,59 i 75,19 ± 4,64% d'eficàcia, respectivament). En condicions de microcosmos a baix nivell d'infestació (10 ous), *B. tarsalis*, va produir la màxima mortalitat a la densitat de cinc individus amb una eficàcia del 91,67 ± 8,33%. En canvi, a l'alt nivell d'infestació (50 ous) el màxim de mortalitat es va assolir a la densitat de 10 individus amb una eficàcia del 98,52 ± 1,48%. En l'assaig d'elecció es va observar que *B. tarsalis* no mostra preferència entre *T. solanivora* i *Phthorimaea operculella*, suggerint la utilitat de l'àcar en infestacions mixtes d'ambdos arnes. Els resultats obtinguts denoten que *B. tarsalis* és molt bon candidat com a agent de biocontrol en condicions d'emmagatzematge, inclús en cas d'infestacions combinades de *T. solanivora* i *P. operculella.*

Resumen

Tecia solanivora es la plaga más dañina en el cultivo de patatas en campo en las Islas Canarias, no obstante, las mayores pérdidas se producen en postcosecha en el periodo de almacenamiento. Actualmente no hay productos fitosanitarios autorizados para instalaciones de almacenaje. En el presente trabajo se ha llevado a cabo un análisis de viabilidad del ácaro *B. tarsalis* como agente de control biológico. El estudio del efecto de la temperatura, mostró que *B. tarsalis* mantiene su capacidad depredadora en el rango de 10 a 25 °C. A este respecto, si bien se observó que la actividad predatoria se incrementó con la temperatura, no hubo diferencias en las tasas de mortalidad observadas entre 10 y 20 °C (33,52 ± 2,44 y 40,14 ± 3,54% de eficacia, respectivamente), tampoco entre los 25 and 27 °C (59,26 ± 4,59 i 75,19 ± 4,64%de eficacia, respectivamente). Bajo condiciones de microcosmos, al nivel bajo de infestación (10 huevos), *B. tarsalis* produjo la máxima mortalidad a la densidad de cinco individuos con una eficacia del 91,67 ± 8,33%. En cambio, al alto nivel de infestación (50 huevos) el máximo de mortalidad se alcanzó a la densidad de 10 individuos 98,52 ± 1,48%. El ensayo de elección no mostró preferencias por parte de *B. tarsalis* respecto a *T. solanivora* i Phthorimaea operculella, sugiriendo que puede ser útil en condiciones de infestación mixta con las dos polillas. Los resultados obtenidos denotan que

B. tarsalis es muy buen candidato como agente de biocontrol en condiciones de almacén, incluso cuando se den infecciones mixtas.

1. Introduction

Potato crops (Solanum tuberosum L.) worldwide suffer important yield losses due to the potato moth complex (Lepidoptera: Gelechiidae). This pest complex consists of three species: the common potato tuber moth, *Phthorimaea operculella* (Zeller 1873); the Andean potato tuber moth, *Symmetrischema tangolias* (Gyen 1913); and the Guatemalan potato tuber moth, *Tecia solanivora* (Povolný 1970) (Sánchez-Aguirre and Palacios 1995) While *P. operculella* is a cosmopolitan pest, *T. solanivora* and *S. tangolias* have more restricted distributions. In fact, the latter is not present in Europe (Carrillo and Torrado-León 2013), and neither is it present in the Canary Islands, where *P. operculella* and *T. solanivora* are currently common potato pests.

Tecia solanivora is originally from Guatemala (1970), from the area between the isthmus of Tehuantepec in Mexico to northern Honduras and El Salvador. In a relatively short period, through successive shipments of seed potatoes, it has spread to Costa Rica (1970), Panamá (1973), Venezuela (1983), Colombia (1985), and Ecuador (1996). This insect was first detected in Tenerife (Canary Islands) in 1999, probably introduced from Venezuela (Villanueva and Saldamando 2013). In 2015, *T. solanivora* was found in Galicia (mainland Spain), probably introduced from the Canary Islands, and spread quickly to the neighbouring region of Asturias (Arias et al. 1996, Puillandre et al. 2008, Corral et al. 2017).

In the Canary Islands, *T. solanivora* produces great yield losses in potato crops, where it can damage more than 50% of harvested tubers in the field and 100% during storage, if the pest is not managed (Gallegos et al. 2005, Trujillo and Perera 2011). Moreover, the Guatemalan tuber moth has led to a reduction in cultivated area and an increase in production costs. If this pest is not controlled, it could cause a collapse in the local potato production, and many local potato varieties could be lost (Carnero et al. 2008, Lobo et al. 2021). In the Iberian Peninsula, the management of the Guatemalan tuber moth is essential to prevent its spread through the rest of the European continent.

The severity of this pest has led to its declaration as a quarantine organism by the European Union, and eradication programs have been established in Galicia and Asturias. However, in the Canary Islands, *T. solanivora* is so well established and widespread, it is assumed it cannot be eradicated (Gavara et al. 2023a) (RD 197/2017).

In the field, the use of phytosanitary treatments to reduce their populations has shown to be ineffective (Perera et al. 2009). This moth spends most of its lifetime under soil in a larval state inside the tuber or in pupal stage near the surface. Therefore, contact

pesticides have a very limited effect, whereas systemic pesticides would be dangerous for both the environment and consumers' health (Torres 1998, Niño 2004). Consequently, at present its management is based on cultural techniques throughout the growing cycle, and on insect population monitoring using pheromone traps.

However, in storage warehouses, larges losses occur due to conditions favouring tuber moth reproduction (i.e., potatoes are exposed without any protection; the typical darkness of these sites benefit adult moths' activity, and the low temperatures promote egg laying, reaching 311 eggs at 15 °C (Herrera 1998, Kroschel and Schaub 2013). In storage, chemical control of the tuber moth has limited potential, as most insecticides cannot be applied to potatoes shortly before they are marketed, as this would make them unsafe for human consumption. In fact, at present, there are no pesticides authorized for storage facilities (MAPA 2024b). Therefore, research efforts are aimed at the use of natural enemies.

For the use of a biological control, it is very important to pay attention to different storage conditions, as temperature is a vital factor that affects biological efficacy (Ferragut et al. 1987, Guzmán 2013). The temperature affects the pest as a natural enemy. Specifically, the development time of *T. solanivora* decreases from 208 days at 10 °C to 37 days at 27 °C (Schaub et al. 2021). Moreover, the development time of *B. tarsalis* decreases from 22 days at 15 °C to 6 days at 27 °C, and it was observed that the predatory activity of mites rises with temperature (Nielsen 1999 and 2001). Therefore, refrigerated and non-refrigerated conditions should be differentiated. Under refrigerated conditions, usual temperatures range between 5 and 12 °C, depending on the variety and destination (fresh consumption, processing, or seed potatoes) of the tubers. Nevertheless, in large refrigerated facilities, higher temperatures can be found, because potatoes are stored between 12 and 16 °C with a relative humidity of 90–95% for two weeks or more to facilitate wound healing and lowering their sugar content (Pinhero et al. 2009).

Surveys of natural enemies in potato storage facilities and crops have been conducted, and a selection of natural enemies have been initiated, focusing on organisms that have already been tested on the common tuber moth, *P. operculella* (Torres 1998, Carrillo and Torrado-León 2013). The use of predators on the Guatemalan tuber moth has been little studied. Even if some works on natural enemies of *T. solanivora* have been carried out (Osorio et al. 2001, Martinelli 2004, Benítez and Duarte 2016, Piedra-Buena et al. 2019), few predators have been found.

Osorio et al. (2001) found two heteropteran, *Lyctocoris campestris* (Parajulee and Phillips 1993) and *Buchananiella contigua* (White 1880), and some other occasional, generalist predators, and suggested the use of both heteropterans in storage conditions. However, these species are not commercially available, and consequently, they are very

difficult to obtain for research and use. In addition, species and strains of other natural enemies have been tested against *T. solanivora*: entomopathogenic fungi, *Beauveria* spp. (Villamil and Martínez 2014); bacteria, *Bacillus thurigiensis* (Berliner 1915) (Rojas Arias et al., 2013); virus, *Phthorimaea operculella* granulovirus (PhopGV) (Villamizar et al. 2006, Gómez-Bonilla et al. 2013); and the egg parasitoid *Trichogramma lopezandinensis* (Sarmiento 1993) (Rubio et al. 2004).

In a warehouse survey, Piedra-Buena et al. (2019) found two egg-predator mite species from the *Blattisocius* genus, *B. mali* (Oudemans 1929) and *B. tarsalis* (Berlese 1918), which have been tested against the common tuber moth, *P. operculella*, showing high efficacy under laboratory conditions (Gallego et al. 2020b, Gallego et al. 2020c, Gavara et al. 2021). Although these species are not commercially available, the *Blattisocius* genus is a cosmopolitan group, and its species often appear in stored plant products and in laboratory cultures, so it is relatively easy to find (Haines 1981). Owing to its relative availability and promising preliminary results in a similar host, the study of these *B. tarsalis* species has been considered interesting for IPM control of *T. solanivora*.

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

2. Materials and Methods

2.1.Biological Materials and Experimental Conditions

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

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

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

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

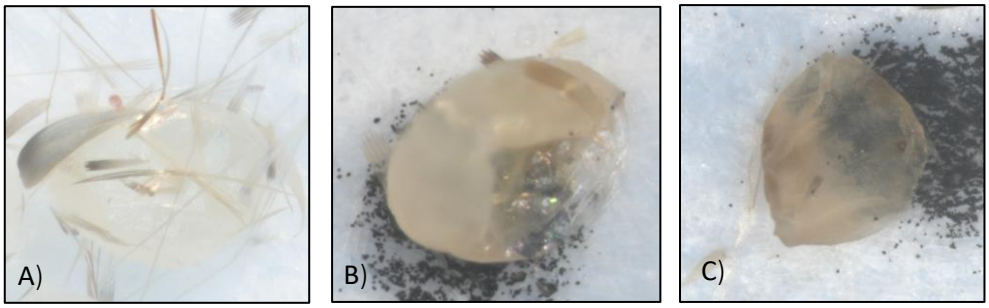

Figure 1. (**A**) Healthy egg. (**B**) Partially consumed egg. (**C**) Totally consumed egg.

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

Wallis one-way analysis with a level of significance of p = 0.05. All the analyses were carried out with IBM SPSS version 25 statistical software.

Moreover, the effectiveness of *T. solanivora* egg control by the mite was evaluated using the modified Abbott's equation (Robertson et al. 2009):

$$EP = \left(\frac{M - M'}{100 - M'} \right) x\ 100 \qquad \textbf{(Equation 1)}$$

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

2.3. Microcosm

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

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

After this period, the surviving individuals, larvae, pupae, and adult moths of each treatment were counted. In both bioassays, the experimental design was univariate and completely randomised, with only one factor, predatory mite density at four levels: 0 (control), 5, 10, and 20 mites released once per container. In each bioassay, there were five replicates per treatment. The values obtained of the number of surviving *T. solanivora* were analysed statistically through a generalised linear model (GLM) with normal distribution and the identity link function. The average values were compared in pairs through a Wald test at p = 0.05. These analyses were carried out with IBM SPSS version 25 statistical software. Finally, the results were corrected for the mortality in the control with the modified Abbott's equation (Robertson et al. 2009).

2.4. Host Choice: Tecia solanivora (Tcs)-Phthorimaea Operculella (PTH)

For the choice bioassay, the same methodology of "no-choice" test was used, with two exceptions: (1) only the standard temperature of 25 °C was tested, and (2) instead of five *T. solanivora* eggs, three *T. solanivora* and three *P. operculella* eggs were offered simultaneously to each isolated female mite. At the end of tanhe predation period (48 h), female mites were extracted, and the eggs were returned to the climatic chamber (25 ± 1 °C, 70–80% RH, total darkness) until hatching. Finally, the surviving larvae of both species were counted, and the number of killed eggs was calculated. Mite preference for hosts was determined through the Manly preference index (β_2) (Manly et al. 1972). In accordance with Chesson (1983), the expression of this index is:

$$\beta_2 = \frac{\ln \frac{n_i - r_i}{n_i}}{\sum_2^1 \ln \frac{n_i - r_i}{n_i}} \qquad \textbf{(Equation 2)}$$

where (β_2) is the Manly preference index (without replacement); r_i is the number of preyed eggs; n_i is the number of offered eggs; and i = 1 or 2 represents the host eggs of each species tested. When the value of the preference index is (β_2) > 0.5, this means preference; indifference when (β_2) = 0.5; and rejection if (β_2) < 0.5 (Manly et al. 1972). The values of the Manly index obtained for both moth species were analysed statistically by the Wilcoxon test with IBM™ SPSS™ version 25 statistical software.

2.5. Functional Response: Predatory Behaviour at Different Prey Densities

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

$$\frac{N_e}{N_o} = \frac{\exp\left(P_0 + P_1N_0 + P_0N_0N_0 + P_2N_0^2\right) + P_3N_0^3}{1 + \exp\left(P_0 + P_1N_0 + P_2N_0^3 + P_0^3\right)} \quad \textbf{(Equation 3)}$$

Where N_e is the number of preys eaten; N_o the initial value of prey offered; and P_0, P_1, P_2, and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively, estimated using the maximum likelihood method. Statgraphics version 18 software was used for the adjustments. P_0–P_3 parameters were obtained from a logistic regression. If the P_1 coefficient was not significantly different from zero, it corresponded to a type I functional response. It was considered a value to be different from zero when zero was not included in its confidence interval. If the P_1 value was significantly negative, this would demonstrate type II functional response, while a significantly positive P_1 value would demonstrate a type III functional response. A more exact statistical analysis was then conducted, and mortality data were fitted to the equations proposed by Hassell (1978) (**Eq. 4** and **5**) and Cabello and Gamez (2007) (**Eq. 6**) for predators when there is no prey replacement:

- Type I:

$$N_a = N\left(1 - \exp\left(-a'TP\right)\right) \quad \textbf{(Equation 4)}$$

- Type II:

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

- Type III:

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

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

3. Results

3.1. Evaluation of the Predatory Potential at Different Temperatures

The number of surviving eggs was significantly lower ($p < 0.05$) in treatments with the female mite than in control treatments without it, at all the temperatures tested. This indicates that the mite can predate on *T. solanivora* eggs at the four assayed temperatures. Results also show the influence of temperature on mortality caused by *B. tarsalis*. The lowest mortality rates were registered at 10 and 20 °C (40.00 ± 2.34% and 45.33 ± 4.06%, respectively), whereas at higher temperatures (25 and 27 °C), the mortality increased markedly (64.67 ± 4.15% and 76.67 ± 4.40%, respectively (**Table 1**). When Abbott's equation was applied, it was observed that the efficacy percentage also increased with temperature: 33.52 ± 2.44%, 40.14 ± 3.54%, 59.26 ± 4.59%, and 75.19 ± 4.64% at 10, 20, 25, and 27 °C, respectively.

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

Table 1. Average values (±SE) of the predatory potential evaluated at 10, 20, 25, and 27 ºC.

Temperature °C	Surviving Eggs (nº ± SE)		Mortality (% ±SE)		Efficacy %
	Treatment	Check	Treatment	Check	
10	3.15 ± 0.35 **a**	4.30 ± 0.39 **b**	40.00 ± 2.34	13.10 ± 1.80	33.52 ± 2.44
20	2.87 ± 0.31 **a**	4.53 ± 0.18 **b**	45.33 ± 4.06	3.45 ± 1.43	40.14 ± 3.54
25	1.90 ± 0.25 **a**	4.62 ± 0.40 **b**	64.67 ± 4.15	7.33 ± 1.83	59.26 ± 4.59
27	1.17 ± 0.20 **a**	4.4 ± 0.38 **b**	76.67 ± 4.40	12.41 ± 2.51	75.19 ± 4.64

In **Table 1**, the statistical analysis showed a highly significant treatment effect in the number of surviving eggs at the four temperatures tested (10 ± 1, 20 ± 1, 25 ± 1 and 27 ± 1 °C) using omnibus tests ($X^2 = 4.911$, d.f. = 1, $p < 0.0001$; $X^2 = 14.261$, d.f. = 1, $p < 0.0001$; $X^2 = 35.384$ and d.f. = 1; likelihood ratio chi-squared test = 60.035 and d.f. = 1, $p < 0.0001$, at 10, 20, 25, and 27 °C, respectively).

In **Figure 2** the statistical analysis showed a highly significant effect of temperature in partially consumed eggs using the omnibus tests (X^2 = 154.784, d.f. = 3, p < 0.0001). The comparison in pairs using the Wald test at p = 0.05 did not reveal significant differences at temperatures of 10 and 20 °C, nor between 25 °C and 27 °C. Significant differences were only observed with temperatures over 20 °C (p > 0.05 in all case). The Kruskal–Wallis test results showed the same pattern for the mean of killed eggs with p-value = 0.05.

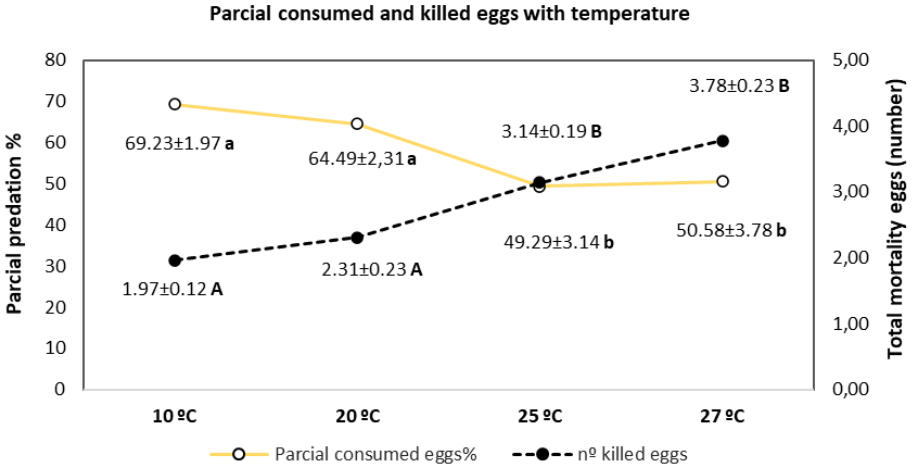

Figure 2. Mean percentage values of partial killed eggs (±SE) and number killed eggs (±SE) in the predatory potential bioassays. Values with different letters mean significant differences at *p* = 0.05, using the Wald test in the case of number of killed eggs and Kruskal–Wallis test for partially consumed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

3.3. Microcosm

In the bioassay at low infestation level (10 eggs), a highly significant effect was observed in the number of surviving eggs with significant variance (omnibus test: X^2 = 17.177, d.f. = 3, $p < 0.001$). The efficacies obtained for the mite densities of 5, 10, and 20 were 91.67 ± 8.33%, 82.29 ± 17.73%, and 81.25 ± 11.97%, respectively.

At a prey density of 50 eggs, statistical analysis of the number of surviving eggs found a highly significant effect (significant variance with omnibus test: X^2 = 41.233, d.f. = 3, $p < 0.0001$). The efficacies obtained for mite densities of 5, 10, and 20 were 67.02 ± 13.53%, 82.29 ± 5.77%, and 98.52 ± 1.48%, respectively.

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

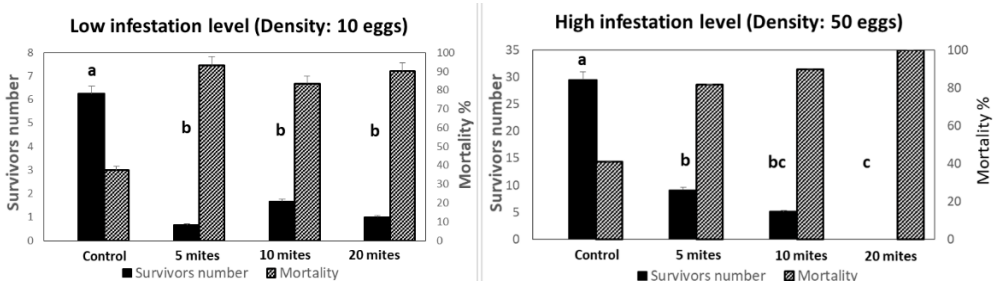

Figure 3. Mean values of surviving eggs (±SE) and mortality rate (±SE) at the infestation levels of 10 and 50 *T. solanivora* eggs and doses of 0, 5, 10, and 20. Values with different letters denote significant differences at $p = 0.5$, using Wald test.

In the bioassay at low infestation level (10 eggs), a highl significant effect was observed in the number of surviving eggs with significant variance (omnibus test: X^2 = 17.177, d.f. = 3, $p < 0.001$). The efficacies obtained for the mite densities of 5, 10, and 20 were 91.67 ± 8.33%, 82.29 ± 17.73%, and 81.25 ± 11.97%, respectively. At a prey density of 50 eggs, statistical analysis of the number of surviving eggs found a highly significant effect (significant variance with omnibus test: X^2 = 41.233, d.f. = 3, $p < 0.0001$). The efficacies obtained for mite densities of 5, 10, and 20 were 67.02 ± 13.53%, 82.29 ± 5.77%, and 98.52 ± 1.48%, respectively.

3.3. Host Choice

The number of killed eggs in the choice bioassay showed that there was not a significant difference in egg predation of TCS and *P. operculella* by the mite ($p = 0.482$; omnibus test: $X^2 = 0.495$; d.f. = 1; $p = 0.482$), nor was the difference in the Manly index significant ($p = 0.293$). These results indicate indifference in the hunt of *B. tarsalis* mite between TCS and *P. operculella* eggs.

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

Table 2. Mean number (±SE) of killed eggs in the "choice" trials.

Host	Eggs Mortality	Manly Index
T. solanivora	1.77 ± 0.43 **a**	0.57 ± 0.07 **a**
P. operculella	1.46 ± 0.23 **a**	0.43 ± 0.07 **a**

3.4 Functional Response: Predatory Behaviour at Different Prey Densities

Table 3 shows the adjustment parameters to the polynomial function (equation 3) of the number of TCS eggs killed by *B. tarsalis* female mites. As mentioned above (Materials and Methods section), the P1 value is significantly negative, therefore, in this previous analysis it is shown that this mite shows a type II functional response (when considering a value different from zero, when zero is not included in its confidence interval, as is the case).

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

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

The above result is consistent with the results of the fits carried out with the three types of functional responses shown in **Table 4**. These confirm type II (Equation (**5**)), because the corrected Akaike information criterion (AICC) shows the lowest value. Therefore, **Fig. 4** shows the type II functional response of the predatory mite. This figure

also shows a comparison of the number of killed eggs, where a highly significant effect was found (omnibus tests: $X^2 = 61.361$, d.f = 5, $p < 0.0001$), the maximum value of *B. tarsalis* predation was for the offer of nine eggs, without significant differences as to the number of offered eggs increased ($p > 0.05$).

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

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

In **Figure 4** the mean number of killed *T. solanivora* eggs at all tested densities is shown.

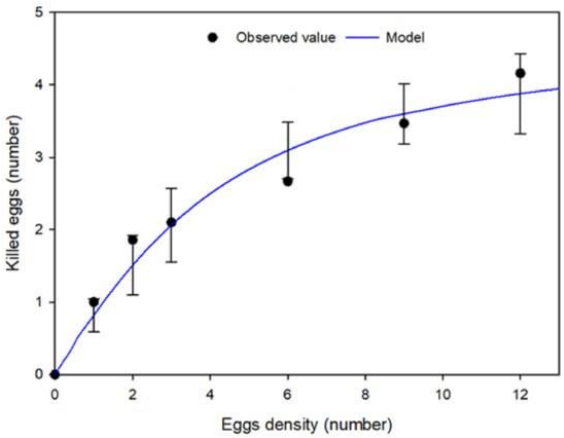

Figure 4. Functional response (number of pest eggs killed), type II, of the adult female of *B. tarsalis* when different densities of *T. solanivora* eggs were offered as prey for 24 h under laboratory conditions (whisker plot: 95% confidence intervals).

4. Discussion

Currently, *Tecia solanivora* management in the field relies on cultural practices and population monitoring using pheromone traps, as there are not effective phytosanitary products. As for storage conditions, there are not authorised products for chemical control nor commercial biological control agents available. The use of *B. tarsalis* has already been analysed in laboratory conditions on other storage pests: two pyralids, *Cadra cautella* (Walker 1863) and *Ephestia kuehniella* (Zeller 1879) (Nielsen 1999, Pinhero et al. 2009), and most recently, on other gelechiid, *P. operculella,* with promising results under non-refrigerated conditions (Gallego et al. 2020b, Gavara et al. 2021).

This study reports results of the efficacy and suitability of *B. tarsalis* as an egg predator on *T. solanivora* in storage, as evaluated in a series of laboratory assays. This is the first attempt to evaluate a natural enemy against *T. solanivora* in postharvest conditions. It is known that temperature is one of the main factors in the predator–prey relationship (Rall et al., 2010). In this sense, the temperature bioassays performed showed that *B. tarsalis* maintained its predatory activity on *T. solanivora* at the range of 10 to 27 °C. Additionally, it was observed that, while the predator mite increased prey mortality significantly between 20–27 °C, there were no differences at the lowest assayed temperatures (10–20 °C; **Table 1**). Evaluation at low temperatures is important to determine the viability in refrigerated conditions. At 10 °C, the number of *T. solanivora* killed eggs by *B. tarsalis* was 1.25 ± 0.11 in 48 h, which is equivalent to 0.63 eggs/day. This result is similar to Nielsen (1999), who also found a mortality rate less than 1 egg/day on *Ephestia kuehniella*, but contrasts with the result of Gavara et al. (2021), who found a higher predatory activity of *B. tarsalis* on *P. operculella,* the common moth, with a rate of 1.44 eggs/day. The differences in our results could be explained by difference in size between preys, because it has been observed that a greater size of eggs favours partial consumption, since the greater the size, the earlier the mite would be satisfied and the greater the survival possibilities of the prey eggs, although other chemical and mechanical factors may be involved (Haines 1981, Gallego et al. 2020b, Gallego et al. 2020c). The *T. solanivora* eggs at around 0.41 × 0.53 mm (RD 197/2017) (Niño 2004; Kroschel and Schaub 2013) are bigger than *E. kuehniella* and *P.operculella* , which are approximately 0.57 × 0.30 (Richards and Thomson 1932) and 0.5 × 0.35 (Kroschel and Schaub 2013), respectively.

This theory was confirmed by our experiment, where we could observe that partially consumed egg percentage remained high at all evaluated temperatures. This value was around 65–70% at low temperatures and was significantly lower, around 50%, at 25–27 °C. Conversely, the number of killed eggs increased with increasing temperature (**Fig. 2**). The

mortality rate at 10 °C yields an efficacy of 33.52 ± 2.44%. It is a lower value than that obtained by Gavara et al. (2021) for *P. operculella* under the same conditions (49.66 ± 5.06%). This result is interesting for large, refrigerated facilities during the recommended period of 12–16 °C mentioned in the introduction.

In addition, the results obtained at the temperature range of 20–27 °C are promising for most small farmers, who keep their harvest in small storage facilities at room temperature. At 25 °C (no difference with 27 °C (**Table 1**)), our results showed surviving egg mean values of between 1.17 ± 0.20 and 1.90 ± 0.25 in 48 h (**Table 1**). This value is lower than that found by Gallego et al. (2020b), who reported a mean value of 0.05 ± 0.05 for *P. operculella* eggs under the same conditions. Efficacies were 59.26 ± 4.59 – 75.19 ± 4.64% for *T. solanivora* against 98.86% for *P. operculella* (Gallego et al. 2020b). These results contrast with those obtained in the microcosm bioassay (**Fig. 3**), where we obtained mortality rates for *T. solanivora* similar to those reported for *P. operculella* under the same conditions (Gavara et al. 2021). As *B. tarsalis* mainly preys on the egg stage (Haines 1981) differences in acceptance tests but similar results in microcosm assays could be partially explained by differences in egg stage duration between the two moths. While *T. solanivora* eggs in our experimental conditions (25 °C, 70% RH) hatch between the sixth and seventh day (Andreadis et al. 2017, Gavara et al. 2023a), *P. operculella* eggs hatch between the fourth and fifth day. This means additional time for predation activity by *B. tarsalis* on *T. solanivora* eggs against *P. operculella* eggs. The effect of this additional time could not be observed in the acceptance assay because the predation period was interrupted at 48 h.

At a low infestation level in the microcosm assay (10 eggs; **Fig. 3A**), higher mortality was reached at minimum predator density, with an efficacy of 91.67 ± 8.33%. At high infestation level (50 eggs; **Fig. 3B**), mortality increased with mite density until the highest efficacy, 98.52 ± 1.48%, when 10 mites were released. These values show the same pattern reported by Gavara et al. (2021) on *P. operculella* with similar efficacy values, 96.97 ± 3.03% and 92.31 ± 2.74%, at low and high infestation levels, respectively.

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

growers in the Canary Islands, as mixed infestations by *T. solanivora* and *P. operculella* in potatoes are frequent.

For predators, the most commonly found response is Holling's type II, with a negatively accelerating rise to an upper limit, above which further increases in prey density do not influence attack rate (Mills 1982). This is the case of *B. tarsalis*, who presents a type-II functional response at 25 °C using *T. solanivora* eggs as prey (**Fig. 4**). This type of functional response has been previously corroborated for *B. tarsalis* with eggs of several species of insects, such as *Lasioderma serricorne* (Coleoptera: Ptinidae), *Plodia interpunctella* (Lepidoptera: Pyralidae), and *P.operculella* (Riudavets et al. 2002, Gallego et al. 2020b).

B. tarsalis has shown a consumption rate (α') in *T. solanivora* of 2.0502 days$^{-1.}$ These values are higher than those reported for *L. serricorne* and *P. operculella* eggs (1.032 days^{-1} and 1.776 days^{-1}, respectively), and similar to those obtained by Gallego et al. (2020b) with *P. operculella* eggs as prey (α' = 2.1258 days^{-1}).Regarding handling time (T_h), with *T. solanivora* eggs (T_h = 0.2091 days^{-1}), it was higher than values previously reported for *P. operculella* and *L. serricorne* eggs (T_h = 0.1014 days^{-1} and T_h = 0.1626 days^{-1}, respectively) (Riudavets et al. 2002, Gallego et al. 2020b). By contrast, the values were lower than those reported for *P. interpunctella* (T) = 0.3467 days−1) (Riudavets et al. 2002). Changes in T_h intrinsically involve several biological parameters, such as digestion time, attack time, gut content, hunger threshold, prey weight, and proportion of prey eaten, in place of a single parameter (Mills 1982). Thus, differences in predators' T_h may be due to one or more of these causes at the same time. For example, larger prey egg size may be related to higher food content, leading to earlier satiation of *B. tarsalis*, which would imply an increase in T_h. In this case, the egg size of *T. solanivora* is larger than the species mentioned above (0.41 × 0.53 mm, compared to 0.50 × 0.35 mm for *P. operculella*, 0.45 × 0.27 mm for *P. interpunctella* and 0.38 × 0.20 mm for *L. serricorne*) (Arbogast et al. 1980, Díaz 2013, Kučerová and Stejskal 2010, Martínez et al. 2019), which seems to support the results obtained for *P. operculella* and *L. serricorne*. However, it would not explain the values for *P. opercuella*. Therefore, in addition to size, differences could be attributed to other causes, for example: the hardness and/or thickness of the chorion, which would imply a longer time to access the egg's content; the presence of chemical compounds with protective functions (unpalatable or toxic) against predators (Blum and Hilker 2002) and even differences in the nutritional value of the egg.

Some works suggested predatory mites could be used as biological control agents for lepidopteran pests (Nielsen 1999, Thomas et al. 2010; Ghoneim 2014). In the case of the potato moth complex (*P. operculella*, *T. solanivora*, and *S. tangolias*), recent works in Spain reported the effectiveness of *B. mali* and *B. tarsalis* against *P. operculella*, the common potato moth, in storage conditions (Gallego et al. 2020b, Gallego et al. 2020c, Gavara et al. 2021). Similar results have been obtained in this work on the effectiveness of *B. tarsalis* against *T. solanivora*, the Guatemalan potato moth, also present in Spain. Our work completes the evaluation of *B. tarsalis* as a biological control agent for both potato moths

under laboratory conditions and, considering the promising results obtained, encourages proceeding with evaluation in real storage conditions.

5. Conclusions

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

Chapter II B.

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

Jorge Gavara[1,2], Mihaela Paris[1], Estrella Hernández-Suárez[1], Tomás Cabello [3], Juan Ramón Gallego[3] and Ana Piedra-Buena [1].

1. Instituto Canario de Investigaciones Agrarias (ICIA), Finca Isamar, Ctra. de El Boquerón, s/n, 38201 San Cristóbal de la Laguna, Spain.
2. Department d'Ecosistemes Agroforestals, Universitat Politècnica de València, Camino de Vera, s/n, 46022 València, Spain.
3. Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAMBITAL), Universy of Almeria, 04120 Cañada de San Urbano (La), Spain.

Abstract

Tecia solanivora (Povolný 1973), 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 °C. 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 mines 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.

Resum

Tecia solanivora (Povolný 1973), és una plaga de quarantena en l'Europa. Fou identificada en Guatemala el 1970, des d'on es va disseminar al llarg del Centre i Sud-amèrica, aplegant a les Illes Canàries l'any 1999 i l'Espanya peninsular el 2015. Esta plaga està causant pèrdues econòmiques inassumibles tant en camp com en magatzem, on poden suposar la pèrdua total de la collita. L'absència de tractaments fitosanitaris autoritzats en este àmbit ha comportat l'estudi de solucions alternatives com l'ús de l'àcar depredador d'ous *Blattisocius tarsalis* (Berlese 1918) i del parasitoide d'ous *Trichogramma euproctidis* (Girault 1911). Investigacions prèvies de laboratori han confirmat el potencial d'ambdós enemics naturals per al seu possible ús en magatzems a temperatura ambient, normalment entre els 15 i 20 °C. En el present treball, hem comparat la eficàcia dels dos agents de control biològic en condicions de semi-emmagatzematge i hem observat que si bé tant T. euproctidis com B. tarsalis redueixen les poblacions *de T. solanivora* (amb unes eficàcies del 82,95 ± 7,32% i el 49,06 ± 2,69%, respectivament) i el nombre de galeries, sols l'àcar depredador va aconseguir obtindre tubercles sans (65%). Per esta raó l'àcar va ser seleccionat i assajat en condicions d'emmagatzematge obtenint resultats reeixits en la protecció dels tubercles, suggerint per tant potencial per futures investigacions en les quals s'haurà de procedir a la adaptació i estandardització del seu ús en condicions reals.

Resumen

Tecia solanivora (Povolný 1973), es una plaga de cuarentena en Europa que fue identificada en Guatemala en los años 70, diseminándose a lo largo de centro y Sudamérica, llegando a las Islas Canarias en 1999 y alcanzando el territorio peninsular español en 2015. Esta plaga, causa pérdidas económicas inasumibles tanto en campo como en almacén, donde las mismas pueden suponer el 100% de la cosecha. Ante la ausencia de productos fitosanitarios autorizados para estas instalaciones, se está estudiando el uso del ácaro depredador de huevos, *Blattisocius tarsalis* (Berlese 1918), y el parasitoide de huevos, *Trichogramma euproctidis* (Girault 1911). En este sentido, los estudios previos de laboratorio ya han confirmado su potencial para su posible aplicación en almacenes no refrigerados, habitualmente con temperaturas entre 15 y 20 °C. En el presente trabajo, primero se comparó la eficacia de los dos enemigos naturales bajo condiciones de semialmacén y se observó que si bien ambos candidatos, *T. euproctidis* and *B. tarsalis* redujeron la población de *T. solanivora* (con eficacias del 82.95 ± 7.32% y 49.06 ± 2.69%, respectivamente) y el número de galerías, solo *B. tarsalis* proporciono tubérculos sanos (65%). Por esta razón, el ácaro fue seleccionado y puesto a prueba en condiciones de almacenaje real donde mostró buenos resultados que sugieren su potencial para futuras investigaciones de adaptación y estandarización de su uso en condiciones reales.

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 (Villanueva et al., 2013). Its spread has occurred mainly through the illegal trade and shipment of seed potatoes and tubers to several countries in Central and South America (Puillandre et. al., 2008). 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 (Puillandre et al. 2008).

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 (Trujillo and Perera 2011). 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 (RD 197/201).

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 (Herrera 1998). 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 (Lobo et al. 2021).

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 (Gavara et al. 2023a, Kroschel et al. 2013). 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 (Lobo et al. 2021; MAPA 2023d). Therefore, research efforts are now focused on finding effective natural enemies of *T. solanivora* (Piedra-Buena et al. 2019, Osorio et al. 2021).

On this subject, Solà (2017) 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 (Villamil et al. 2013); 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 (Rojas-Arias et al. 2013). For its part, the virus *Phthorimaea operculella*-granulovirus (PhopGV) has been tested in experimental conditions in warehouses with promising results (Gómez-Bonilla et al. 2013), 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 (Solà, 2017). 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 (Schöller et al. 2006). On this matter, Osorio et al. (2001), proposed the use of two generalist predators, the heteropterans *Lyctocoris campestris* (Fabricious 1794) and *Buchananiella contigua* White (1880), based on laboratory experiments. Rubio et al. (2004) tested *Trichogramma lopezandinensis* (Sarmiento 1993) under semi-storage conditions, a *Trichogramma* species not present in The Canary Islands (Biota Database, https://www.biodiversidadcanarias.es (accessed on 21 November 2023). Gavara et al. (2022, 2023) 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 (Haines 1987, Schöller et al. 2000, Cherif et al. 2021). This is an advantage because the larvae die before hatching, which is crucial as it prevents the larvae from feeding and protects the tubers (Gavara et al. 2023b). 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 ($d1$ = 11.5 cm, $d2$ = 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 °C, 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 *B. 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. (2020b)'s methodology (**Fig. 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.

j

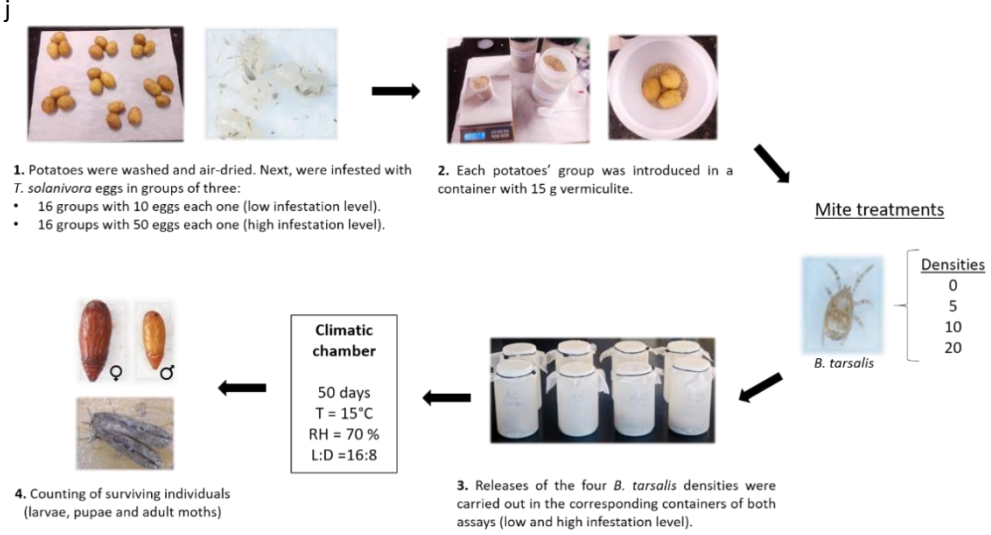

Figure 1. Summary steps of microcosm assay.

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 60×7560×75 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.

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™ SPSS™ version 25 software. Finally, the efficacy was corrected with respect to the control with the modified Abbott equation (Robertson et al. 2009):

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

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 (62×73.562×73.5 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 (**Fig. 2**).

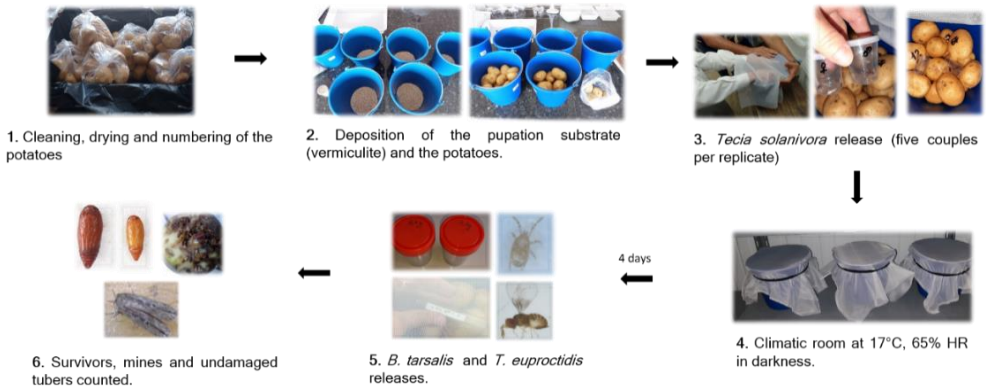

Figure 2. Summary steps of semi-storage assay.

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. (2022) results) of *B. tarsalis* and 120 *T. euproctidis* (based on Gavara et al. (2023) 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.

2.3.2. Release Preparation of Natural Enemies

Based on the sex ratio of *T. euproctidis* obtained by Gavara et al. (2023), 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−1−1, 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 mines 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™ 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 **Fig. 3.**

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.

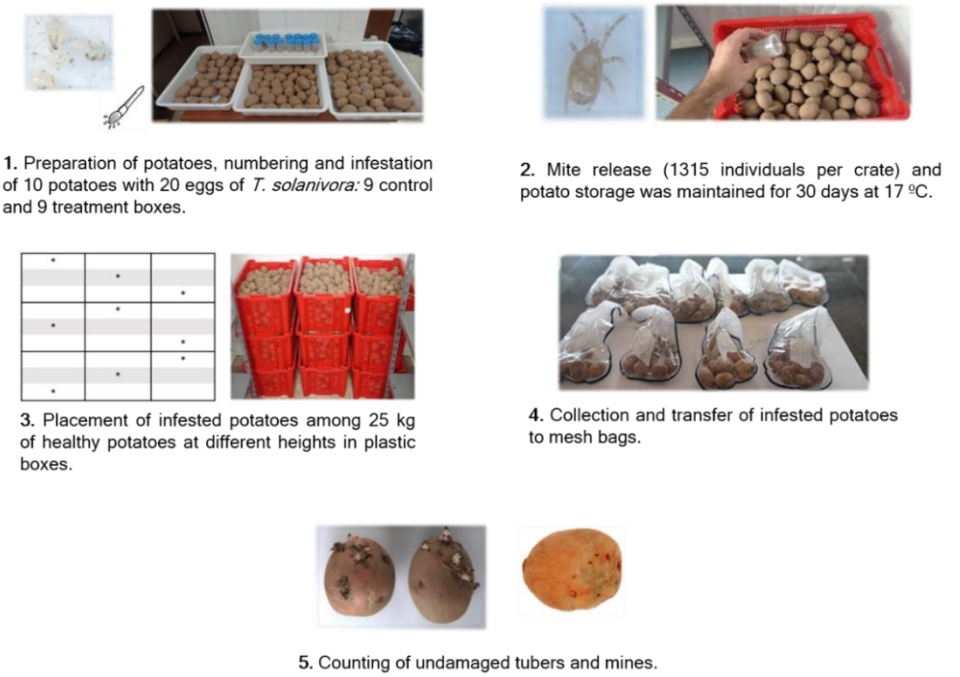

Figure 3. Experimental design of treatments in the storage assay: In 3, "*" indicates the location of the ten infested tubers in the fruit plastic boxes (up, middle or bottom).

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 mines 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, **Fig. 4A**) and high (50 eggs, **Fig. 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, $X2$= 161.42, df = 3, p < 0.001 at 10 eggs and $F3,2$ = 13.38; p < 0.001 at 50 eggs).

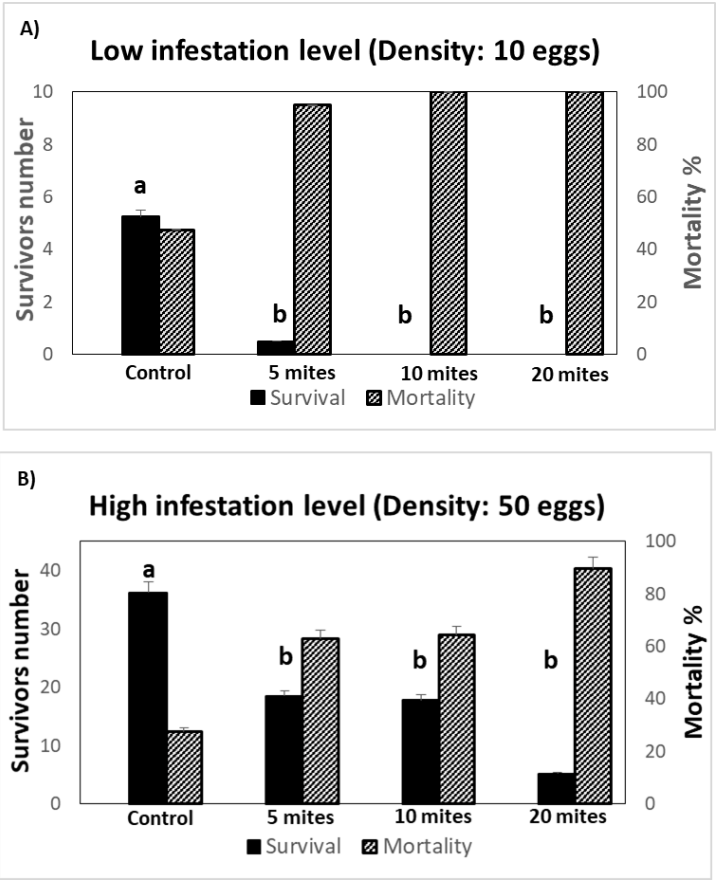

Figure 4. Mean values of surviving eggs (±SE) and mortality rate (±SE) at the infestation levels of 10 (**A**) and 50 (**B**) *T. solanivora* eggs, and doses of 0, 5, 10 and 20 mites. Different letters denote significant differences (Omnibus test, p = 0.05 (**A**); one-way ANOVA, Tukey test, p = 0.05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

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 **Fig. 4**.

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 (**Fig. 5**).

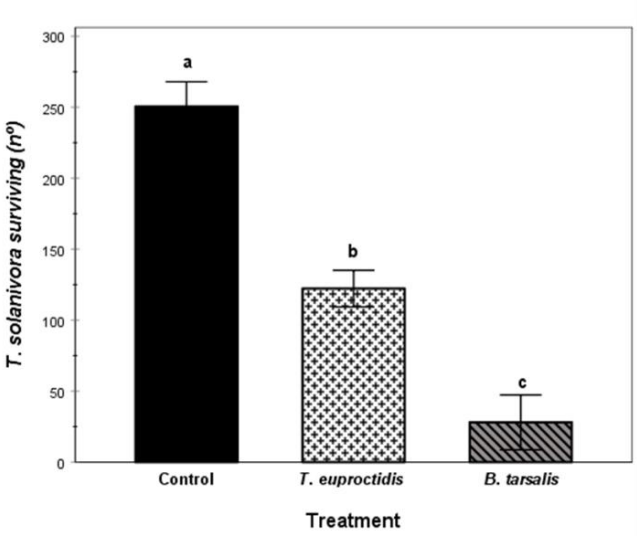

Figure 5. Mean number of surviving *T. solanivora* (±SE). Different letters indicate significant differences (one-way ANOVA, Tukey test, p = 0.05).

3.2.2. Tuber Damage

The number of mines per tuber showed the same pattern as the number of surviving *T. solanivora*. Both natural enemies, *B. tarsalis* and *T. euproctidis*, showed significant differences 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) (**Fig. 6A**).

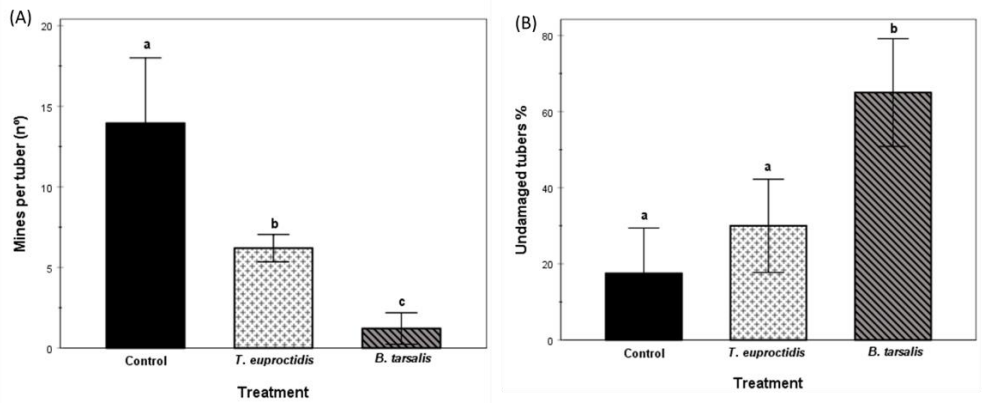

Figure 6. Mean number of mines per tuber (±SE) (**A**) and mean percentage of undamaged tubers (±SE) (**B**). Different letters indicate significant differences (one-way ANOVA, Tukey test, p = 0.05)

The analysis of undamaged tubers (**Fig. 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).

3.3. Storage

Statistical analyses showed that the mean number of mines per tuber (**Fig. 7A**) and the percentage of undamaged tubers (**Fig. 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).

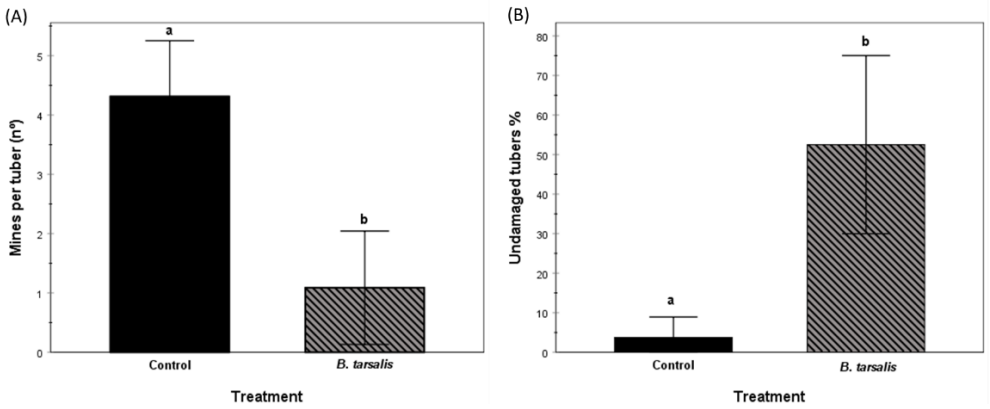

Figure 7. Mean number of mines per tuber (±SE) (**A**) and mean percentage of undamaged tubers (±SE) (**B**) of Control and Mite (*B. tarsalis*) treatment at storage conditions. Different letters denote significant differences (one-way ANOVA, Tukey-test, *p* = 0.05 and Omnibus test, *p* = 0.05; respectively).

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). 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 (Nielsen 1999 and 2001). 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. (2023), 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. (2022) applied the same experimental method to compare the efficacy of *Trichogramma cacoeciae* (Marchal 1927) and *B. tarsalis 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 (1998) 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 (Van Alebeek, 1996; Schöller et al. 2000). 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. (2023) 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 (Murali-

Baskaran et al, 2018). 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, **Fig. 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. (2022), 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 (Rall et al. 2022; Gavara et al. 2021, Schausberger 2021). 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 **Fig. 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 (Flinn et al. 2012). 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 (Mullen et al. 2012).

The choice of the storage system plays a crucial role in the use of natural enemies (Schöller et al. 2000). 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.

General discussion

General discussion

The present work aims to select new biocontrol agents as tools for integrated pest management of the Guatemalan potato tuber moth, *T. solanivora*. The potential application of the parasitoids *T. achaeae* and *T. euproctidis* under field and storage conditions is evaluated in **Chapter 1**. **Chapter 2**, studies the potential of natural enemies *B. tarsalis* and *T. euproctidis* under storage conditions. This chapter is divided into: **Chapter 2A**, laboratory analyses to determine the suitability of *B. tarsalis* as a biocontrol agent for storage conditions, and **Chapter 2B**, testing of the two natural enemies under semi-storage conditions, followed by the testing under storage conditions of the most effective of the two.

Tecia solanivora is an invasive pest whose management is very complex. Almost its entire biological cycle occurs under the soil and inside the tuber, which offers protection to the pest, and the adult stage is the only moment in which it is exposed (**Introduction: 3.2 Biology and Ecology**). When no management strategies are applied, harvest losses can reach around 50% in the field and 100% in warehouses (Trujillo and Perera 2011). Cultural measures aim to reduce the pest population in the field during the growing cycle to prevent harvest losses (**Introduction: 5.1 Cultural control measures**). These measures are combined with pheromone traps (**Introduction: 5.2 Biotechnical control measures**), which can significantly reduce harvest losses. Nevertheless, pest damage frequently causes unacceptable economic losses (Trujillo and Perera 2011). In addition, currently there is a lack of effective phytosanitary treatments to be used in the field or approved for storage (Trujillo and Perera 2017, MAPA 2023c). Given this situation, research into biological control agents has become particularly important. In this regard, the study of oophagous natural enemies, as is the case of *T. achaeae*, *T. euproctidis* and *B. tarsalis*, is of particular interest for the biological control of the Guatemalan potato moth, as they prevent egg hatching. Once the eggs hatch, the larvae migrate to the soil, making it difficult to remove them before they enter the tubers, where they are protected. Therefore, the use of oophagous biocontrol agents removes the pest before the potatoes are damaged.

In **Chapter 1**, an experimental methodology was applied to analyze and monitor the four basic steps in the parasitoid selection process: host habitat location, host location, host acceptance and host suitability (Vinson, 1976). As both *Trichogramma* species are obtained from local potato crops, it can be assumed that the habitat location is appropriate for field conditions. However, it would not be the same for storage conditions, where the parasitoids are exposed to an artificial environment.

For the next step, host acceptance, it should be taken into account that the presence of an alternative host of a target pest can reduce the efficacy of the parasitoid. In the Canary

Islands, mixed infestations of *T. solanivora* and the common potato tuber moth, *P. operculella*, are common. Hence, a host acceptance study was carried out using non-choice and choice assays of *T. achaeae* and *T. euproctidis* against eggs of the two potato moths. Our experiments showed that both parasitoids had a good acceptance on both moths, with a strong preference for *T. solanivora* eggs. Regarding *T. euproctidis*, it showed higher parasitism levels than *T. achaeae* against *T. solanivora*, *P. operculella* and a mixture of both moths (**Chapter 1, Fig. 3 and 4**). These results are consistent with previous observations pointing out that several factors (egg age, chorion thickness and hardness, etc.) are involved in the target preference of *Trichogramma* spp. This parasitoid usually selects the largest available host, as was the case in our study: about 0.53 × 0.41 mm for *T. solanivora* eggs compared to 0.50 × 0.35 mm for *P. operculella* eggs (Mackauer et al. 1993, Mansfield et al. 2004, Samara et al. 2011, Kroschel et al. 2013, Martínez et al. 2019, Myint et al. 2022). The preference results obtained are interesting because, under real conditions, *P. operculella* eggs are more accessible on leaves than *T. solanivora* eggs laid in the soil. Further studies under field conditions would be required to determine if the parasitoid shows a preference for larger eggs and thus might choose *T. solanivora* eggs.

Host suitability is concerned with biological factors related to the development of the parasitoid in the potential host, i.e. survival, parasitism rate, offspring sex ratio and emergence. The functional response is also a fundamental factor in understanding host interactions and is one of the main influences on the stability of the system. All these factors are strongly affected by temperature (Oaten and Murdoch 1975, Berryman 2009, Andrade et al. 2011, Samara et al. 2011, del Pino et al. 2020). For the study of suitability and functional responses, studies were performed at temperatures of 15, 20, 25 and 27°C. The test results revealed greater preliminary potential of *T. euproctidis* as a biocontrol agent than *T. achaeae,* as it obtained the highest egg parasitism capacity and maintained a type III functional response at all the temperatures tested (**Chapter 1: Fig. 7, Tab. 1**, respectively). *Trichogramma achaeae* maintained a type II response, except at 27°C, where it showed type III (**Chapter 1: Tab. 2**). Type III is considered the best behavior to obtain a successful biological control of pests. In theory, it is the only response that can achieve a direct density dependence at a low population host level and stabilize the host-parasitoid system (Rincón 1999). One possible cause for the observed parasitization behaviour of *T. euproctidis* in relation to *T. achaeae* is the lower egg manipulation times (Th), which ranged from 0.0372 to 0.0418 days for *T. euproctidis* compared to 0.0540 to 0.0845 days for *T. achaeae*. This behavior gives the parasitoid more time to seek and find more host eggs. In particular, handling time was previously identified as an important factor in parasitization by *Trichogramma* species (Pak et al. 1986).

Our results show that temperature does not impact fecundity or female offspring, with the only exception being *T. achaeae* at 27°C, where the number of females was higher.

Different conclusions on the effect of temperature have been mentioned in previous works (Andrade et al. 2011, Bari et al. 2015, del Pino et al. 2020, Tabebordbar et al., 2020), perhaps indicating a greater influence of the parasitoid-host species relationship than temperature. Both parameters, fecundity and sex ratio, exceeded the minimum values of 50% and 80% recommended by the IOBC for biological control parasitoids (**Chapter 1: Fig. 8 and Tab. 5**) (Van Lenteren et al. 2003).

Despite the better results of *T. euproctidis* for most of the biological parameters analyzed in the laboratory trials, this species did not reduce *T. solanivora* populations or prevent tuber damage under semi-field conditions. Nevertheless, *T. achaeae* reduced the pest population, reaching an efficacy of about 50% and yielding 20% of undamaged tubers (**Chapter 1: Figs. 10 and 11**). This low search ability of *T. euproctidis* in relation to *T. achaeae* could be explained by a low stimulus in response to the kairomones of *T. solanivora* eggs, as reported for other species and habitat conditions for this group of egg parasitoids (Murali-Baskaran et al. 2018). Thus, only *T. achaeae* has emerged as a potentially effective biocontrol agent for testing under field conditions. Higher densities of the parasitoid should be evaluated, and more releases should be taking place in order to improve its efficacy, especially at the beginning of the tuberization period.

In contrast to the semi-field conditions, the illumination assays showed the inability of *T. achaeae* to parasitize in the dark, compared to *T. euproctidis*, which maintained its parasitism capacity in both light and dark conditions (**Chapter 1: Fig. 9**). These results suggest that *T. achaeae* is not suitable for storage conditions, and that *T. euproctidis* has the potential to be evaluated under storage conditions.

Regarding the suitability of the mite *B. tarsalis*, it is known that temperature is one of the most important factors in the predator-prey relationship. It is particularly important to pay attention to storage conditions for the biological control of this pest, as it affects the biological efficacy of both organisms (Nielsen 1999, 2001; Rall et al., 2010) (**Chapter 2A: Introduction**). In our bioassays, *B. tarsalis* maintained its predatory activity on *T. solanivora* in the range of 10 to 27°C (**Chapter 2A: Tab. 1**). While the predatory mite significantly increased prey mortality between 20-25°C, there were no differences at the lowest temperatures tested (10-20°C), nor the highest (25-27°C) (**Chapter 2A: Fig. 2**)**.** The efficacy values obtained for *T. solanivora* were lower than those found in another previous study on *P. operculella* under the same experimental conditions. Specifically, we obtained an efficacy of about 34% on *T. solanivora* compared to the 50% found by Gavara et al. (2021) on *P. operculella* at 10°C. We also observed between 40 - 75% efficacy on *T. solanivora* compared to the 75 - 98% in the 20 - 30°C range for *P. operculella* found by Gallego et al. (2020b) and Gavara et al. (2021). These differences cannot be explained by prey preferences since the choice test did not show any preference in consumption between the two hosts (**Chapter 2A: Table 2**). Therefore, it could be caused by the difference in prey size. Given that it has

been observed that larger egg sizes favor partial consumption, the larger the size, the sooner the mite would be satisfied. Hence, the prey eggs would have a greater chance of survival, although other chemical and mechanical factors may be involved (Haines 1987; Gallego et al. 2020b). Our results support this theory, as we observed that the percentage of partially consumed eggs decreased and egg mortality increased at the highest temperatures tested (25 - 27°C) (**Chapter 2A: Fig. 2**). The predatory indifference shown by the mite between *T. solanivora* and *P. operculella* could be an advantage in mixed infestations where *B. tarsalis* will feed on both preys.

Another factor that may influence the outcome of biological control measures is the density of prey, as well as the interaction between the density of predators (conspecific interactions) and the density of available prey. *B. tarsalis* displayed a type II functional response, in accordance with previous studies on prey eggs of other insect species, such as *Lasioderma serricorne* (Coleoptera: Ptinidae), *Plodia interpunctella* (Lepidoptera: Pyralidae) and *P. operculella* (Riudavets et al. 2002; Gallego et al. 2020b). This means that the mite increases its predatory activity with the increase of available *T. solanivora* eggs up to a certain prey density level, at which point predatory efficacy stabilizes. Further increases in prey eggs do not improve predator activity (Mills 1982) (**Chapter 1: Fig. 4**). In our microcosm bioassays, no negative effect was observed on the predator efficacy as the number of released mites increased (**Chapter 2A: Fig. 3; Chapter 2B: Fig. 4**). At 25°C, the predatory mite showed similar efficacy to that revealed in previous studies on *P. operculella* (Gallego et al. 2020b, Gavara et al. 2021), with values between 92and 99%. These results are in contrast with those of the acceptance trials, where *B. tarsalis* achieved lower mortality rates on *T. solanivora* than on *P. operculella*. This could be partially explained by the longer duration of the egg stage on the common tuber moth, which means there is more time available for consumption by the mite (Martinelli 2004; Andreadis et al. 2017).

When low levels of *T. solanivora* egg infestation were tested, the results at 15°C (storage temperature in smallholder facilities) and 25°C gave 100% efficacy. However, at high levels of *T. solanivora* infestation, efficacy was lower, with values around 50 - 71% at 15°C compared to 67 - 98% at 25°C (at densities of 5, 10 and 20 mites, respectively). These results indicate that the predatory activity of *B. tarsalis* increases with temperature. This could be an advantage in non-refrigerated stores subject to changing temperatures, where pest control would be improved. Conversely, this predatory mite would not be suitable for cold stores, where temperatures are usually between 5 - 12°C depending on the end use of the potatoes, given the low efficacy observed at 10°C. This is in line with previous studies showing that *B. tarsalis* has very low activity and development at low temperatures (Nielsen 1999, 2001). Nevertheless, this predatory mite showed good potential for non-refrigerated facilities, such as those of small farmers in the Canary Islands, where the typical temperature is around 15 - 20°C.

For this reason, *B. tarsalis* was trialed under semi-storage conditions. *Trichogramma euproctidis* was also included because of its ability to parasitize in the dark and its good parasitization rate within this temperature range (**Chapter 1**). The results of this trial showed that the parasitoid reduced the survival of the Guatemalan potato moth and the number of mines in the tubers, but did not protect a significant number of tubers. In contrast, *B. tarsalis* produced a notable reduction in the *T. solanivora* population and yielded 65% of undamaged potatoes (**Chapter 2B: Fig. 5 and 6**). It should be noted that in all treatments, especially in the control set and that of *T. euproctidis*, most of the infested tubers were found in the middle and lower layers, in contact with the walls and at the bottoms of buckets. This finding is in line with the observations of Torres (1998) on the oviposition preferences of *T. solanivora* in storage conditions. Also, it suggests that *T. euproctidis* has a low detection ability of *T. solanivora* eggs or/and a poor ability to penetrate the gaps between tubers and reach the deeper layers, where adult moths usually lay most of their eggs. The potential role of poor kairomone detection by *T. euproctidis* on *T. solanivora* eggs under semi-field conditions has also been discussed. The same is likely to occur under storage conditions, but further studies would be required to confirm this. The low foraging efficiency shown by the parasitoid means that increasing the parasitoid release density would not significantly improve the results. For this reason, in the evaluation under storage conditions, *T. euproctidis* was discarded and only *B. tarsalis* was tested.

In the storage trial, *B. tarsalis* showed satisfactory efficacy, with a high reduction in tuber damage in comparison to the control set, protecting 50% of the infested potatoes (**Chapter 2B: Fig. 7**). This level of protection was consistently achieved throughout the volume of the plastic boxes used in the trial. This underlines the ability of the mite to move between tuber stacks and efficiently locate *T. solanivora* eggs. One critical aspect to consider is the timing of the release (Flinn et al. 2012). We consider that *B. tarsalis* should be introduced as soon as possible after harvest to prevent hatching of the moth eggs. Based on our results, mite efficacy would improve with higher release densities. Additional mite releases should also be investigated, especially for long-term storage. Furtermore, it is known that the type of storage system plays a crucial role in the use of natural enemies (Schöller and Flinn 2000). In our study, *B. tarsalis* was evaluated in storage boxes, but further trials should assess their performance when potatoes are stored in burlap bags, as is often the case in the Canaries.

In this study, three natural enemies were evaluated and assessed: two egg parasitoids, *T. achaeae* and *T. euproctidis*, and a predatory mite, *B. tarsalis*. Although *T. euproctidis* revealed good preliminary results in laboratory studies to be used under both field and warehouse conditions, semi-field and semi-storage trials indicate that it is not a good biocontrol agent due to its poor ability to locate *T. solanivora* eggs. In contrast, *T. achaeae*, although displaying lower efficacy than *T. euproctidis* under laboratory conditions, showed to be a promising control agent for field conditions. These results highlight the importance

of conducting assays with natural enemies under semi-field/semi-storage conditions before testing them in the field or in storage facilities. In fact, selection of biocontrol agents based on positive laboratory trial results alone could lead to incorrect decision-making and unnecessary expenditure of effort and resources. In the case of *B. tarsalis*, which has been shown to be a good biocontrol agent in experimental storage, there is a need for improvement of mite releases (density of mites per release and number of releases), and evaluations in other storage systems. Also, the economic viability of using and rearing this biocontrol agent should be assessed.

It is important to highlight that the use of biocontrol agents such as the parasitoid *T. achaeae* and the mite *B. tarsalis* are only two of the available tools that exist within the integrated pest management approach. Both natural enemies target moth eggs and cannot attack the pest once the larvae have hatched. Under field conditions, cultural measures and trapping should be maintained to reduce the population of adults and their eggs. Under storage conditions, visual inspection and removal of infested tubers before storage should be maintained.

General conclusions

- Under field conditions, although both parasitoids (*Trichogramma achae* and *Trichogramma euproctidis*) gave good results in laboratory tests, only *T. achaeae* showed promising results for protecting tubers under semi-field conditions, and therefore should be the only one that is tested under field conditions.
- Future field trials of *Trichogramma achaeae* will need to include higher densities and more releases, particularly at the start of the tuberization period, to ensure higher efficacy.
- Under storage conditions, *Trichogramma achaeae* was not suitable due to its inability to parasitize in dark conditions.
- *Trichogramma euproctidis* in the semi-storage trial showed poor searching ability to locate *Teci solanivora* eggs and protect tubers.
- *Blattisocius tarsalis* did not prove to be suitable for normal refrigerated storage conditions due to its very low efficacy in laboratory tests at low temperatures, but showed promising results for room temperature storage, as it was observed under semi-storage and experimental storage trials.

Considerations for future works

- For the use of *Blattisocius tarsalis* in real (room temperature) conditions, the release densities should be standardized and the number of releases should be tested, especially under long-term storage of tubers.
- The use of *Trichogramma achaeae* and *Blattisocius tarsalis* against *Tecia solanivora* could be complementary measures to improve pest control in the context of integrated pest management, but could not solve the problem alone. All of the various management measures should be maintained.
- Selection of biocontrol agents should be based on a comprehensive methodology of assays with natural enemies: laboratory, semi-field/semi-storage, and field/storage trials, to avoid incorrect decision-making and unnecessary expenditure of effort and resources.
- Before recommending new biologicontrol agents such as *Blattisocious tarsalis*, they must not only demonstrate their effectiviness, but should also prove the economic viability of their use and rearing.

Bibliography and legislation

Bibliography

Alphen J.J.M.; Jervis M.A. (1996). Foragin behavior. In: Springer, ed. *Insect Natural Enemies.* DOI: 10.1007/978-94-011-0013-7_1

Andrade, G.S.; Pratissoli, D.; Dalvi, L.P.; Desneux, N.; Dos Santos Junior, H.J. (2011). Performance of four *Trichogramma* species (Hymenoptera: *Trichogramma*tidae) as biocontrol agents of *Heliothis virescens* (Lepidoptera: Noctuidae) under various temperature regimes. *J. Pest Sci.* ; *84*: 313-320. DOI: 10.1007/s10340-011-0364-3

Andreadis, S.; Spanoudis, C.; Zakka, G.; Aslanidou, B.; Noukari, S.; Savopoulou-Soultani, M. (2017). Effect of temperature on rate of development, survival and adult longevity of *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Eur. J. Entomol.* , *114*, 35-41. DOI:10.14411/eje.2017.006

Arbogast, R.T.; Lecato, G.L.; Van Byrd, R. (1980). External Morphology of Some Eggs of Stored-Product Moths (Lepidoptera). *J. Insect Morphol. Embryol*, *9*: 165–167. DOI: 10.1016/0020-7322(80)90013-6

Arias, J.; Jaramillo, J.A.; Arévalo, E.; Muñoz, L.; Rocha, N.R. (1996). Evaluación de la incidencia y severidad del daño de la polilla gigante de la papa *Tecia solanivora* en el departamento de Antioquia. *Ing. Cienc.* Available online: https://repository.agrosavia.co/handle/20.500.12324/31921 (Accessed on 23 October 2023).

Babendreier, D.; Kuske, S.; Bigler, F. (2002). Parasitism of non-target butterflies by *Trichogramma brassicae* Bezdenko (Hymenoptera: *Trichogramma*tidae) under field cage and field conditions. *Biol. Control*, *26*: 139-145.

DOI: 10.1016/S1049-9644(02)00120-2

Báez, F.; Gallegos, P. (2011). Prospección y eficiencia de parasitoides nativos de las polillas de la papa, *Tecia solanivora* Povolný, *Symmetrischema tangolias* Gyen*, Phthorimarea operculla* Zeller . *Memorias 4to Congr Ecuatoriano la Papa*. Available online: https://repositorio.iniap.gob.ec/bitstream/41000/2847/1/iniapsc322pe.pdf (Accessed on 26 October 2023).

Bari, M.N.; Jahan, M.; Islam, K.S. (2015). Effects of Temperature on the Life Table Parameters of *Trichogramma zahiri* (Hymenoptera: *Trichogramma*tidae), an Egg Parasitoid of *Dicladispa armigera* (Chrysomelidae: Coleoptera). *Environ. Entomol, 44*: 368-378. DOI: 10.1093/ee/nvu028

Barragán, A.; Pollet, A.; Prado, M.; Lagnaoui, A.; Onore, G.; Aveiga, I.; Lery, X.; Zeddam J. (2004). La polilla guatemalteca *Tecia solanivora* (Povolný) (Lepidoptera: Gelechiidae) en Ecuador. Diagnóstico y perspectivas de manejo bajo un método de predicción. In: *Memorias II Taller Internacional de La Polilla Guatemalteca. Avances En La Investigación y Manejo Integrado de La Polilla Guatemalteca de La Papa Tecia solanivora,* 5-23.

Available online: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers11-03/010036182.pdf (Accessed on 10 October 2023).

Barreto, N., Espitia, E.; Galindo, R.; Gordo, E.; Cely, L.; Sánchez, J.; López-Ávila, A. (. 2004). Fluctuación de la población de *Tecia solanivora* Povolný, (Lepidoptera: Gelechiidae) en tres intervalos de altitud en Cundinamarca y Boyacá, Colombia. In: *Memorias II Taller Internacional de La Polilla Guatemalteca. Avances En La Investigación y Manejo Integrado de La Polilla Guatemalteca de La Papa Tecia solanivora,*: 25-43. Available online: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers11-03/010036182.pdf (Accessed on 10 October 2023).

Benítez, K.; Duarte, A. del P. (2016). Prospección de patógenos con potencial para el control biològico de la polilla guatemalteca de la papa (*Tecia solanivora*, FAM Gelechiidae). Bachelor's Thesis, Universidad Santo Tomás, Bogotá, Colombia. Available online: https://repository.usta.edu.co/handle/11634/2594 (Accessed on 25 October 2023).

Beramendi M. (2019). La UE certifica que Galicia está a un paso de erradicar la plaga de la patata. *La voz de Galícia*. Available online: https://www.lavozdegalicia.es/noticia/somosagro/agricultura/2019/01/09/ue-certifica-galicia-paso-erradicar-plaga-patata/0003_201901G9P31991.htm (Accessed on 10 October 2023).

Berryman, A.A. (2009). The theoretical foundations of biological control. In Theoretical Approaches to Biological Control; Cambridge University Press: Cambridge, UK, 3-21. DOI: https://doi.org/10.1017/CBO9780511542077.003

Blanco, C. (2013). Experiencias en el control de Batrocera oleae, Ceratitis capitata y otras plagas emergentes, en la zona mediterránea, mediante técnicas de "Atract and Kill." *Phytoma,* 254. Available online: https://www.phytoma.com/images/254_feromonas_plagas_emergentes.pdf (Accessed on 25 October 2023).

Blum, M.; Hilker, M. (2002). *Chemical protection of insect eggs. Chemoecology of Insect Eggs and Egg Deposition*; Hilker, M., Meiners, T., Eds.; Blackwell Publishing: Malden, MA, USA, 61-90.

Bosa, F.; Witzgall, P.; Cotes A.M.; Fukumoto, T.; Barreto, N. (2005). Evaluación de la técnica de la interrupción de la cópula de *Tecia solanivora* Povolný (Lepidoptera: Gelechiidae). *Rev Colomb Entomol*, 31(2):145-150. Available online: http://www.scielo.org.co/pdf/rcen/v31n2/v31n2a07.pdf (Accessed on 23 October 2023).

Caballero, P.; López-Ferber, M.; Williams, T. (2001). *Los Baculovirus y Sus Aplicaciones Como Bioinsecticidas. In: El Control Biológico de Plagas.* Phytoma-España and Universidad Pública de Navarra, España.

Cabello, T.; Gallego, J.; Vila, E., Soler, A.; Soler, A.; Carnero, A.; Hernández-Suárez, E.; Hernández-Suárez, Polaszek, A. (2009). Biological control of the South

American tomato pinworm, *Tuta absoluta* (Lep.: Gelechiidae), with releases of *Trichogramma achaeae* (Hym.: *Trichogramma*tidae. *IOBC/ Bulletin*, (49): 225-230. Available online: https://iobc-wprs.org/publication-category/iobc-wprs-bulletins/2009/ (Accessed on 26 October 2023).

Cabello, T.; Gamez, M. (2007). An improvement of the holling type III functional response in entomophagous species model. *J Biol Syst*, 15(4): 515-524. DOI: 10.1142/S0218339007002325

Cabello, T.; Gámez, M.; Torres, A; Garay, J. (2011) Possible effects of inter-specific competition on the coexistence of two parasitoid species: *Trichogramma brassicae* Bezdenko and *Chelonus oculator* (F.) (Hymenoptera: *Trichogrammatidae*, Braconidae). *Community Ecol*, 12(1): 78-88. DOI:10.1556/ComEc.12.2011.1.10

CABI (2019). *Trichogramma lopezandinensis* Compendium. Available online: https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.54739 (Accessed on 26 October 2023)

Cabrera, R.; Giménez, C.; Prendes, C.; Rincón, A.; Ríos, D.; Reina, M. (2002). Evaluación de la actividad de extractos de plantas de las Islas Canarias sobre *Tecia solanivora* (Povolny) (lep.: Gelechiidae). In: Memorias del II Taller Internacional de polilla guatemalteca. *Avances En La Investigación y Manejo Integrado de La Polilla Guatemalteca de La Papa Tecia solanivora.* Eds, Pollet, A.; Onore, G.; Chamorro, O.; Barrgán, A.; Pontificia Universidad Católica del Ecuador-Institut de Recherche pour le Dévelopement - Centro Internacional de la patata. Quito, Ecuador, 131-141. Available online: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers11-03/010036182.pdf (Accessed on 23 October 2023).

Camargo, C.; Rincón, D.; Valencia, E. (2010). Localización de hospedero por larvas neonatas de *Tecia solanivora* (Lepidoptera: Gelechiidae). *Corpoica Cienc y Tecnol Agropecu*, 11(1): 5-10. Available online: https://revistacta.agrosavia.co/index.php/revista/article/view/189 (Accessed on 10 October 2023).

Cañedo, V.; Carhuapoma, P.; Kroschel, J. (2016). *Copidosoma koehleri* (Blanchard 1940). In: *Pest Distribution and Risk Atlas for Africa. Potential Global and Regional Distribution and Abundance of Agricultural and Horticultural Pests and Associated Biocontrol Agents under Current and Future Climates,* 208-219. Available online: https://cipotato.org/riskatlasforafrica (Accessed on 25 October 2023).

Carnero, A.; Padilla, A.; Perera, S.; Hernández, E.; Trujillo, E. (2008). Pest status of *Tecia solanivora* (Povolný 1973) (Lepidoptera: Gelechiidae), Guatemalan Potato moth, in the Canary Islands. *Rev. Peru Entomol, 31*: 336–339. DOI: 10.2903/j.efsa.2018.5102

Carrillo, D.; Torrado-León, E. (2013). *Tecia solanivora* Povolný (Lepidoptera: Gelechiidae), an invasive pest of potatoes *Solanum tuberosum* L. in the Northern Andes. In: *Potencial Invasive Pest of Agricultural Crops*; Ed, Peña, J.E.; CABI: Boston, MA, USA, 126–136.

Casquet, J.; Thebaud, C.; Gillespie, R.G. (2012). Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Mol Ecol Resour.*, 12(1): 136-141. DOI:10.1111/J.1755-0998.2011.03073.X

Cely-Pardo, N.; Romero Barrera, Y.; Laso Paredes Z.; Barreta-Triana, N.;SantaCruz Guevara, A; Peña Puerto, Y. (2022). Busqueda preliminar de fuentes de resistencia a *T.solanivora* en la Colección Central Comolbinana de papa. *Rev.latinoamericana de la papa.26 (1):17-37k.* DOI: 10.37066/ralap.v26iN1.439

Cherif, A .; Mansour R.; Grissa-Lebdi, K. (2021). The egg parasitoids *Trichogramma*: from laboratory mass rearing to biological control of lepidopteran pests. *Biocontrol Sci Technol*, 31(7): 661-693. DOI: 10.1080/09583157.2020.1871469

Chesson J. (1983). The Estimation and Analysis of Preference and Its Relatioship to Foraging Models. *Ecology*, 64(5): 1297-1304. DOI: 10.2307/1937838

Chulde-La fuente R.I.; Gallegos, P.; Asaquibay, C. (2003). Control de la polilla de la papa *Tecia solanivora* en almacenamiento del tubérculo semilla mediante el uso de baculovirus y asolación. *Inf Anu Exp St Catalina, Dep Nac Protección Veg*, 62-67. Available online: http://181.112.143.123/bitstream/41000/2827/1/iniapsc322est.pdf (Accessed on 25 October 2023).

CIP (2001). Control biológico de la polilla con baculovirus-*Phthorimarea*. *Bol Capacit*, 5-6. Available online: https://books.google.es/books?id=yQ2ueDXKhhgC&printsec=frontcover&hl=ca&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false (Accessed on 25 October 2023).

Corral, S.; Romero, M.D.; Pereira, A. (2017). Assessing the complexity of the spreading processes of agricultural pests: The case of the Guatemalan potato moth in Tenerife. *Land Use Policy*, 69: 338–348. DOI: 10.1016/j.landusepol.2017.09.031

Cuartas P.; Villamizar, L., Espinel, C.; Cotes, A.M. (2009). Infección de granulovirus nativos sobre *Tecia solanivora* y *Phthorimaea operculella* (lepidoptera: Gelechiidae). *Rev Colomb Entomol*., 35(2): 122-129. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-04882009000200003 (Accessed on 25 October 2023).

Cuartas, P. (2016). Estudio del desarrollo de la infección y del efecto de la formulación de aislamiento de granulovirus sobre *Tecia solanivora* (Povolný) y

Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae). Bachelor's Thesis. Pontificia Universidad Javeriana. Bogotá, Colombia.

Cuartas, P.; Villamizar, L.; Espinel, C.; Cotes, A.M. (2009). Infección de granulovirus nativos sobre *Tecia solanivora* y *Phthorimaea operculella* (lepidoptera: Gelechiidae). *Rev Colomb Entomol*., 35(2): 122-129. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-04882009000200003 (Accessed on 25 October 2023).

del Pino, M., Hernández-Suárez, E.; Cabello, T.; Rugman-Jones, P.; Stouthamer, R.; Polaszek, A. (2013). *Trichogramma canariensis* (Insecta: Hymenoptera: *Trichogramma*tidae) a parasitoid of Eggs of the twinspot moth *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) in the Canary Islands. *Arthropod Syst Phylogeny*., 71(3): 169-179. DOI:10.1007/s10526-013-9519-x

del Pino, M.; Gallego, J.R.; Hernández-Suárez, E.; Cabello, T. (2020). Effect of temperature on life history and parasitization behavior of *Trichogramma achaeae* Nagaraja and Nagarkatti (Hym.: *Trichogramma*tidae). *Insects*, *11*: 482. DOI: 10.3390/insects11080482

del Pino, M.; Rugman-Jones, P.; Hernández-Suárez, E.; Polaszek, A.; Stouthamer, R. (2013). Rapid molecular identification of five species of *Trichogramma* occurring in the Canary Islands with notes on their distribution in banana groves. *BioControl, 58*: 515-524.

DOI: 10.1007/s10526-013-9519-x

Díaz, F. (2003). Evaluación del efecto del huevo huésped de cría en la biología del parasitoide *Trichogramma achaeae* (Hym.: *Trichogramma*tidae). Degree Final Project. University of Almería, Almería, Spain. Available online: https://repositorio.ual.es/ (Accessed on 29 October 2023).

EPPO. Data sheets on quarantine pests *Tecia solanivora*. EPPO. *Eur Mediterr Plant Prot Organ Bull OEPP/EPPO*.

Espinel, C.; Cotes A.M.; Villamizar L. (2008). Efecto de la infección con granulovirus en el desarrollo de *Tecia solanivora* (Lepidoptera: Gelechiidae). *Rev.Fac.Na.lAgr.Medellín*, 62(1): 4797-4805. Available online: https://repositorio.unal.edu.co/handle/unal/37055 (Accessed on 25 October 2023).

Espinel, C.; Cotes, M.; Villamizar, L. (2009). Efecto de la infección con granulovirus en el desarrollo de *Tecia solanivora* (Lepidoptera: Gelechiidae). *Rev Fac Nac Agron Medellín*, 62(1):4797-4805. Available online: https://repositorio.unal.edu.co/handle/unal/37055 (Accessed on 10 October 2023).

Fan X.; Maggiorani, A.; Gudiño, S. (2001). Uso de nemátodos entomopatógenos como una alternativa en el control de polilla *Tecia solanivora*, importante plaga de la papa (*Solanum tuberosum*). *Revevista For Venezolana*, 44(1): 115-118.

Felipe Bosa, O.; Pablo Osorio, M., Cotes A.M., Bengtsson, M.; Witzgall, P.; Fukumoto,T. (2008). Control de *Tecia solanivora* (Lepidoptera: Gelechiidae) mediante su feromona para la interrupción del apareamiento. *Rev Colomb Entomol*., 34(1):68-75. Available online: http://www.scielo.org.co/pdf/rcen/v34n1/v34n1a09.pdf (Accessed on 23 October 2023).

Fernández-Arhex, V.; Corley, J.C. (2003). The functional response of parasitoids and its implications for biological control. *Biocontrol Sci. Technol.*, *13*: 403-413. DOI: 10.1080/0958315031000104523

Ferragut, F.; Garcia-Marí, F.; Costa-Comelles, J.; Laborda, R. (1987). Influence of food and temperature on development and oviposition of *Euseius stipulatus* and *Typhlodromus phialatus* (Acari: Phytoseiidae). *Exp Appl Acarol*., 3(4):317-329. DOI:10.1007/BF01193168

Firake D.M.; Khan M.A. (2014). Alternating temperatures affect the performance of *Trichogramma* species. *J Insect Sci*. 14(41): 1-14. DOI:10.1673/031.014.41

Flinn, P.W., Scholler, M. (2012). Biological control: Insect pathogens, parasitoids, and predators. In: Stored Product Protection. Hagstrum, 501 D.W., Phillips, T.W., Cuperus, G., eds. S156. Manhattan, KS: Kansas State University, 203–212

Foerste, M.R.; Foerste L.A. (2009). Effects of temperature on the immature development and emergence of five species of *Trichogramma*. *BioControl*. 54(3): 445-450. DOI: 10.1007/s10526-008-9195-4

Gallego J.R.; Caicedo, O.; Gamez, M.; Hernandez, J.; Cabello, T. (2011). Selection of predatory mites for the biological control of potato tuber moth in stored potatos. Insects, 3: 196. DOI: 10.3390/insects11030196479

Gallego, J.R.; Mellado-López, L.; Cabello T. (2020a). Selección de una especie de *Trichogramma* (Hym., *Trichogramma*tidae) para el control biológico de la polilla de la patata *Phthorimaea operculella* (Lep., Gelechiidae) mediante el estudio del comportamiento de parasitación del huésped . *Inf Tec Econ Agrar*.,1-17. DOI: 12706/itea.2019.013

Gallego, J.R.; Caicedo, O.; Gamez, M.; Hernandez, J.; Cabello, T. (2020b). Selection of predatory mites for the biological control of potato tuber moth in stored potatoes. *Insects*, 11: 196. DOI: 10.3390/insects11030196

Gallego, J.R.; Gamez, M.; Cabello, T. (2020c). Potential of the *B. tarsalis mali* Mite (Acari: Blattisociidae) as a Biological Control Agent of Potato Tubermoth (Lepidoptera: Gelechiidae) in Stored Potatoes. *Potato Res.*, *63*: 241-251.

DOI: 10.1007/s11540-019-09438-9

Gallegos, P.; Asaquibay, C.; Cahmorro F.; Rodríguez, P.; Williams, R. (2005). Asolación de los tubérculos de semilla de papa como método de control para

la polilla, *Tecia solanivora*: Una tecnología tradicional para la solución a un nuevo problema. *Boletín INIAP (Inst Nac Investig Agropecu*., 260:5. Available online: http://repositorio.iniap.gob.ec/handle/41000/2613 (Accessed on 25 October 2023).

García-Martín, M.; Gámez, M.; Torres-Ruiz, A.; Cabello, T. (2008). Functional response of Chelonus oculator (Hymenoptera: Braconidae) to temperature, and its consequences to parasitism. Community Ecol., 9: 45–51. DOI::10.1556/COMEC.9.2008.1.6

Gavara, J.; Piedra-Buena, A.; Hernandez-Suarez, E.; Gamez, M.; Cabello, T.; Gallego, J.R. (2021). Potential for the Postharvest Biological Control of *Phthorimaea operculella* (Lepidoptera, Gelechiidae) by *B. tarsalis* (Mesostigmata, Blattisociidae). *Agronomy*, 11: 288. DOI: https://doi.org/10.3390/agronomy11020288

Gavara, J.; Cabello, T.; Gallego, J.R.; Hernández-Suárez, E.; Piedra-Buena Díaz, A. (2022). Evaluation of the Egg Predator *B. tarsalis* (Mesostigmata: Blattisociidae) for the Biological Control of the Potato Tuber Moth *Tecia solanivora* under Storage Conditions. *Agriculture*, *12*: 920. DOI: 10.3390/agriculture12070920

Gavara, J.; Suárez-Hernández, E.; Piedra-Buena, A. (2023a). *Gestion Integrada de La Polilla Guatemalteca de La Papa (Tecia solanivora)*. *Instituto Canario de Investigaciones Agraias (ICIA)*. Available online: https://www.icia.es/icia/download/publicaciones/Tecia.pdf (Accessed on 25 October 2023).

Gavara, J.; Cabello, T.; Gámez, M.; Bastin, S.; Hernández-suárez, E.; Piedra-Buena A. (2023b). Evaluation and Selection of New *Trichogramma* spp as (*Tecia solanivora*) in Europe. Insects, 14 (679): 107–19. DOI: 10.3390/insects14080679

Germain, J.F.; Povolný, D. (2006). Diagnostic Protocols. *Tecia solanivora* PM7/72(1). *EPPO Bull.,* 36(1):175-178. DOI:10.1111/j.1365-2338.2006.00932.x

Ghoneim, K. (2010). Parasitic insects and mites as potential biocontrol agents for a devastative pest of tomato, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the world: A review. *J. Adv. Res.*, *2*: 81-115.

Gómez, J.; Villamizar, L.; Espinel, C.; Cotes A.M. (2009). Comparación de la eficacia y la productividad de tres granulovirus nativos sobre larvas de *Tecia solanivora* (Povolný) (Lepidoptera: Gelechiidae). *Corpoica Cienc y Tecnol Agropecu*., 10(2): 152. DOI:10.21930/rcta.vol10_num2_art:137

Gómez-Bonilla, Y.; López-Ferber, M.; Caballero, P.; Murillo, R.; Muñoz, D. (2013). Granulovirus formulations efficiently protect stored and field potatoes from *Phthorimaea operculella* and *Tecia solanivora* in Costa Rica. *BioControl*., *58*: 215-224. DOI: 10.1007/s10526-012-9473-z

Gómez-Bonilla, Y.; López-Ferber, M.; Caballero, P-; Murillo, R.; Muñoz D. (2013). Granulovirus formulations efficiently protect stored and field potatos from *Phthorimaea operculella* and *Tecia solanivora* in Costa Rica. BioControl., 58 (2): 215-224. 460

Guerra-Luran, E.; Acero-Godoy J. (2021). Principales microorganismos entomopatógenos de *Tecia solanivora* y sus mecanismos de acción. *Rev Tecnol en Marcha*, 34:137-146. DOI: 10.18845/tm.v34i2.4981

Guzmán C. (2014). Cambio climático y control biológico de plagas: efecto de las condiciones abióticas en las interacciones entre enemigos naturales presentes en el agro-ecosistema del aguacate. Doctoral Thesis. Universidad de Málaga, Spain. Available online: http://hdl.handle.net/10630/6927 (Accessed on 29 October 2023).

Haines, C.P. (1987). Laboratory studies on the role of an egg predator, *B. tarsalis* (Berlese) (Acari: Ascidae), in relation to the natural control of *Ephestia Cautella* (Walker) (Lepidoptera: Pyralidae) in Warehouses. *Bull. Entomol. Res.*, 71: 557574. DOI:10.1017/S0007485300010075

Hassan S.A. (1989). Selection of suitable *Trichogramma* strains to control the codling moth *Cydia pomonella* and the two summer fruit tortrix moths *Adoxophyes orana*, *Pandemis heparana* [Lep.: Tortricidae]. *Entomophaga*, 34(1): 19-27. DOI:10.1007/BF02372583

Hassan, S.A. (1993). The mass rearing and utilization of *Trichogramma* to control lepidopterans pests: Achievements and outlook. *Pestic. Sci.*, *37*: 387-391.

DOI: 10.1002/ps.2780370412

Hassell, M.P. (1978). *Arthropod Predator-Prey System*; Princeton University Press: Princeton, NJ, USA.

Hassell, M.P. (2000). *The Spatial and Temporal Dynamics of Host-Parasitoid Interactions*. Eds, May, Robert M. and Harvey, Paul H.; Oxford University Press: Oxford, UK.

Hayden, J.E.; Lee, S.; Passoa, S.C.; Young, J.; Landry, J.-F.; Nazari V.; Mally R.; Somma L. A.; Ahlmark, K.M. (2013). Digital Identification of Microlepidoptera on Solanaceae, USDA-APHIS-PPQ Identification Technology Program (ITP). Fort Collins, CO. Available online: https://idtools.org/microlep_solan/ (Accessed on 10 October 2023).

Hernández-Fernández, J.; López-Pazos, S. (2011). *Bacillus Thuringiensis*: Soil microbial insecticide, diversity and their relationship with the entomopathogenic activity. In: *Soil Microbes and Environmental Health*. Ed., Miransari M.; Nova Science Publishers, Inc. Hauppauge, United States, 59-80.

Herrera, F. (1998). La Polilla Guatemalteca de La Papa. Biología, Comportamiento y Practicas de Manejo Integrado. Available

online: https://es.scribd.com/document/22245296/La-polilla-guatemalteca-de-la-papa-Biologia-comportamiento-y-practicas-de-manejo-integrado (Accessed on 10 October 2023).

Hilje L. (1994). Characterization of the damage by the potato moths *Tecia solanivora* and *Phthorimaea operculella* (Lepidoptera: Gelechiidae) in Cartago, Costa Rica. *Manejo Integr Plagas*., 31:43-46. Available online: https://www.cabdirect.org/cabdirect/abstract/19951108446 (Accessed on 10 October 2023).

Holling, C.S. (1959). The Components of Predation as Revealed by a Study of Small-Mammal Predation of the European Pine Sawfly. *Can. Entomol.*, *91*: 293-320. DOI:10.4039/Ent91293-5

ISTAC IC de estadística. Estadística Agraria de Canarias / Series anuales de agricultura. Municipios, islas y provincias de Canarias. 1999-2017. Available online: Sector primario (gobiernodecanarias.org) (Accessed on 10 October 2023).

Jiménez, G.; Poveda K. (2009).Synergistic effects of repellents and attractants in potato tuber moth control. *Basic Appl Ecol*., 10(8): 763-769. DOI:10.1016/j.baae.2009.06.009.

Juliano, S.A. (2001). Nonlinear Curve Fitting: Predation and Functional Response Curves. In: *Design and Analysis of Ecological Experiments*; Eds, Scheiner, S., Gurevitch, J.; Oxford University Press: Oxford, UK, 178–196.

Karlsson M.F.; Proffit M.; Birgersson G. (2017). Host-plant location by the Guatemalan potato moth *Tecia solanivora* is assisted by floral volatiles. *Chemoecology,* 27(5):187-198.DOI:10.1007/s00049-017-0244-2

Keasar, T.; Sadeh, A. (2007). The parasitoid *Copidosoma koehleri* provides limited control of the potato tuber moth, *Phthorimaea operculella*, in stored potatoes. *Biol. Control*.*42*: 55-60. DOI:10.1016/J.BIOCONTROL.2007.03.012

Knutson A. (1998). The *Trichogramma* Manual: A Guide to the Use of *Trichogramma* for Biological Control with Special Reference to Augmentative Releases for Control of Bollworm and Budworm in Cotton. Texas Agricultural Extension Service, Texas A&M University System.

Available online: https://hdl.handle.net/1969.1/160453 (Accessed on 25 Oct 2023).

Kroschel, J.; Schaub, B. (2013). Biology and ecology of potato tuber moths as major pests of potato. In Insect Pests of Potato: Global Perspectives on Biology and Management. Eds, Vincent, P. and Alyokhin, C.A.; Elsevier: Amsterdam, The Netherlands, 165-192. DOI:10.1016/B978-0-12-386895-4.00006-5

Kroschel, J.; Zegarra O. (2010). Attract-and-kill: A new strategy for the management of the potato tuber moths *Phthorimaea operculella* (Zeller) and *Symmetrischema tangolias* (Gyen) in potato: Laboratory experiments towards

optimising pheromone and insecticide concentration. *Pest Manag Sci.,* 66(5): 490-496. DOI:10.1002/ps.1898

Kučerová, Z.; Stejskal, V. (2010). External egg morphology of two stored-product anobiids, *Stegobium paniceum* and *Lasioderma serricorne* (Coleoptera: Anobiidae). *J. Stored Prod. Res.*, *46*: 202–205. DOI: 10.1016/j.jspr.2010.05.002

Lobo, M.G.; González-García, C.; Cabrera, R.; Ríos, D. (2021); Development of a Quarantine Postharvest Treatment against Guatemalan Potato Moth (*Tecia solanivora* Povolný). *Agriculture*, 11(8): 801. DOI:10.3390/agriculture11080801

López-Pazos S.A.; Rojas Arias A.C.; Ospina S.A; Cerón, J. (2010). Activity of *Bacillus thuringiensis* hybrid protein against a lepidopteran and a coleopteran pest. *FEMS Microbiol Lett*., 302(2): 93-98. DOI:10.1111/j.1574-6968.2009.01821.x

Mackauer, M.; Sequeira, R. (1993). Patterns of development in insect parasite. In *Parasites and Pathogens of Insects*; Academic: New York, NY, USA, 1-23.

Manly, B.F.J.; Miller, P.; Cook, L.M. (1972) Analysis of a Selective Predation Experiment. *Am. Nat.,* 106: 719–736. DOI:10.1086/282808

Manohar, T.N.; Sharma, P.L.; Verma, S.C.; Sharma, K.C.; Chandel, R.S. (2020) Functional response of indigenous *Trichogramma* spp. to invasive tomato leafminer, *Tuta absoluta* (Meyrick) under laboratory conditions. *Int. J. Trop. Insect Sci.*, *40*: 101-107. DOI: 10.1007/S42690-019-00057-Y

Mansfield, S.; Mills, N.J. (2004). Host Selection by Insect Parasitoids. A comparison of methodologies for the assessment of host preference of the gregarious egg parasitoid *Trichogramma platneri*. *Biol. Control*, *29*: 332-340.

DOI: 10.1016/j.biocontrol.2003.09.004

Marínez, P.; Martínez, W. (2009). Toxicidad biológica de cepas nativas de *Bacillus thuringensis* Berliner en larvas de *Tecia solanivora* Povolný. *Cienc y Agric*., 7(2): 7-16. Available online: https://dialnet.unirioja.es/servlet/articulo?codigo=4986452 (Accessed on 25 October 2023).

Martinelli C. (2004). Resultados de investigación acerca de la biología de *Tecia solanivora* Povolný. *Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife*.

Martínez, C.; Soria, C.A.; León, V.R. (2019). Morfología comparativa del corión del huevo de *Symmetrischema tangolias* (Gyen 1913) y *Tecia solanivora* (Povolný 1973), polillas del cultivo de *Solanum tuberosum*. *Rev. Ecuat. Med. Cienc. Biol.*, *40*: 11–22. Available online: https://dialnet.unirioja.es/servlet/articulo?codigo=7189742 (Accessed on 26 October 2023).

Mills, N.J. (1982). Satiation and the functional response: A test of a new model. *Ecol. Entomol.*, *7*, 305-315. DOI: 10.1111/j.1365-2311.1982.tb00671.x

Mills, N.J.; Lacan, I. (2004). Ratio dependence in the functional response of insect parasitoids: Evidence from *Trichogramma minutum* foraging for eggs in small host patches. *Ecol. Entomol, 29*, 208-216. DOI: 10.1111/j.0307-6946.2004.00584.x

Ministerio de Agricultura, Pesca y Alimentación. (2015). Sanidad vegetal. Guía de Gestión Integrada de Plagas. Patata. Available online: https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/guiadepatata_tcm30-57961.pdf (Accessed on 7 February 2024).

Ministerio de Agricultura, Pesca y Alimentación. (2022). Anuario de estadísticas. Tubérculos para el consumo humana- papa. Análisis provincial de superficie y producción según épocas de recolección. Available online: 2 (Accessed on 10 October 2023).

Ministerio de Agricultura, Pesca y Alimentación. (2024a). Sanidad vegetal. Organismos nocivos. *Tecia solanivora*. Available online: https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/organismos-nocivos/tecia-solanivora/ (Accessed on 7 February 2024).

Ministerio de Agricultura, Pesca y Alimentación. (2024b). Sanidad vegetal. Registro de productos fitosanitarios. Available online: https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/productos-fitosanitarios/registro/menu.asp (Accessed on 7 February 2024).

Motulsky, H.; Christopoulos, A. (2003). *Fitting Models to Biological Data Using Linear and Nonlinear Regression. A Practical Guide to Curve Fitting*; GraphPad Prism Softw Inc.: San Diego, CA, USA. Available online: https://www.facm.ucl.ac.be/cooperation/Vietnam/WBI-Vietnam-October-2011/Modelling/RegressionBook.pdf (Accessed on 26 October 2023).

Mullen, G.R.; Oconnor, B.M. Mites (Acari). In *Medical and Veterinary Entomology*; Mullen, G.R., Durden, L., Eds.; Academic Press: Cambridge, MA, USA, 2002. DOI: 10.1016/B978-012510451-7/50025-6

Murali-Baskaran, R.K.; Chander-Sharma, K.; Kaushal, P.; Kumar, J.; Parthiban, P.; Senthil-Nathan, S.; Mankin, R.W. (2018). Role of kairomone in biological control of crop pests: A review. *Physiol. Mol. Plant Pathol.*, *101*: 15–93. DOI: 10.1016/j.pmpp.2017.07.004

Myint Y.Y.; Bai, S.; Zhang, T.; Babendreier, D.; He, K.; Wang, Z. (2022). Oviposition preference of *Trichogramma dendrolimi* and *Trichogramma ostriniae* strains from Myanmar on different host egg ages of Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae). *Biocontrol Sci Technol*., 32(6): 700-714. DOI:10.1080/09583157.2022.2044015

NAS (Nacional Academy of Science). (1978). Resistencia de las plantas y animales. In: *Manejo y Control de Plagas de Insectos, Control de Plagas de Plantas y Animales*.,: 91- 125.

Nielsen P.S. (1999). The impact of temperature on activity and consumption rate of moth eggs by *Blattisocius tarsalis* (Acari: Ascidae). *Exp Appl Acarol*., 23(2):149157. DOI:10.1023/A:1006054627912

Nielsen, P.S. (2001). Developmental time of *B. tarsalis* (Acari: Ascidae) at different temperatures. *Exp Appl Acarol.,* 25(7): 605-608. DOI:10.1023/A:1014736526463

Niño L. (2004). Revisión sobre la polilla de la papa *Tecia solanivora* en Centro y Suramérica. *Supl Rev Latinoam la Papa*,18.

Notz, A. (1996). Influencia de la temperatura sobre la biología de *Tecia solanivora* (Povolný) (Lepidoptera: Gelechiidae) criadas en tubérculos de papa *Solanum tuberosum* L. *Boletín Entomol Venez*., 11:49-54.

Oaten, A.; Murdoch, W.W. (1975). Functional Response and Stability in Predator-Prey Systems. *Am. Nat.*, *109*: 289-298. DOI: 10.1086/282998

Osorio, P.; Espitia, E.; Luque, E. (2001). Reconocimiento de enemigos naturales de *Tecia solanivora* (Lepidoptera:Gelechiidae) en localidades productoras de papa en Colombia. *Rev. Colomb. Entomol, 27*: 177-185. DOI: 10.25100/socolen.v27i2.9685

Pak, G.A.; Buis, H.C.E.M.; Heck, C.C.; Hermans, M.L.G. (1986). Behavioural variations among strains of *Trichogramma* spp.: Host-age selection. *Entomol. Exp. Appl.,* 40: 247-258. DOI: 10.1111/j.1570-7458.1986.tb00508.x

Perera, S.; Cubas, F.; Padilla, Á.; Carnero, A.; Hernández, J.; Asensio, M. (2009). Ensayo de eficacia en el control de la polilla guatemalteca de la papa (*Tecia solanivora*) mediante la aplicación en campo de nemátodos entomopatógenos. *Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife*. Available online: https://www.icia.es/icia/download/pvegetal/Ensayo_control_%20polilla.pdf (Accessed on 25 October 2023).

Perera, S.; Trujillo, E.; Lobo, G.; Ríos, D.; Bentabol A. (2011). Evaluación de la aplicación de atmósfera controlada para el sanemiento de tubérculos de papas afectadas por la polilla gutemalteca de la papa (*Tecia solanivora*). *Inf Cabil Insul Tenerife*., 14. Available online: https://www.agrocabildo.org/publica/publicaciones/papa_371_atmosferacontrolada.pdf (Accessed on 25 October 2023).

Piedra-Buena A.; Gavara, J.; Bastin, S.; Martín-Toledo, T.; Hernández-Suárez E.; Cabello, T. (2019). Resultados de las prospecciones para la búsqueda de nuevos enemigos naturales de las polillas de la papa en Canarias. In: *Memorias IV Taller Internacional de la Polilla Guatemalteca,* 81-89. Available online: https://www.agrocabildo.org/publica/Publicaciones/papa_709_Libro%20de%2

0resumenes%20del%20IV%20Taller%20Internacional%20de%20Tecia%20solanivora.pdf (Accessed on 25 October 2023).

Pinhero R.G.; Coffin, R.; Y Yada, R. (2009). Post-Harvest Storage of Potatoes. In: Advances in Potato Chemistry and Technology. Eds, Singh, J.; Kaur, L., Academic Press. DOI: 10.1016/b978-0-12-374349-7.00012-x

Polaszek, A.; Rugman-Jones, P.F.; Stouthamer, R.; Hernández-Suárez, E.; Cabello, T.; Del Pino, M. (2012). Molecular and morphological diagnoses of five species of *Trichogramma*: Biological control agents of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae) in the Canary Islands. *BioControl*, *51*: 21–35. DOI: 10.1007/s10526-011-9361-y

Povolný, D. (1973). *Scrobipalpopsis solanivora* sp. a new pest of potato (*Solanum tuberosum*) from Central America. *Acta Univ Agric Fac Agron*, 21(1):133-146.

Press release. (2021) Preocupación entre los productores de papas en Tenerife por la polilla guatemalteca, que temen pérdidas del 50% en las cosechas. *Eldiario.es*. Available online: https://www.eldiario.es/canariasahora/tenerifeahora/economia/preocupacion-productores-papas-tenerife-polilla-guatemalteca-producir-perdidas-50-cosecha_1_7047401.html (Accessed on 25 October 2023).

Puillandre, N.; Dupas, S.; Dangles, O. (2008). Genetic bottleneck in invasive species: The potato tuber moth adds to the list. *Biol. Invasions*, 10: 3 19–333. DOI: 10.1007/s10530-007-9132-y

Rall, B.C.; Vucic-Pestic, O.; Ehnes, R.B.; Emmerson, M.; Brose, U. (2010). Temperature, predator-prey interaction strength and population stability. *Glob. Change Biol., 16*: 2145-2157. DOI: 10.1111/j.1365-2486.2009.02124.x

Ramírez, J.E.; Gómez, M.I.; Cotes, J.M.; Ñústez, C.E. (2010). Efecto insecticida de los aceites esenciales de algunas lamiáceas sobre *Tecia solanivora* Povolný en condiciones de laboratorio. *Agron Colomb*., 28(2):255-263. Available online: https://repositorio.unal.edu.co/handle/unal/31503 (Accessed on 23 Oct 2023).

Reay-Jones, F.P.F.; Rochat, J.; Goebel, R.; Tabone, E. Functional response of *Trichogramma chilonis* to *Galleria mellonella* and *Chilo sacchariphagus* eggs. *Int. J. Entomol. Exp. Appl.* 2006; *40*: 229-236. DOI:10.1007/S42690-019-00057-Y

Richards, O.W.; Thomson, W.S. (1932). A contribution to the study of the genera *Ephestia*, gn. (including strymax, dyar), and *Plodia*, gn. (lepidoptera, phycitidae), with notes on parasites of the larvae. *Transactions of the Royal Entomological Society of London*, 80 (2): 169-247. DOI: 10.1111/J.1365-2311.1932.TB03306.X

Ricón, A. (2002). Cría en Laboratorio de *Copidosoma* sp. Para el Control de la Polilla de la Papa. Bachelor's Thesis, Universidad de La Laguna, Canary Islands, Spain.

Rincón, C. (1999). Estudios Biológicos del Parasitoide *Trichogramma lopezandinensis* Sarmiento, Hymmenoptera *Trichogramma*tidae, Orientados al Control de la Polilla Guatemalteca de la Papa *Tecia solanivora*. Bachelor's Thesis, Universidad Nacional de Colombia, Bogotà, Colombia, Available online: https://repository.agrosavia.co/handle/20.500.12324/16223 (Accessed on 26 October 2023).

Rincón, D.F.; López-Avila, A. (2004). Dimorfismo sexual en pupas de *Tecia solanivora* (Povolný) (Lepidoptera: Gelechiidae). *Artic técnico, CORPOICA*, 5:41-42. Available online: https://revistacta.agrosavia.co/index.php/revista/article/view/20 (Accessed on 10 October 2023).

Rincón, D.F.; García, J. (2007). Frecuencia de cópula de la polilla guatemalteca de la papa *Tecia solanivora* (Lepidoptera: Gelechiidae). Revista Colombiana de Entomologia, 33(2), 133140.

Ríos D. (2012a). Las Papas Antiguas de Tenerife. Introducción al cultivo y principales variedades. *Centro Conservación de la Biodiversidad Agrícola de Tenerife. Servicio Técnico de Agricultura y Desarrollo Rural. Cabildo de Tenerife*. ISBN 978-84-15012-01-6 Available online:https://www.agrocabildo.org/publicaciones_detalle.asp?id=623 (Accessed on 20 February 2024).

Ríos D. (2012b). Las papas antiguas de Canarias: origen y diversidad. In Agricultura en Canarias: conciliando tradición y ciència. Ed Afonso-Carrillo, J. Actas VII Semana CientíficaTelesforo Bravo. Instituto de Estudios Hispánicos de Canarias. Puerto de la Cruz, Tenerife. pp. 11-41. ISBN: 978-84-616-0641-2.

Ríos, D. (2012c). La Polilla Guatemalteca de La Papa (*Tecia solanivora*) En Tenerife. *Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife*. Available online: https://www.agrocabildo.org/publica/Publicaciones/papa_482_polilla_guatemalteca_red.pdf (Accessed on 10 October 2023).

Riudavets, J.; Maya, M.; Monserrat M. (2002). Predation by *B. tarsalis* (Acari: Ascidae) on stored product pests. *IOBC WPRS Bull.,* 121–126.

Riudavets, J.; Quero, R. (2002). Prey preference of the predatory mite *B. tarsalis* (Acari: Ascidae). In *Advances in Stored Product Protection*; Eds, Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E.; CABI Publishing: Wallingford, UK, 297-299.

Robertson, J.L.; Russell, R.M.; Preisler, H.K.N.E.S. 2009. Pesticide Bioassays with Arthropods. Ed., Bruck D., CRC Press.

Roblero, E.N.C.; Vera, A.C.; Malo, E.A. (2011). First report of *Tecia solanivora* (Lepidoptera: Gelechiidae) attacking the potato *solanum tuberosum* in Mexico. *Florida Entomol*., 94(4):1055-1056. DOI: 10.1653/024.094.0445

Rojas-Arias, A.C.; López Pazos, S.A.; Chaparro-Giraldo, A. (2013). Actividad biológica de *Bacillus thuringiensis* sobre la polilla guatemalteca de la papa, *Tecia solanivora* Povolný (Lepidoptera: Gelechiidae). *Rev. Mutis.*, *3*: 31-42. Available online: https://revistas.utadeo.edu.co/index.php/mutis/article/view/883 (Accessed on 25 October 2023).

Rubio, S.A.; Vargas, B.I.; López-Ávila, A. (2004). Evaluation of the efficiency of *Trichogramma lopezandinensis* (Hymenoptera: Irichogrammatidae) to control *Tecia solanivora* (Lepidoptera: Gelechiidae) in storage potato. *Rev. Colomb. Entomol.*, *30*: 107-114. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-04882004000100016 (Accessed on 25 October 2023).

Sáenz, A. (2003). Eficacia de invasión de *Tecia solanivora* y *Clavipalpus ursinus* por el nematodo *Steinernema feltiae*. *Manejo Integr Plagas y Agroecol*, 67:35-43.

Salazar, C.G.; Betancourth, C.G. (2009). Evaluación de extractos de plantas para el manejo de polilla guatemalteca (*Tecia solanivora*) en cultivos de papa en Nariño, Colombia. *Agron Colomb.*, 27(2):219-226. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-99652009000200010 (Accessed on 23 October 2023).

Samara, R.; Monje, J.C.; Qubbaj, T.; Zebitz, C.P.W. (2011). Studies on host preference and oviposition behaviour of *Trichogramma aurosum* Sugonjaev and Sorokina strains in choice and non-choice tests. *Arab. J. Plant Prot., 29*: 259-266.

Sánchez-Aguirre, R.; Palacios, M.L. (1995). Eficacia del parasitismo de *Copidosoma koehleri* en el complejo polilla de la papa. *Rev Peru Entomol*., 38: 59-62. Available online: https://www.revperuentomol.com.pe/index.php/rev-peru-entomol/article/view/28 (Accessed on 26 October 2023).

Sánchez-León, G.; Cely-Pardo, N.; Barreta-Triana, Sotelo-Cardona, P. (2021). Parámetros reproductivos de *Tecia solanivora* (Povolný, 1973) (Lepidoptera: Gelechiidae) en genotipos de papa (*Solanum tuberosum* L.). *Rev.latinoamericana de la papa.* 25 (1): 71-90. DOI: 10.37066/ralap.v25i1.420

Schäfer, L.; Herz A. (2020). Suitability of european *Trichogramma* species as biocontrol agents against the tomato leaf miner *Tuta absoluta*. *Insects*, 11(6): 1-18. DOI: 10.3390/insects11060357

Schaub, B.; Carhuapoma, P.; Kroschel, J. (2021). Guatemalan potato tuber moth, *Tecia solanivora* (Povolný 1973). In: Pest Distribution and Risk Atlas for Africa, 24-38.

Schausberger, P.; Walzer, A. (2021). Combined versus single species release of predaceous mites: Predator-predator interactions and pest suppression., Biol Control., 20 (3):269–278. DOI: 10.1006/bcon.2000.0908 500

Schöller, M.; Flinn, E. (2000). Parasitoids and predators. In: Alternatives to Pesticides in Stored-Product IPM. Heaps JW, ed. Springer. DOI: 10.1007/978-1-4615-4353-4 472

Schöller, M.; Flinn, P.; Grieshop, M.; Žd'árková, E. (2006). Biological Control of Stored-Product Pests. In: Insect Management for Food Storage and Processing. Ed, Heaps J.W.; AACC International Press. DOI: 10.1016/B978-1-891127-46-5.50014-3 462

Segoli, M.; Bouskila, A.; Harari, A.R.; Keasar, T. (2009). Developmental patterns in the polyembryonic parasitoid wasp *Copidosoma koehleri*. *Arthropod Struct Dev*., 38(1): 84-90. DOI:10.1016/j.asd.2008.05.003

Smith, S.M. (1996). Biological control with *Trichogramma*: Advances, successes, and potential of their use. *Annu Rev Entomol*., 41(1): 375-406. DOI: 10.1146/ANNUREV.EN.41.010196.002111

Solà M. (2017). Approaches for the biological control of stored product pests. Doctoral thesis. Universitat Autònoma de Barcelona, Barcelona, Spain. Available online: https://www.tdx.cat/bitstream/handle/10803/461300/msc1de1.pdf?sequence=1&isAllowed=y (Accessed on 14 November 2023).

Solano-Rojas, Y.; Gallego, J.R.; Gámez, M.; Garay J.; Hernández J.; Cabello, T. (2022). Evaluation of *Trichogramma cacaeciae* (Hymenoptera: *Trichogramma*tidae) and *B. tarsalis mali* (Mesostigmata: Blattisociidae) in the Post-Harvest Biological Control of the Potato Tuber Moth (Lepidoptera: Gelechiidae): Use of Sigmoid Functions. Agriculture, 129:519. DOI: 10.3390/agriculture12040519

Sporleder, S. (2003). El granulovirus de *Phthorimarea operculella* (PoGV) características biológicas, su interacción con el medio ambiente y su posible uso en el manejo de *Tecia solanivora* (Lepidoptera: Gelechiidae). In: *Memorias III Taller Nacional Tecia solanivora*, 37-48. Available online: https://books.google.es/books?id=SQQKGLRNBEAC&printsec=frontcover&hl=ca&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false (Accessed on 25 October 2023).

Stouthamer, R.; Hu, J.; Van Kan; F.J.P.M.; Platner, G.R.; Pinto, J.D. (1999). Utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *BioControl,* 43(4):421-440. DOI:10.1023/A:1009937108715

Tabebordbar, F.; Shishehbor, P.; Ebrahimi, E.; Polaszek, A.; Ugine, T.A. (2020). Effect of Different Constant Temperatures on Life History and Life Table Parameters of *Trichogramma euproctidis* (Hymenoptera: *Trichogramma*tidae). *J. Econ. Entomol.*, *2*: 474-481.

DOI: 10.1093/jee/toac007

Tascón, C.; Perera S. (2021). Identificación y control de la polilla de la papa (*Phthorimaea operculella*). *Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife*. Available online: https://www.agrocabildo.org/publicaciones_detalle.asp?id=694 (Acceced on 23 January 2023).

Tai H.H.; Vickruck, Y. (2022). Potato resistance against insect herbivores: resources and opportunities. In: *Insect Pests of Potato. Global Perspectives on Biology and Management.* Eds, Alyokhin, A., Rondon, S.I. *Gao Y.* Academic Press, 277-296. DOI: 10.1016/B978-0-12-821237-0.00014-7

Thomas, H.Q.; Zalom, F.G.; Nicola, N.L. (2011). Laboratory studies of *B. tarsalis keegani* (Fox) (Acari: Ascidae) reared on eggs of navel orangeworm: Potential for biological control. *Bull. Entomol. Res.*, 101: 499-504. DOI: 10.1017/s0007485310000404

Torres, F. (1998). Biología y manejo integrado de la polilla centroamericana de la papa *Tecia solanivora* en Venezuela. *Fondo Nac Investugaciones Agrioecuarias/Fundación para el dessarrollo la Cienc y la Tecnol del estado Tachira*., 60. Available online: https://repositorio.iica.int/handle/11324/2745 (Accessed on 10 October 2023).

Trujillo E.; Perera S. (2011a). Estudio comparativo de dos feromonas sexuales y de dos tipos de trampas para la captura de adultos de la polilla guatemalteca de la papa (*Tecia solanivora*). *Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife*. Available online: https://www.agrocabildo.org/publica/publicaciones/papa_382_informe_fer_pol_tra.pdf (Accessed on 23 October 2023).

Trujillo, E.; Perera, S. (2017a). Control de la polilla guatemalteca de la papa gestión de la papa bichada recolección, almacenamiento y gestión de la papa*. Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife.* Available online: https://www.agrocabildo.org/publica/Publicaciones/papa_215_polilla.pdf (Accessed on 23 October 2023).

Trujillo, E.; Perera, S. (2017b). Polilla guatemalteca de la papa (*Tecia solanivora*). Identificación y control de daños. *Serv Técnico Agric y Desarro Rural Cabil Insul Tenerife*. Available online: https://www.agrocabildo.org/publica/Publicaciones/papa_567_papa.pdf (Accessed on 10 October 2023).

Universidad de la Laguna. (2017). Establecimiento y seguimiento de estrategias de control de las polillas de la papa en la comarca de Icod el Alto (T.M. Los Realejos) y la búsqueda de enemigos naturales de las polillas de la papa de Tenerife (2015-2017). Internal technical report.

Van Alebeek FAN. (1996). Foraging behaviour of the egg parasitoid *Uscana lariophaga* towards biological control of bruchid pests in stored cowpea in West Africa. Doctoral thesis. Wageningen University & Research, Wageningen,

Netherlands. Available online: https://research.wur.nl/en/publications/foraging-behaviour-of-the-egg-parasitoid-uscana-lariophaga-toward (Accessed on 14 November 2023).

Van Lenteren, J.; Hale, A.; Klapwijk, J.; Van Schelt, J.; Steinberg, S. (2003). Guidelines for quality control of commercially produced natural enemies. In *Quality Control and Production of Biological Control Agents: Theory and Testing Procedures*; CABI: London, UK, 278–316. Available online: mrqa.eu/images/guidelines.pdf (Accessed on 29 October 2023).

Vargas, P.; Cabello, T. (1989). Resistencia de los estados de desarrollo de *Trichogramma* cordubensis Vargas y Cabello y T. pintoi Voegelé (Hym.: *Trichogramma*tidae) a las altas temperaturas. *Boletín Sanid Veg Plagas*, 15(3): 263-266. Available online: https://www.mapa.gob.es/app/publicaciones/art_datos.asp?articuloid=1262&codrevista=Plagas (Accessed on 25 October 2023).

Vigil-Cando, F.J. (2017) Expansión de la Polilla Guatemalteca (Lepidoptera: Gelechiidae) en el Norte de la Península Ibérica: *Tecia solanivora* (Povolný 1973). Master's Thesis, Universitat de València, València, Spain.

Villamil, J.E.; Martínez, J.W. (2014). Evaluación de aislamientos nativos de *Beauveria* spp. sobre *Tecia solanivora* (Lepidoptera: Gelechiidae) in vitro. *Rev Ciencias Agrícolas*, 31 (1): 92-105. Available online: https://dialnet.unirioja.es/servlet/articulo?codigo=5104168 (Accessed on 25 October 2023).

Villamizar, L.; Espinel, C.; Grijalba, E.; Torres, L.; Costes A. B.; López Ávila, A.; Barrera, G. P.; Zeddam, J.L.; Herrera, L.; Gómez, J. (2006) Reconocimiento, selección y evaluación de aislamientos nativos de virus de la granulosis para el control biológico de la polilla guatemalteca de la papa. *Boletín Técnico CORPOICA*, 24. Available online: https://1library.co/document/y96o15ry-reconocimiento-seleccion-evaluacion-aislamientos-nativos-granulosis-biologico-guatemalteca.html (Accessed on 25 October 2023).

Villanueva, D.; Torres, J.; Rivera, H.; Núñez, V.; Arango, R.; Ángel, F. (2014). Colombian genetically modified potato lines resistant to *Tecia solanivora* (Lepidoptera: Gelechiidae) under a confined field. *Rev Colomb Entomol*., 40(2):148-157. Available online: http://www.scielo.org.co/scielo.php?pid=S0120048820140002000003&script=sci_abstract (Accessed on 25 October 2023).

Villanueva, D.F.; Saldamando, C.I. (2013). *Tecia solanivora*, Povolný (Lepidoptera: Gelechiidae): Una revisión sobre su origen, dispersión y estrategias de control biológico. *Ing. Cienc.,* 9: 197-214. DOI: 10.17230/ingciecia.9.18.11

Vinson, S.B. (1976). Host Selection by Insect Parasitoids. *Annu. Rev. Entomol., 21*: 109-133. DOI: 10.1146/annurev.en.21.010176.000545

Wang, B.; Ferro, D.N. (1998). Functional Responses of *Trichogramma ostriniae* (Hymenoptera: *Trichogramma*tidae) to *Ostrinia nubilalis* (Lepidoptera: Pyralidae) under Laboratory and Field Conditions. *Environ. Entomol.,* 27: 752-758. DOI:10.1093/ee/27.3.752

Weber D.C.; Blackburn M.B.; Jaronski S.T. (2022). Biological and behavioral control of potato insect pests. In: *Insect Pests of Potato: Global Perspectives on Biology and Management*. second Edi. Academic Press, 231-276. DOI:10.1016/B978-0-12-821237-0.00013-5

Zeddam, J.; Vasquez Soberon R., Vargas Ramos Z.; Lagnaoui A. (2003). Producción viral y tasas de aplicación del granulovirus usado para el control biológico de las polillas de la papa *Phthorimaea operculella* y *Tecia solanivora* (Lepidoptera:Gelechiidae). *Boletín Sanid Veg Plagas*., 29(4):659-668.

Legislation

Diario Oficial de Galicia (DOG) Núm. 209. 3 de noviembre de 2015. Resolución de 16 de octubre de 2015, de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que se declara la presencia de la plaga de cuarentena denominada *Tecia solanivora* Povolný o polilla guatemalteca de la papa, se establecen las zonas demarcadas para esta plaga y se adoptan medidas urgentes para su erradicación y control en la Comunidad Autónoma de Galicia.

Diario Oficial de Galicia (DOG) Núm. 209. 25 de febrero de 2016. RESOLUCIÓN de 2 de febrero de 2017, de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que se amplían las zonas demarcadas por la presencia de la plaga de cuarentena denominada *Tecia solanivora* Povolný o polilla guatemalteca de la patata.

Diario Oficial de Galicia (DOG) Núm.25. 6 de febrero de 2017. Resolución de 2 de febrero de 2017, de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que se amplían las zonas demarcadas por la presencia de la plaga de cuarentena denominada *Tecia solanivora* Povolný o polilla guatemalteca de la papa. (P. 5426-5427). Diario Oficial de Galicia (DOG) Núm.38. 25 de febrero de 2016. Resolución de 11 de febrero de 2016, de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que se amplían las zonas demarcadas por la presencia de la plaga de cuarentena denominada *Tecia solanivora* Povolný o polilla guatemalteca de la papa.

Diario Oficial de Galicia (DOG) Núm.200. 20 de octubre de 2017. Resolución de 17 de octubre de 2017, de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que se amplían las zonas demarcadas por la presencia de la plaga de cuarentena denominada *Tecia solanivora* Povolný o polilla guatemalteca de la papa.

Diario Oficial de Galicia (DOG) Núm.11. 17 de enero de 2020. Resolución de 13 de enero de 2020. de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que modifican las medidas fitosanitarias establecidas en la resolución de 8 de marzo de 2017, de la Dirección General de Ganadería, Agricultura e Industrias Agroalimentarias, por la que se establecen las zonas infestadas y las zonas tampón y se implementan las medidas para la erradicación y

el control con respecto al organismo de cuarentena *Tecia solanivora* Povolný, o polilla guatemalteca de la patata, en la comunidad autónoma de Galicia.

Boletín Oficial del Principado de Asturias (BOPA) Núm. 33 de 10 de febrero del 2017. Resolución de 8 de febrero de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales, por la que se declara la presencia de la plaga de cuarentena denominada *Tecia solanivora* (Povolný) o polilla guatemalteca de la papa en diversas zonas del Principado de Asturias y se establecen actuaciones transitorias para esta plaga. 4 pp.

Boletín Oficial del Principado de Asturias (BOPA) Núm. 69 del 24 de marzo del 2017. Resolución de 21 de marzo de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales, por la que se establecen zonas demarcadas adicionales a las contempladas en la Resolución de 13 de marzo de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales por la que se establecen las zonas infestadas y zonas tampón, y se fijan los baremos de indemnización derivados de la erradicación del organismo de cuarentena denominado Tecia (Scrobipalpopsis) solanivora (Povolný) o polilla guatemalteca de la papa en el Principado de Asturias.

Boletín Oficial del Principado de Asturias (BOPA) Núm. 126 del 2 de junio del 2017. Resolución de 26 de mayo de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales, por la que se modifica la Resolución de 13 de marzo de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales por la que se establecen las zonas infestadas y zonas tampón, y se fijan los baremos de indemnización derivados de la erradicación del organismo de cuarentena denominado Tecia (Scrobipalpopsis) solanivora (Povolný) o polilla guatemalteca de la papa en el Principado de Asturias.

Boletín Oficial del Principado de Asturias (BOPA) number 268, on 20 of November 2017. Resolución de 13 de noviembre de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales, por la que se actualiza la delimitación de las zonas demarcadas establecidas en la Resolución de 13 de marzo de 2017, de la Consejería de Desarrollo Rural y Recursos Naturales, por la que se establecen las zonas infestadas y zonas tampón, y se fijan los baremos de indemnización derivados de la erradicación del organismo de cuarentena denominado Tecia (Scrobipalpopsis) solanivora (Povolný) o polilla guatemalteca de la patata en el Principado de Asturias.

Boletín Oficial del Principado de Asturias (BOPA) number 24, on 5 of Febrary 2020. Resolución de 31 de enero de 2020, de la Consejería de Desarrollo Rural, Agroganadería y Pesca, por la que se actualiza la delimitación de las zonas demarcadas establecidas en la Resolución de 13 de noviembre de 2017 dentro del programa de erradicación de Tecia (scrobipalpopsis) solanivora (Povolný) o polilla guatemalteca de la patata en el Principado de Asturias.

Boletín Oficial del Principado de Asturias (BOPA) number 33, on 7 of February 2023. Resolución de 7 de febrero de 2023, de la Consejería de Medio Rural y Cohesión Territorial, por la que se actualiza la delimitación de las zonas demarcadas establecidas en la Resolución de 29 de enero de 2021, de la Consejería de Medio Rural y Cohesión Territorial, dentro del programa de erradicación de Tecia (scrobipalpopsis) solanivora (Povolný) o polilla guatemalteca de la patata, en el Principado de Asturias.

Real Decreto 197/2017, de 3 de marzo, por el que se establece el Programa Nacional de control y erradicación de Tecia (Scrobipalpopsis) solanivora (Povolný). (P. 15749-15760). Boletín Oficial del Estado (BOE) Núm. 54 de 4 de marzo de 2017.

Doctoral thesis
Jorge Gavara Vidal

The management of *Tecia solanivora* is a highly intricate process. The majority of its biological cycle occurs below the soil and within the tuber, which protects the pest. Only the adult stage is exposed. Without management strategies crop losses can reach as high as 50% in the field and 100% in storage. Current measures to prevent crop loss are often insufficient and result in unacceptable economic losses. Furthermore, there is currently a lack of effective phytosanitary treatments that can be used in the field or approved for storage. In this context, research into biological control agents has become particularly important. The study of oophagous natural enemies such as *Trichogramma achaeae*, *Trichogramma euproctidis* and *Blattisocius tarsalis* is of particular interest as they prevent the eggs from hatching, thereby removing the pest before the potatoes are damaged.

In the present study, an experimental methodology was employed to assess the efficacy of both *Trichogramma* species in field and storage conditions, as well as the predatory mite *B. tarsalis* in storage conditions. The results demonstrated that *T. euproctidis* is unsuitable for use against *T. solanivora*, both in the field and in storage. In contrast, *T. achaeae* exhibited promising results when tested in semifield conditions. The predatory mite has also demonstrated promising results in experimental storage conditions. Further research is necessary to enhance its efficacy through improvements in release densities and numbers, as well as to assess its suitability for use in other storage systems. Additionally, a comprehensive economic analysis is essential to determine the viability of this approach, considering the costs associated with mass rearing.

April 2024