UNIVERSIDAD
DE ALMERÍA

TESIS DOCTORAL

Estudio de modelos logísticos: aplicaciones en artrópodos plagas y su control

Study of logistic models: Applications in arthropods pest and their control

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Departamento de Biología y Geología

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Tesis Doctoral presentada para optar al Grado de Doctor por la Universidad de Almería. Programa de Doctorado en Agricultura Protegida

DIRECTORES

Prof. Dr. Tomás Cabello García
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Autorización de los directores

Este trabajo de investigación titulado "Estudio de modelos logísticos: aplicaciones en artrópodos plagas y su control", para aspirar al grado de Doctor que presenta **Yohan Alexander Solano Rojas**, bajo la dirección del Prof. Dr. **Tomás Cabello García** y Prof. Dr. **Manuel Gámez Cámara**.

En Almería, a 7 de octubre de 2022.

Prof. Dr. Tomás Cabello García

Prof. Dr. Manuel Gámez Cámara

A Dios, por su bendición, su compañía y su misericordia
A mis padres María Rojas de Solano y José Solano Bustos
A mi Tía Alma Solano Bustos
A mis hermanas Jennifer Solano y Mónica Solano
A mi familia y amigos

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Resumen

Los modelos matemáticos permiten representar el comportamiento poblacional de los insectos en función de los cambios del entorno, lo cual es útil dentro de programas de manejo integrado de plagas para analizar, predecir y/o simular la dinámica poblacional de las plagas y/o sus organismos de control biológico en función del tiempo y de las estrategias de control o de las condiciones climáticas. Esta tesis se enfocó en desarrollar y estudiar la aplicación de los modelos logísticos en el control de plagas, tanto aplicados a la especie plaga como a los métodos de control químico y biológico.

En el capítulo II se estudió la fenología y control químico óptimo del picudo de las palmeras *Rhynchophorus ferrugineus*. El modelo fenológico se desarrolló a partir de datos experimentales obtenidos en Almuñecar, Granada, España. La fenología del picudo ajustó a una doble función sigmoide en la que cada ecuación representó una cohorte, debido a que la diapausa larvaria, inducida por bajas temperaturas, dividió la población del picudo. Este modelo puede aplicarse en otras zonas con presencia de *R. ferrugineus* y con temperaturas entre 10 °C y 15 °C por 80 días aproximadamente. El modelo de control óptimo reveló que la aplicación de insecticidas es efectiva durante el máximo poblacional larvario ($\leq$ 50 días de inicio de la infestación), y según la curva solución, el coste de control de larvas es menor que el de pupas y/o adultos.

A su vez, el capítulo III se refiere a la determinación de la efectividad de los entomófagos *Trichogramma cacaeciae* y *Blattisocius mali* en el control de la polilla *Phthorimaea operculella* utilizando la fórmula de Abbot y un modelo logístico. Para ello, tanto el parasitoide como el depredador se liberaron en recipientes que contenían patatas recién infestadas, y se dejaron actuar durante 49 días. Al finalizar el estudio, *B. mali* (Abbot: 86,53%; Modelo logístico: 94,85%) ejerció un mayor control de la polilla que *T. cacaeciae* (Abbot: 43,88%; Modelo logístico: 73,77%). El modelo logístico fue más acurado en la determinación de la efectividad de control que la ecuación de Abbot, ya que considera los valores intermedios de la población de la plaga en el tiempo y bajo la regulación de los entomófagos, mientras que la fórmula de Abbott carece de la fiabilidad que aporta la varianza de los datos.

Finalmente, en el capítulo IV se estudió un sistema novedoso de control biológico en cultivos de invernaderos. El capítulo está dividido en dos subapartados, el primero se enfocó en la estandarización de la metodología de evaluación que incluyó la comparación del método de extracción de embudo de Berlese-Tullgren con un nuevo método por flotación en hexano, y se estudió la dinámica poblacional del depredador *A. swirskii* y su presa *C. lactis* en el interior de sobres de liberación lenta, y la tasa de liberación del depredador. El nuevo método extrajo 3,7 más ácaros que el embudo. La correlación positiva entre la población interna del depredador y la presa indicó que la dinámica poblacional fue óptima para mantener la cría de *A. swirskii*. La tasa máxima de liberación ocurrió entre los días 7 y 13, cuando la población de la presa disminuyó en el interior del sobre.

En el segundo subapartado se estudió el efecto del microclima sobre la dinámica poblacional de *A. swirskii* y *C. lactis* y la efectividad del sistema mediante modelos logísticos. El modelo reflejó que en humedad relativa (HR) baja la liberación acumulada del depredador fue inferior a 300 ácaros/sobre en un periodo de 2 a 3 días, mientras que en HR media y alta la liberación fue cercana a 500 ácaros/sobre en un periodo de 10 y 15 días, respectivamente. No se registró crecimiento poblacional de *C. lactis* en ningún régimen de HR, y esto influyo en la tasa de crecimiento del depredador, particularmente en HR baja.

Palabras clave: control biológico, control químico, dinámica poblacional, entomófagos, modelos matemáticos.

Abstract

Mathematical models allow to represent the population behavior of insects depends on the changes of the environment, which is useful into the management integrated pest to analyze, to predict and/or to simulate the population dynamics of pest and/or its biological control organisms in accordance with the time and the control strategies or environmental conditions. This thesis focused on developing and study the application of logistic models in pest control, both applied to the pest species and to chemical and biological control methods.

In the chapter II the phenology and optimal chemical control of the palm weevil *Rhynchophorus ferrugineus* was studied. The phenological model was developed based on experimental data gathered in Almuñecar, Granada, Spain. The phenology of RPW was fitted by a double sigmoid function, which each logistic function represents one cohort, due to larval diapause, induced by low temperatures, which divided the RPW population. This model could be applied elsewhere with RPW occurrence and with temperatures between 10 °C – 15 °C for approximately 80 days. The optimal chemical control model shown that insecticide application is effective during the maximum larvae population (≤ 50 days from infestation), and according to solution curve, the cost of larval control is lower than of pupae and/or adults.

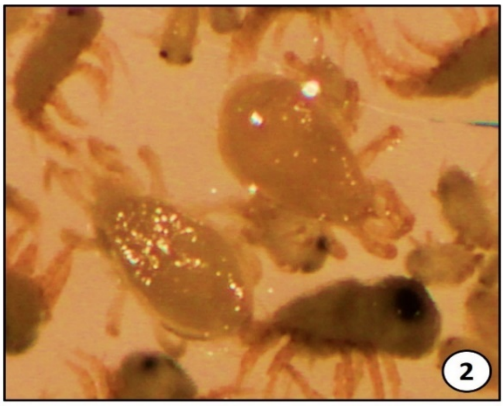
In turn, Chapter III deals with the determination of the effectiveness of the entomophagous *Trichogramma cacaeciae* and *Blattisocius mali* in the control of the moth *Phthorimaea operculella* using the Abbott equation and a logistic model. For this, the parasitoid and predatory mite was released in containers with freshly infested potatoes and allowed themselves to act for 49 days. At the end of the study, *B. mali* (Abbot: 86,53%; logistic model: 94,85%) exerted greater control of the moth than *T. cacaeciae* (Abbot: 43,88%; logistic model: 73,77%). The logistic model was more accurate in determining control effectiveness than the Abbott equation, as it considers the intermediated values of the pest population over time and under entomophagous control, while the Abbott's formula lacks the reliability provided by the variance of the data.

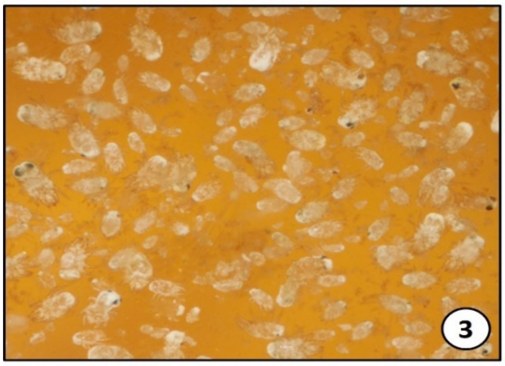
Finally, in Chapter IV, a novel biological control system for greenhouse crops was studied. The chapter is divided into two sub-sections, the first one focused on the standardization of the evaluation methodology that included the comparison of the extraction method using Berlese-Tullgren with a new method based on the flotation in hexane, and we study the population dynamics of *A. swirskii* and *C. lactis* into the slow-release sachets, and the release rate of predator. The new method extracted 3.7 more mites than the funnel. The positive correlation between the internal population of the predator and the prey indicated that the population dynamics were optimal for maintaining *A. swirskii*. The maximum release rate occurred between days 7 and 13, when the population of the prey population decreased inside the sachet, showing a negative correlation between predator that left the sachet and internal prey population.

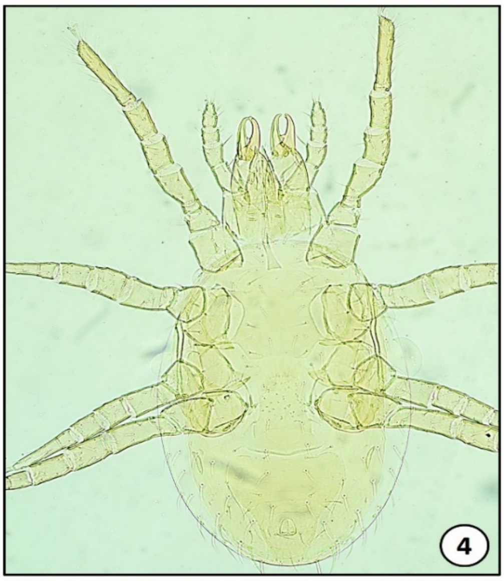
The second subpart studied the effect of microclimate on the population dynamics of *A. swirskii* and *C. lactis* and the effectiveness of the systems were studied using logistic models. The model showed that at low relative humidity (RH) the cumulative predator release was less than 300 mites/sachet over a period of 2 to 3 days, while at medium and high RH the release was close to 500 mites/sachet over a period of 10 and 15 days, respectively. No population growth of *C. lactis* was recorded in any RH regime, and this influenced the predator growth rate, particularly at low RH.

Key words: biological control, chemical control, entomophagous, mathematical models.

Capítulo I







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1. Adulto de *Rhynchophorus ferrugineus* (Fuente: Tomás Cabello).
2. Adultos del ácaro depredador *Amblyseius swirskii* y ninfa y adulto de su presa, *Carpoglyphus lactis*.
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Capítulo I

Introducción y Objetivos

Capítulo I. Introducción y objetivos

I.1. Introducción

I.1.1. Los modelos matemáticos y sus aplicaciones en el manejo integrado de plagas

La intensificación de los sistemas agrícolas ha promovido la necesidad de profundizar el conocimiento sobre la interacción entre los componentes del sistema suelo – planta – atmosfera, la dinámica poblacional de insectos y las estrategias de protección vegetal, con el propósito de diseñar planes de manejo que optimicen el control de plagas y garanticen una mejor producción agrícola (Carreño 1996, Gámez *et al*. 2000, Dourado-Neto *et al*. 1998, Tonnang *et al*. 2017). Es por ello que diversos marcos conceptuales de modelización matemática han sido propuestos para analizar, controlar, describir, explicar, predecir o simular los cambios que ocurrirían en el funcionamiento de los sistemas agrícolas como respuesta a variaciones del entorno (France 1988, Ingalls 2015), y a las interacciones multitróficas entre los cultivos, las plagas y los insectos benéficos (depredadores y/o parasitoides) (Tonnang *et al*. 2017).

En la producción de cultivos, la modelización matemática ha sido empleada para describir y/o simular el crecimiento vegetativo y/o reproductivo de las plantas en función del tiempo y de la variación de parámetros climáticos (p. ej. von Fircks & Verwijst 1993, Zavalloni *et al*. 2006, Candir *et al*. 2009, Tudela & Santibáñez 2016) o de la modificación de estrategias de manejo (p. ej. Roose & Fowler 2004, Shabani *et al*. 2018, Silva *et al*. 2018), mientras que en el contexto de la protección vegetal han permitido el estudio y/o simulación de la dinámica poblacional de las plagas bajo control químico o en los sistemas plaga – enemigo natural (p.ej. Vendite & Ghini 1999, Rafikov *et al*. 2007, Tang & Cheke 2008, Grysperit & Grégoire 2012, Li & Tang 2018, Tian *et al*. 2019, Santra 2020), para encontrar tasas y momentos óptimos de aplicación de insecticidas o de liberación de enemigos naturales para alcanzar un control eficaz de la plaga (Gallego *et al*. 2013, Molnár *et al*. 2016, Sánchez *et al*. 2018, Téllez *et al*. 2020).

Dentro del contexto del manejo integrado de plagas (MIP), Getz & Gutierrez (1982) indicaron que la modelización matemática ha estado enfocada en tres aspectos principales: a) la simulación, a través de la cual se explora la estructura y funcionamiento de los sistemas de control (p. ej. Liu *et al*. 2005, Bhattacharyya & Bhattacharya (2006), Kumari *et al*. 2020), b) el análisis, en el que se destacan las estructuras básicas del subsistema y en los que los modelos son usados para la gestión de recursos (p. ej. Cabello & Carreño 2002, Meng & Song 2007, Chowdhury *et al*. 2019), y c) la investigación operativa, en donde los modelos permiten gestionar y solucionar problemas específicos (p. ej. Zhang & Swinton 2012, Cabello *et al*. 2012, Téllez *et al*. 2020). Al respecto, Barlow (2004) recogió un conjunto de investigaciones orientadas al uso de modelos matemáticos para simular, analizar o solucionar problemas específicos de plagas a través del uso de entomopatógenos o parasitoides.

Entre los principales modelos usados para estudiar el crecimiento y dinámica poblacional de los insectos plaga, destacan los modelos matriciales, especialmente los modelos de Leslie, que describen cambios en la densidad poblacional, los modelos fenológicos los cuales simulan la estructura de estadios de una plaga bajo determinadas condiciones climáticas, los modelos en tiempo continuo donde las ecuaciones diferenciales permiten estudiar la dinámica poblacional a través de la estimación de determinados parámetros del modelo, los modelos de competencia que son usados para explorar los efectos de los recursos y la competencia entre insectos plaga, y los ajustes de modelos que contrastan las propias predicciones del modelo con la trayectoria de los datos experimentales (Tonnang *et al*. 2017).

Los modelos matemáticos también han sido utilizados para medir o predecir el impacto de los enemigos naturales en las poblaciones de plagas, estimar la distribución espacial y la capacidad de dispersión de los insectos o evaluar la respuesta estimada de los insectos a la temperatura, tales como los modelos de distribución de especies, los modelos de nichos ecológicos y el modelo teórico Climex (Tonnang *et al*. 2017). Otros modelos matemáticos no lineales como los exponenciales, polinomiales de 3er orden, funciones de Stinner, Logan y Pradhan-Taylor, Sharpe & DeMichele, Lactin, Briere y logística de Davidson (Stedinger & Shoemaker 1985, Cabello & Carreño 2002, Sporleder *et al*. 2004, Yurk & Powell 2009, Golizadeh y Zalucki 2012, Kroschel *et al*. 2013, Arbab *et al*. 2016) también se han empleado para estudiar la fluctuación poblacional de diversas plagas frente a variaciones en las condiciones climáticas.

En la agricultura, las funciones logísticas se han utilizado a través de regresiones no lineales o de la modelización de respuestas bimodales (Lipovetsky 2010), con el propósito de representar el crecimiento poblacional de plantas cultivadas, microorganismos y/o insectos, que generalmente sigue un patrón sigmoidal caracterizado por un aumento en la tasa de crecimiento hasta alcanzar un punto de inflexión o capacidad de carga, a partir del cual la población decrece

(Meyer 1994, Shabani *et al*. 2018, Kawano *et al*. 2020). Estas funciones permiten representar el crecimiento autolimitado de las poblaciones en el que la tasa de crecimiento de los individuos (*r*) y la capacidad de carga (*K*) del sistema (Meyer, 1994, Kawano *et al*. 2020), reflejaran el efecto de parámetros de regulación tales como las relaciones tróficas, el comportamiento biológico y fenológico, la disponibilidad de recursos, las variaciones en las condiciones climáticas y los cambios en las estrategias de manejo de los cultivos o de las plagas y enfermedades (Watt 1960, Tonnang *et al*. 2017, Kawano *et al*. 2020).

De allí que las ecuaciones sigmoides singulares, o las de tipo compuesto, como aquellas que comprenden la suman de dos o más funciones logísticas (Amorim *et al*. 1993, Hau *et al*. 1993, Meyer 1994, Carreño 1996), se utilicen para analizar la dinámica poblacional de plantas, microorganismos o insectos, especialmente cuando existen eventos o estados de desarrollo que ocurren en dos o más etapas (p. ej. Cabello & Carreño 2002, Battude *et al*. 2016, Fernandes *et al*. 2017, Shabani *et al*. 2018, Rustia & Lin 2019), en las que se manifiestan dos o más puntos de inflexión y saturación (Meyer 1994, Lipovetsky 2010), por lo que el abordaje de los modelos logísticos incluirá dos o más ecuaciones simples, cada una de las cuales representará un evento, etapa o estado de desarrollo. Entre las ecuaciones más utilizadas para la construcción de estos modelos se encuentran las de Verhulst-Pearl, Mitscherlich o monomolecular, Bertalanffy, Richards, Gompertz y Carreño (Carreño 1996, Cabello & Carreño 2002, Tsoularis & Wallace 2002), y cuya selección depende del ajuste de los datos, de la bondad del ajuste y de los parámetros que definen el tipo de curva solución que se usará en la predicción o simulación de la dinámica poblacional de dos o más organismos en tiempo y espacio (Carreño 1996, Irvine 2011).

Los modelos logísticos pueden abordarse desde ecuaciones diferenciales (tiempo continuo), cuando los organismos tienen una reproducción constante, o con ecuaciones en diferencias (tiempo discreto), cuando la reproducción es estacional y/o el organismo presenta etapas de desarrollo bien definidas, como es el caso de la mayoría de las plantas cultivadas (p. ej. semilla, plántula, periodo de crecimiento vegetativo, floración y fructificación) y de los insectos holometábolos (p. ej. huevo, larva, pupa y adulto) (Allman & Rhodes 2004, Berryman 2004), cuyas etapas también se caracterizan por tener diferentes periodos de duración y/o variación en las tasas de mortalidad, respectivamente (Allman & Rhodes 2004). Sin embargo, a pesar de la tendencia de uso de estas ecuaciones y de la advertencia de que las ecuaciones de diferencias son generalmente menos estables que las diferenciales, diversos autores sostienen que el uso de cualquiera de ellas no afecta la interpretación de los modelos matemáticos (Allman & Rhodes 2004, Berryman 2004, Santra 2020).

En la protección vegetal, los modelos de control óptimo han permitido analizar la respuesta poblacional de las plaga frente a la aplicación de control químico, con el propósito de optimizar el tiempo y coste de aplicación de los insecticidas (p. ej. Chowdhury *et al*. 2019), sincronizar la implementación del control químico con la estructura de edad de la población de la plaga (p. ej. Marsolan & Rudd 1976, Chi 1990), o minimizar el número de liberaciones de parasitoides y/o depredadores necesario para mantener las plagas debajo del umbral económico (p. ej. Gallego *et al*. 2013, Sánchez *et al*. 2018), particularmente cuando el momento de aplicación de las estrategias coincide con el estado de vida de la plaga más susceptible al control (Sporleder *et al*. 2013). Otro tipo de modelos de control, son los denominados modelos presa-dependiente del consumo con una estrategia de control impulsivo, los cuales permiten analizar el efecto combinado de estrategias de control químico y/o biológico en diferentes periodos fijos, con el propósito de sincronizar y establecer frecuencias de aplicación de ambas estrategias en el tiempo, evitar la mortalidad de los enemigos naturales por acción de los insecticidas e incrementar la eficiencia de ambas estrategias (p. ej. Liu *et al*. 2005, Zhang *et al*. 2005, Tang *et al*. 2010, Fu & Wang 2012, Li & Tang 2018).

De igual forma, la fecundidad de los insectos también ha sido analizada a través de modelos matemáticos como el clásico de Lotka – Volterra y las ecuaciones logísticas (Watt 1960). Al respecto, Baring-Gould (1998) revisó diferentes modelos aplicados al estudio de la fecundidad como los de optimización de la oviposición (p. ej. Kindlmann & Dixon 1993) y modelos de elección de la presa (p. ej. Iwasa *et al*. 1984), y modelos con un factor de mortalidad que sustentan que las hembras invierten menos recursos reproductivos en la medida que incrementa su edad (p. ej. Parker & Courtney 1984, Begon & Parker 1986).

En el ámbito del control biológico, las funciones logísticas de Verhulst-Pearl y Lotka-Volterra para el sistema depredador-presa, y el modelo de Nicholson & Bailey, para el sistema huésped-parasitoide, destacan entre las más utilizadas para estudiar la dinámica poblacional de las plagas con sus enemigos naturales (Berryman 2004). De estas ecuaciones, la de Verhulst-Pearl es dependiente de la proporción de depredadores respecto a los recursos disponibles, mientras que las otras son dependientes de la densidad de la presa/huésped, respectivamente (Berryman 2004). El uso de estas funciones ha permitido clarificar, simular y/o predecir el efecto de factores decisivos en la inclusión del control biológico en programas MIP tales como la proporción presa/huésped – depredador/parasitoide, densidad inicial, tasa y tiempo de liberación, la selección de agentes de control (p. ej. Cabello *et al*. 2012, Gallego *et al*. 2013, Garay *et al*. 2015, Sánchez *et al*. 2018, Téllez *et al*. 2020), y la sincronización de estos factores con la aplicación de insecticidas (Tang & Cheke 2008, Tang *et al*. 2010). De igual forma, la eficiencia de los parasitoides y depredadores en la búsqueda y captura de sus huéspedes o presas respectivas se ha estudiado a través de modelos de forrajeo óptimo que permiten analizar las estrategias que adoptan los enemigos naturales para encontrar, consumir y aprovechar la energía del alimento al menor coste (P. ej. Comins & Hassell 1979, Kindlmann & Dixon 1993, Weisser 1993, Schofield *et al*. 2002, Garay *et al*. 2018).

Barlow (2004) destacó que el desarrollo de investigaciones sobre modelización matemática de sistemas de control biológico contribuirá al alcance de beneficios futuros tales como: predecir resultados de la introducción de organismos de control biológico, predecir el impacto sobre las especies no objetivo, seleccionar organismos de control biológico antes de su introducción a través de la modelización prospectiva del impacto de los enemigos naturales considerando su comportamiento y/o efectividad en el control de la plaga, predecir la liberación óptima y las estrategias de manejo de los organismos de control biológico, e incrementar el conocimiento sobre el sistema de control biológico a través del estudio de casos y/o modelos ajustados a la realidad.

I.1.2. Características biológicas e importancia de las especies estudiadas

I.1.2.1. Insectos plaga

a. *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae)

R. ferrugineus, conocido como el picudo rojo de la palmera (**Figura I.1d**), es una especie plaga originaria del sur de Asia y Melanesia, desde donde se ha expandido alrededor del mundo causando daños a los cultivos de palma aceitera, coco, sagú y palmera datilera (Ferry & Gómez 2002, EPPO 2008, El-Shafie & Faleiro 2020). Casi todos los estados de desarrollo de *R. ferrugineus* se encuentran ocultos en el interior de las palmeras, con excepción de los adultos, los cuales salen eventualmente para el apareamiento o la búsqueda de nuevos huéspedes (El-Shafie & Faleiro 2020). El ciclo de vida de *R. ferrugineus* ha sido estudiado por diversos autores sobre sustratos de alimentación y condiciones climáticas diferentes, por lo que la duración de los estados de desarrollo del picudo varía en función de esas condiciones tal como resume Dembilio & Jacas (2011).

La principal causa de la diseminación de *R. ferrugineus* en el mundo ha sido el transporte de palmeras infestadas, ya sean jóvenes o adultas, desde zonas contaminadas a zonas no infectadas (Ferry & Gómez 2002). Su dispersión también ha sido facilitada por la cercanía geográfica de las plantas huéspedes y la capacidad de vuelo de los adultos, los cuales son atraídos por el efecto sinergético que produce su propia feromona de agregación (ferruginol) con las kairomonas de la planta huésped (Ferry & Gómez 2002, Cabello 2012). De acuerdo con El-Shafie & Faleiro (2020), el picudo afecta a cerca de 40 especies de palmeras, entre las que destacan *Phoenix dactylifera* y *P. canariensis*, que son las principales palmas cultivadas y de uso ornamental en el área mediterránea de África y Europa (Barranco *et al*. 2000).

Las hembras de *R. ferrugineus* colocan sus huevos (**Figura I.1a**) en la base de las hojas jóvenes o en las heridas de los tallos de las palmas, en el interior de agujeros hechos con sus rostrum. Una vez que emergen las larvas (**Figura I.1b**), estas se alimentan de las fibras blandas y/o tejidos de las yemas terminales, y luego se internan en el tallo haciendo túneles, y luego pupan (**Figura I.1c**) (Ferry & Gómez 2002, Faleiro 2006, Dembilio & Jacas 2011, El-Shafie & Faleiro 2020). La naturaleza criptica de las larvas del picudo hacen que su control sea complejo, ya que reduce la probabilidad de que los insecticidas, y otras estrategias de manejo, alcancen las larvas en estado de desarrollo avanzado (Dembilio & Jacas 2011, El-Shafie & Faleiro 2020). Adicionalmente, la existencia de un desarrollo asincrónico de las larvas, promovido por baja temperatura ambiental, dificulta su control ya que dentro de una misma palmera puede haber generaciones superpuestas (Ferry & Gómez 2002, Faleiro 2006), lo que conlleva a un rebrote repentino de la cohorte no controlada.

Los primeros síntomas del daño causado por las larvas de *R. ferrugineus* se observan después de un largo tiempo de infestación (Ferry & Gómez 2002), de manera que la detección temprana del picudo a través del monitoreo o el uso de trampeo masivo con feromonas de agregación o kairomonas semisintéticas (El-Sabea *et al*. 2009, Faleiro *et al*. 2010, Faleiro *et al*. 2011, Dembilio & Jacas 2011) es fundamental para planificar y aplicar estrategias de control como la aplicación de insecticidas a través de la perfusión o inyección dentro del tronco (Faleiro

2006, Dembilio & Jacas 2012), el uso de control biológico y de machos estériles (Faleiro 2006), optimización del manejo agronómico de los cultivos de palmas (El-Shafie & Faleiro 2020) y/o la eliminación completa de las palmas infestadas para evitar los focos de infestación (Dembilio & Jacas 2012, El-Shafie & Faleiro 2020).

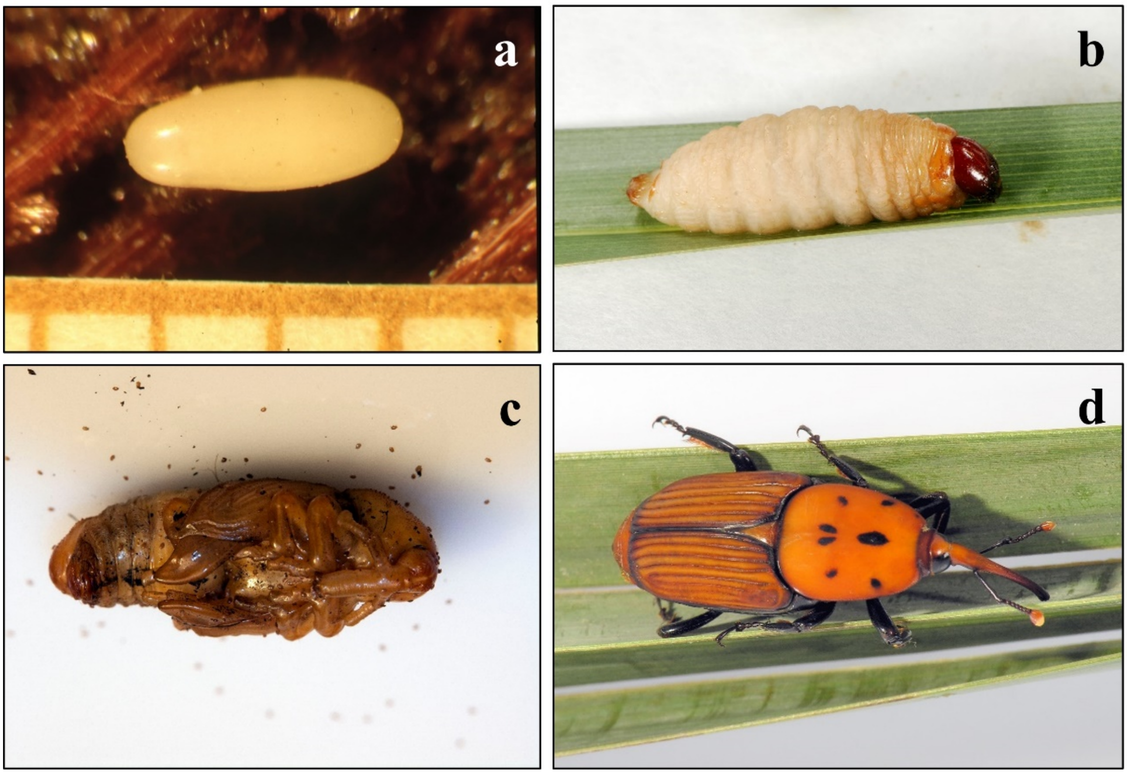

Figura I.1 Estados de (a) Huevo, (b) Larva, (c) Pupa y (d) Adulto de *Rhynchophorus ferrugineus*.
Fuente: Tomás Cabello

b. *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)

P. operculella, conocida como polilla de la patata o gusano del tabaco, es una plaga principal del cultivo de la patata (*Solanum tuberosum* L.) pero también ataca el tomate (*S. lycopersicum* L.) y el tabaco (*Nicotiana tabacum* L.) (Rondon *et al*. 2007, Rondon 2010, Gill *et al*. 2014). Sin embargo, su rango de hospedantes puede alcanzar las 60 especies, entre las que se incluyen cultivos de la familia Chenopodiaceae, la berenjena (*Solanum melongena* L.), los pimientos (*Capsicum* spp.) y la remolacha azucarera (*Beta vulgaris* L.) (Gill *et al*. 2014, Kroschel *et al*. 2020). La polilla tiene una distribución cosmopolita y se ha reportado en más de 90 países, muchos de los cuales se encuentran en zonas tropicales y subtropicales (Kroschel & Sporleder 2006, Kroschel & Schaub 2013, Rondon 2020). Respecto a la biología de *P. operculella*, Rondon (2010) y Kroschel & Schaub (2013) enfatizaron que la influencia de la temperatura en la duración de los estados de desarrollo permite que la polilla puede completar varias generaciones por año, particularmente cuando el umbral térmico mínimo de sus huevos, larvas y pupas esta entre 5 °C y 8 °C, con lo cual puede sobrevivir al invierno suave y/o adaptarse a cualquier lugar, tal como lo resume Rondon (2010) en su compendio de investigaciones sobre la variación del ciclo de vida de la polilla.

La polilla de la patata (**Figura I.2.a**) coloca sus huevos sobre el follaje o sobre las yemas axilares de los tubérculos, a los que accede internándose en el suelo a través de grietas (Kroschel & Schaub 2013, Gill *et al*. 2014, Kroschel *et al*. 2020). Una vez que el huevo completa su desarrollo, las larvas emergen para alimentarse de las hojas, tallos y pecíolos haciendo minas irregulares, mientras que en los tubérculos excavan túneles (**Figura I.2.b**) (Rondon 2010, Kroschel & Schaub 2013, Kroschel *et al*. 2020). Los daños típicos en los tubérculos son las galerías producidas por las larvas (**Figura I.2.c**) y la putrefacción que causan sus heces y los hongos y bacterias que se desarrollan en el interior de las galerías (Rondon *et al*. 2007, Rondon 2010, Kroschel & Schaub 2013, Kroschel *et al*. 2020). Las larvas que van a pupar dejan el tubérculo para formar un capullo de seda y pupar en el suelo o en los restos de cultivo (Rondon 2007, Rondon 2010). Los daños al follaje reducen los rendimientos en la formación de tubérculos cuando la infestación ocurre al principio de temporada (Kroschel *et al*. 2020), mientras que los tubérculos infestados representan grandes pérdidas económicas en campo que luego pueden magnificarse en los almacenes, especialmente en aquellos no refrigerados (Rondon 2010)

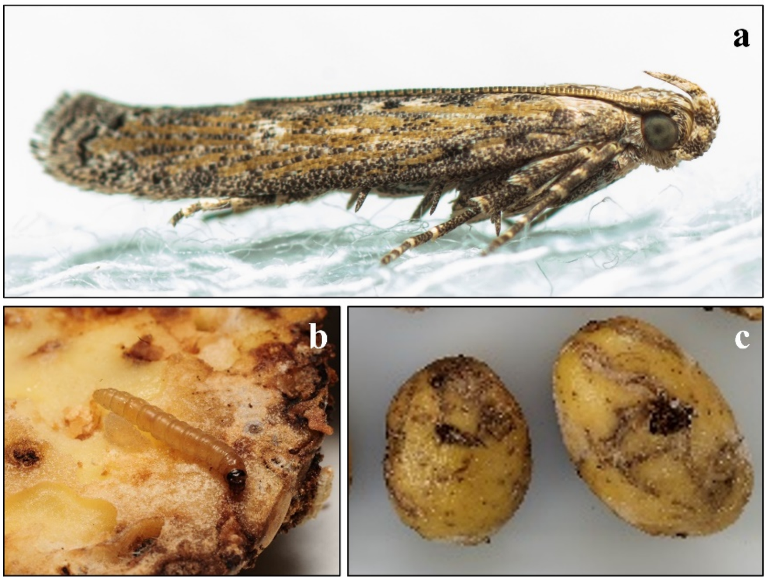

Figura I.2 (a) Adulto de *Phthorimaea operculella*, (b) Larva, (c) Daño de las larvas en tubérculos de patata
Fuente: Juan Ramón Gallego

En el control de la polilla de la patata se han adoptado diversas estrategias dentro de programas MIP, entre las que destacan el control químico y el monitoreo a través de trampas con feromonas que atraen y controlan a los machos, y con los cuales se puede estimar la población y distribución de la plaga, y planificar la aplicación de insecticidas (Rondon 2010, Gill *et al*. 2014). Así mismo, las prácticas culturales como el uso de hospedantes alternativos, siembra profunda y buena cobertura de semillas, uso de tubérculos sanos para la siembra, cosecha oportuna, eliminación de restos de plantas luego de la cosecha, uso de variedades resistentes y suficiente riego para evitar grietas en el suelo (Rondon 2010, Kroschel *et al*. 2020), han resultado efectivas en la regulación de las poblaciones de *P. operculella*.

A pesar de que el control químico es la estrategia más rápida para disminuir las poblaciones de la polilla, su uso indiscriminado promueve la aparición de resistencia, contamina el ambiente y afecta las poblaciones de organismos no objetivo (Rondon 2010, Kroschel *et al*. 2020). En este sentido, el control biológico ha resultado la estrategia más adecuada dentro de programas MIP para reducir la dependencia de insecticidas, y en su lugar, incluir el uso de cepas específicas de entomopatógenos como *Bacillus thuringiensis* (Berliner), *Beauveria bassiana* y el granulovirus PhopGV (Baculoviridae), para el control de larvas de *P. operculella* (Gao 2018, Kroschel *et al*. 2020). De igual forma, se han utilizado especies parasitoides y depredadores como los enlistados por Rondon (2010) y Kroschel & Schaub (2013), las cuales han sido exitosamente introducidas dentro de programas de control biológico clásico en países de América, África, Asia y Europa. Entre estos parasitoides, destacan las especies *Copidosoma koehleri* (Blanchard), *Apanteles subandinus* (Blanchard) y *Orgilus lepidus* (Muesebeck) (Kroschel *et al*. (2020), mientras que entre los depredadores se encuentran *Chrysoperla carnea* Stephens y *Orius albidipennis* (Reuter) (Rondon 2010).

En los almacenes de patata, las prácticas de control de la polilla están enfocadas en la limpieza de las instalaciones, el uso de mallas finas en las ventanas para evitar el ingreso de polillas adultas, destrucción de tubérculos infestados, uso de trampas cebadas, e inclusive, el uso de bioinsecticidas a base de *B. thuringiensis* y el granulovirus PhopGV, que se espolvorean a las patatas antes de almacenarlas (Schaub & Kroschel 2017, Gao 2018, Kroschel *et al*. 2020).

c. *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae)

El ácaro de los frutos secos, *C. lactis* (**Figura I.3a**), es una especie que infesta productos almacenados ricos en sacáridos como las frutas secas o deshidratadas como las ciruelas, uvas, higos, dátiles y caquis, frutas confitadas, productos lácteos, mermeladas, azúcar, polen, miel, jamón curado, cerveza y vino los cuales son ricos en ácido acético, cúprico o láctico (Chmielewski 1971, Baker & Delfinado 1978, Wang *et al*. 2008, Hubert *et al*. 2014, Zhan *et al*. 2017), pero también puede encontrarse en granos almacenados tales como el arroz, el maíz y el trigo (**Figura I.3b**) (Taha *et al*. 2019). Además de dañar y/o contaminar alimentos, *C. lactis* puede causar acariasis cutánea, pulmonar e intestinal, y también ulcera duodenal, las cuales son ocasionadas por la irritación mecánica inducida por los quelíceros de los ácaros, así como por sus secreciones corporales o los microorganismos asociados (Wang *et al*. 2008, Zhan *et al*. 2017).

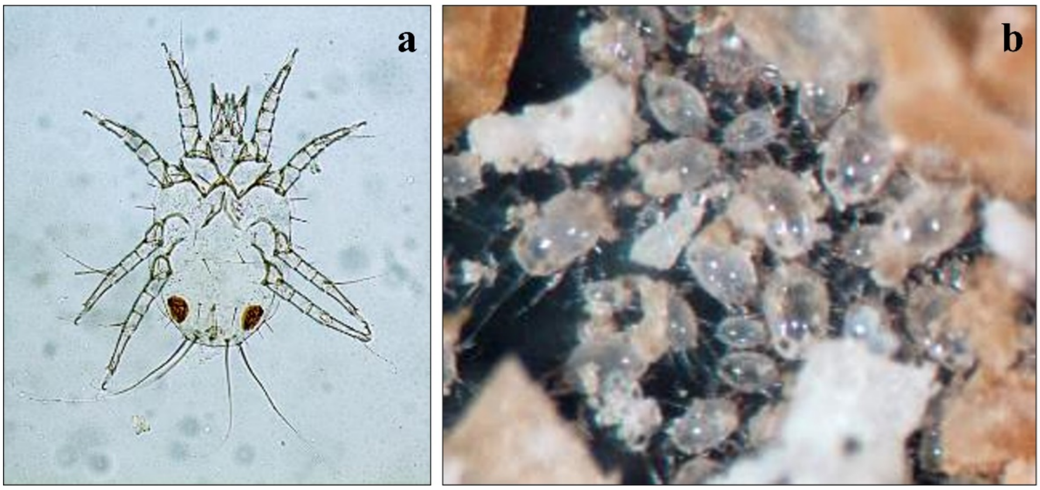

Figura I.3 (a) Adulto de *Carpoglyphus lactis*, (b) Inmaduros y adultos
Fuente: Juan Ramón Gallego

C. lactis tiene una distribución cosmopolita (Hagstrum *et al*. 2013), con una fuerte predilección por entornos húmedos (≥ 90%) y con temperaturas entre los 25 °C y 29 °C (Hubert *et al*. 2014). Al respecto, diversos estudios señalan que la humedad es determinante en la biología de este ácaro (p. ej. Ibrahim 2006, Guldali & Cobanoglu 2010, Taha *et al*. 2019, Bakr *et al*. 2021), cuya sobrevivencia o reproducción no ocurren cuando la humedad es inferior a 50% (Okamoto 1984). De allí que una estrategia para controlar sus poblaciones es mantener ambientes con una humedad relativa inferior al 50%, ya que los ácaros son vulnerables a la desecación (Collins 2006). Sin embargo, la estrategia más utilizada para controlar las poblaciones de *C. lactis* es la fumigación con insecticidas como el bromuro de metilo y la fosfina, entre otros productos (Ferizli *et al*. 2004, Turgu & Kumral 2019), pero esta forma de control está asociada a problemas ambientales y de salud pública que han estimulado la búsqueda de alternativas como el uso de extractos vegetales, ozono o atmosferas modificadas con CO_2 a presión (Emekci *et al*. 2004, Turgu & Kumral 2019, Bakr *et al*. 2021).

Esta especie ha sido recibido especial interés dentro del control biológico debido a su uso como alimento alternativo para ácaros depredadores de la familia Phytoseiidae criados masivamente como agentes de control (Baxter *et al*. 2011, Zhang 2012), especialmente en la producción y liberación de *A. swirskii*, *Phytoseiulus persimilis* (Athias-Henriot) y *Neoseiulus californicus* McGregor, entre otros (Bolckmans & van Houten 2006, Buitenhuis *et al*. 2014, Calvo *et al*. 2015). Adicionalmente, *C. lactis* también se distribuye de forma comercial como complemento alimenticio para mantener las poblaciones de ácaros depredadores en campo.

I.1.2.2. Enemigos naturales

a. *Trichogramma cacaeciae* (Marchal) (Hymenoptera: Trichogrammatidae)

Los parasitoides del género *Trichogramma* (Hymenoptera: Chalcidoidea: Trichogrammatidae) son importantes agentes de control biológico que se han usado con éxito en más de 32 millones de hectáreas anuales alrededor del mundo para el control de varias plagas del orden Lepidóptera (Özder & Kara 2010). En España, se han identificado 15 especies atacando diversas plagas agrícolas y forestales (Gallego 2017), entre las que destaca *T. cacaeciae* (**Figura I.4**), la cual ha sido estudiada por su habilidad para desarrollarse en condiciones de cría masiva (Pizzol *et al*. 2010) y usada en programas de control biológico en viñedos en el país (Moreno *et al*. 2009).

Figura I.4 Hembra adulta de *Trichogramma cacaeciae*
Fuente: Tomás Cabello

T. cacaeciae se encuentra presente en América, Asia, Europa y el norte de África (Tunisia, Morocco y Egipto) (Moreno *et al*. 2010), en donde se ha reportado parasitando huevos de lepidópteros plagas como *Lobesia botrana* Den. & Schiff., *Laspeyresia pomonella* (L.), *Grapholita molesta* (Busck), *Ectomyelois ceratoniae* Zeller, *Prays oleae* Bernard, *Tuta absoluta* (Meyrick) y *P. operculella*, en uva, manzana, durazno, dátiles, olivos, tomate y patata, respectivamente (Grutzmacher *et al*. 2004, Saour 2004, Pintureau 2008, Pizzol & Pintureau 2008, Pizzol 2004, Gallego *et al*. 2020, Schafer & Herz 2020). Esta especie tiene una reproducción partenogénetica de tipo telitóquica en la que las hembras ovipositan huevos diploides que dan lugar a hembras (Vavre *et al*. 2004), lo que además representar una ventaja en la búsqueda y parasitación de huéspedes frente a otras especies del género con reproducción arrenotóquica, ya que *T. cacaeciae* no necesita decidir si ovipositan un huevo fecundado o no (Gallego *et al*. 2020). La biología de *T. cacaeciae* sobre diversos hospederos y condiciones climáticas ha sido estudiada por Özder & Kara (2010) y Pizzol *et al*. (2010), con el propósito de comprender la influencia de los factores abióticos sobre la efectividad de las liberaciones del parasitoide, toda vez que las variaciones de la temperatura y la humedad relativa afecta parámetros como la tasa de emergencia, fecundidad, longevidad y sex ratio de la progenie (Cherif *et al*. (018).

Los aspectos ecológicos de *T. cacaeciae* también han sido abordados con el propósito de conocer su potencial para la cría masiva y su dinámica poblacional en campo en liberaciones aumentativas o por inundación (Sigsgaard *et al*. 2017, Cherif *et al*. 2018), de modo que las investigaciones se han centrado en estudiar la adaptabilidad del parasitoide a la conservación en frío (Özder 2008, Özder & Kara 2010, Pizzol *et al*. 2010) y/o la inducción de diapausa para almacenarlos y liberarlos en el momento oportuno (Pizzol & Pintureau 2008). Así mismo, se ha estudiado su supervivencia al efecto de insecticidas botánicos o químicos (Grutzmacher *et al*. 2004, Hassan *et al*. 1998, Saber *et al*. 2004) y su efectividad en el parasitismo de diversas plagas (Saour 2004, Moreno *et al*. 2009, Scheffer & Herz 2020).

T. cacaeciae ha sido liberada con éxito en cultivos de tomate, manzana, dátiles y viñedos, en donde ha sido eficiente en alcanzar altas tasas de parasitismo de huevos de plagas como *T. absoluta*, *Cydia pomonella* (L.), *E ceratoniae* y *L. botrana*, respectivamente (Hommay *et al*. 2010, Cherif & Kaouthar 2013, Sigsgaard *et al*. 2017, Cherif *et al*. 2018, Zougari *et al*. 2021, Zouba *et al*. 2022). De igual forma, el potencial de *T. cacaeciae* en el control de *P. operculella* sobre patatas en condiciones experimentales de almacenamiento también ha sido estudiado por

Saour (2004) quien indicó que el parasitoide mostró una óptima actividad de parasitismo, siendo capaz de moverse dentro de los tubérculos de patata en busca de los huevos de la polilla.

b. *Blattisocius mali* (Oudemans) (Mesostigmata: Blattisociidae)

El género *Blattisocius* está conformado por quince especies cosmopolitas comúnmente asociadas a productos almacenados (Esteca *et al*. 2014, Gallego *et al*. 2019), pero sus especies también pueden encontrarse en la naturaleza como depredadores de vida libre en el suelo, en donde se alimentan de pequeños artrópodos, huevos de insectos y nematodos (Hagstrum *et al*. 2013, Athanassiou & Rumbos 2018). Debido a su potencial como agentes de control biológico, algunas especies del género tales como *B. mali* (**Figura I.5**), *B. tarsalis* (Berlese), *B evertii* Britto y *B. keegani* Fox han sido estudiadas para conocer su efectividad en el control de plagas como las polillas *Amyelois transitella* Walker, *Ephestia kuehniella* Zeller y *P. operculella*; los gorgojos *Lasioderma serricorne* (F.) y *Zabrotes subfasciatus* (F.); y el ácaro *Rhyzoglyphus robini* Claparéde (Athanassiou & Palyvos 2006, Thomas *et al*. 2011, Esteca *et al*. 2014, Gallego et al. 2019, Abbas *et al*. 2020, Gavara *et al*. 2021). Sin embargo, *B. tarsalis* y *B. keegani* destacan por el número de investigaciones relacionadas con su biología en diversas condiciones experimentales para el control de insectos y ácaros tal como sintetizaron Thomas *et al*. (2011).

Figura I.5 Adulto de *Blattisocius mali* y huevos de *P. operculella*
Fuente: Juan Ramón Gallego

De acuerdo con Hagstrum *et al*. (2013), *B. mali* se encuentra distribuido en Asia (China, India, Taiwán), África (Egipto) y Europa (Inglaterra, Grecia, Holanda, Polonia, y Turquía), en donde recientemente fue registrada en España (Gallego *et al*. 2019). Este depredador suele encontrarse en almacenes de cereales y/o alimentos procesados como el arroz, la avena, el girasol, el maíz, el trigo y las harinas, pero también en aquellos que contienen frutos secos o deshidratados como la avellana, el higo, las uvas pasas y verduras deshidratadas (Cakmak *et al*. 2011, Hagstrum *et al*. 2013), los cuales ofrecen condiciones óptimas de humedad y temperatura para su desarrollo. El registro de presas de *B. mali* incluye los ácaros *Acarus siro* L., *Carpoglyphus lactis* (L.) y *Tyrophagus putrescentiae* (Schrank), y sobre ésta última presa se han dirigido estudios experimentales para determinar aspectos biológicos y reproductivos de *B. mali* (Hughes 1976, Pirayeshfar *et al*. 2021, Pirayeshfar *et al*. 2022).

La literatura sobre la actividad depredadora de *B. mali* es escasa, particularmente sobre condiciones de campo y/o almacenamiento a gran escala. Sin embargo, el estudio de Gallego *et al*. (2019) sobre el potencial de este depredador en el control de la polilla de la patata abre la posibilidad de nuevas investigaciones que complemente su perfil como agente de control de plagas en condiciones de almacenamiento. En su trabajo, Gallego *et al*. (2019) destacaron que *B. mali* mostró una alta efectividad como depredador y un buen desempeño para dispersarse entre los tubérculos en busca de huevos de *P. operculella*. Así mismo, el potencial de producción masiva de *B. mali* también ha sido explorado por Pirayeshfar *et al*. (2021) sobre *T. putrescentiae*, lo que a juicio de Thomas *et al*. (2011) representaría una innovación en el uso de ácaros no fitoseidos para el control biológico de lepidópteros. Dentro de este contexto, Gallego *et al*. (2020) y Gavara *et al*. (2021) también señalaron la importancia de otra especie del género *Blattisocius* en el control de la polilla de la patata, al estudiar el comportamiento y actividad de depredación de *B. tarsalis* sobre huevos de *P. operculellla*, respectivamente.

c. *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae)

El ácaro depredador *A. swirskii* (**Figura I.6a**) es un importante agente de control biológico de ácaros y de insectos plagas como los recopilados por Buitenhuis *et al*. (2014) y Calvo *et al*. (2015). Esta especie es originaria de la costa mediterránea oriental (Israel) y se ha diseminado a países de Oriente medio (Gaza, Egipto, Israel), del sur de Europa (Azerbaijan, Georgia, Italia, España), África (Benin, Burundi, Cabo Verde, Congo, Ghana, Kenia) y América (Argentina, USA) (Calvo *et al*. 2015, Demite *et al*. 2022), en donde se ha registrado en cultivos de algodón, cítricos, hortalizas y viñedos. La biología de *A. swirskii* ha sido estudiada sobre diversas dietas y condiciones climáticas con el propósito de comprender su eficiencia como depredador, adaptabilidad a medios de cría y a las condiciones de campo (p. ej. Park *et al*. 2010, Lee & Gillespie 2011, Asgari *et al*. 2020, Farazmand & Amir-Maafi 2021).

Desde el año 2005, *A. swirskii* se produce comercialmente y se usa en el control biológico aumentativo para regular las poblaciones de diversas especies de ácaros, moscas blancas y trips (**Figura I.6b**) (Buitenhuis *et al*. 2014, Knapp *et al*. 2018). Este depredador destaca entre los ácaros más utilizado en el control biológico ya que puede usarse simultáneamente en la lucha contra varias plagas debido a su carácter generalista (Buitenhuis *et al*. 2014, Calvo *et al*. 2015), pero también porque puede liberarse de forma preventiva aún en ausencia de presas, ya que es capaz de desarrollarse y reproducirse cuando se alimenta de polen (Calvo *et al*. 2015). Adicionalmente, *A. swirskii* tiene un buen perfil de acción en zonas con amplia fluctuación de los niveles de humedad y temperatura como ocurre dentro de los invernaderos durante el verano (Buitenhuis *et al*. 2014, Buitenhuis *et al*. 2015), lo cual refuerza su utilidad y adaptación en condiciones que serían extremas para otros ácaros depredadores (Ferrero *et al*. 2010).

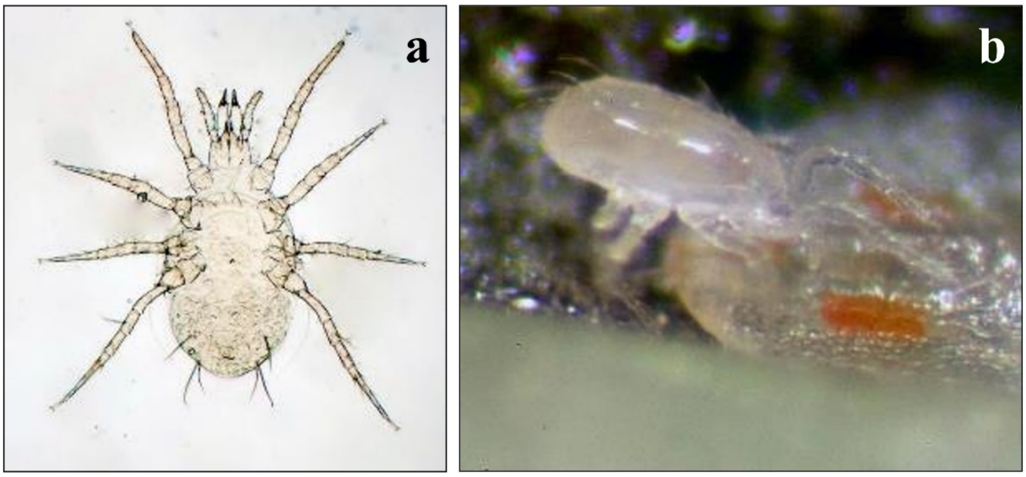

Figura 6 (a) Vista ventral de *Amblyseius swirskii*, (b) Adulto depredando a ninfa de *Bemisia tabaci*
Fuente: Juan Ramón Gallego, Tomás Cabello

La liberación de *A. swirskii* inicialmente se realizó utilizando envases con salvado de trigo como sustrato de soporte, y su distribución entre las plantas se realizaba de forma manual (Calvo *et al*. 2015) o con el uso de sopladores mecánicos (Takano-Lee & Hoddle 2001, Opit *et al*. 2005), pero actualmente la suelta del ácaro se ha optimizado a través de diversos mecanismos como el uso de fuentes suplementarias de alimentación (polen y/o huevos de polillas) (Delisle *et al*. 2015, Kumar *et al*. 2015), banker plants (Buitenhuis *et al*. 2014, Kumar *et al*. 2015) y los sobres de liberación controlada (Midthassel 2015, Addesso *et al*. 2018), o también llamados sobres de cría porque son dispositivos que contienen tanto a *A. swirskii* como a una presa (p. ej. *C. lactis*, *Suidasia medanensis* (Oudemans), *Thyreophagus entomophagus*

(Laboulbene and Robin) que permite el desarrollo del depredador y su liberación progresiva hacia el cultivo (Bolckmans & van Houten 2006).

Investigaciones recientes avalan el impacto positivo de los sobres de liberación de *A. swirskii* en la regulación de la población de plagas en campo (Buitenhuis *et al*. 2014, Lanzoni *et al*. 2017, Abou-Haidar *et al*. 2021), sin embargo, existen aspectos que aún deben ser abordados para mejorar la tecnología de los sobres y hacer que su uso sea más rentable y eficiente, entre tales aspectos se encuentran la humedad interna de los sobres para mantener la hidratación de los ácaros durante su estancia en el campo, la dinámica poblacional del depredador y su presa en el interior de los sobres y el perfil de liberación del depredador en función de las condiciones climáticas (Buitenhuis *et al*. 2014, Midthassel 2015).

I.2. Objetivos

I.2.1. Objetivos generales

El objetivo general de esta tesis doctoral fue estudiar la aplicación de modelos logísticos en poblaciones de artrópodos plaga y sus estrategias de control. Para este propósito, se eligieron las especies plaga *Rhynchophorus ferrugineus* (Olivier), *Phthorimaea operculella* (Zeller) y *Carpoglyphus lactis* (L.), y los agentes de control biológico *Trichogramma cacaeciae* (Marchal), *Blattisocius mali* (Oudemans) y *Amblyseius swirskii* (Athias-Henriot).

I.2.2. Objetivos específicos

- Desarrollar un modelo logístico del comportamiento fenológico de *R. ferrugineus* en climas de invierno frío.

- Fijar un modelo de control óptimo para simular el momento oportuno de aplicación del control químico de larvas de *R. ferrugineus*.

- Evaluar la efectividad del control biológico de *T. cacaeciae* y *B. mali* sobre *P. operculella* en condiciones de almacenamiento de patatas.

- Analizar la efectividad de *T. cacaeciae* y *B. mali* en el control de *P. operculella* desde el enfoque de un modelo logístico.

- Desarrollar una metodología para evaluar la dinámica poblacional del depredador *A. swirskii* y su presa *C. lactis* en el interior de sobres de liberación lenta, y en la tasa de liberación del depredador.

- Evaluar el efecto del microclima sobre la dinámica poblacional de *A. swirskii* y *C. lactis* en el contexto de sobres de liberación lenta, y fijar un modelo matemático para representar el efecto de la humedad relativa en la velocidad y periodo de liberación del depredador.

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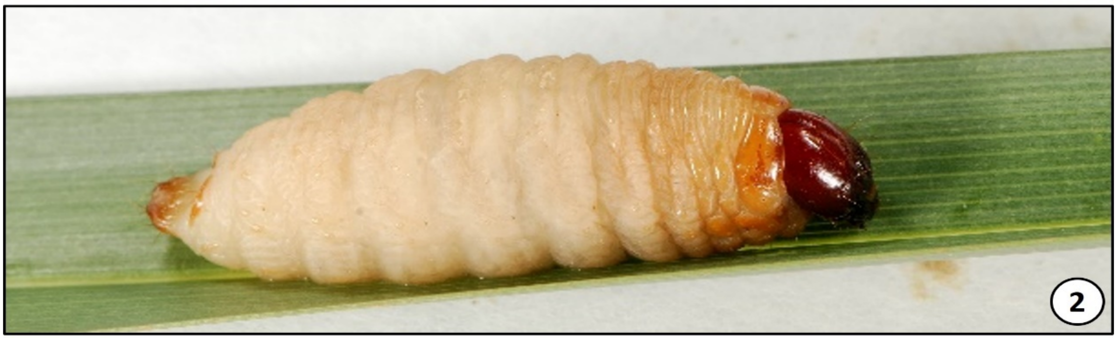
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Ilustraciones página anterior:

1. Tallo de palmera dañado por larvas de *R. ferrugineus* (Fuente: Tomás Cabello).
2. Larva de *R. ferrugineus* (Fuente: Tomás Cabello).

Capítulo II

Conservation strategy for palm groves: Optimal chemical control model for red palm weevil, *Rhynchophorus ferrugineus*

Y Solano-Rojas, M Gámez, I López, J Garay, Z Varga, T Cabello. 2021. Conservation strategy for palm groves: Optimal chemical control model for red palm weevil, *Rhynchophorus ferrugineus*. Agronomy 11(11): 920. DOI: 10.3390/agronomy11050920

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Capítulo II. Conservation strategy for palm groves: optimal chemical control model for red palm weevil, *Rhynchophorus ferrugineus*

Abstract

Rhynchophorus ferrugineus (Olivier, 1790) is an invasive pest species that constitutes one of the most important problems around the Mediterranean region and has been responsible for the loss of over 100,000 palm trees with an estimated annual cost of EUR several hundred million since its introduction into Europe. Methodological approaches of conservation ecology, such as multidisciplinary modelling, also apply in the management of cultural landscapes concerning ornamental plants, such as palm trees of the area. In this paper, we propose a dynamic model for the control of the red palm weevil, contributing in this way to the sustainability of an existing cultural landscape. The primary data set collected is a sample from the density-time function of a two-cohort pest population. This data set suggests a bimodal analytic description. If, from this data set, we calculate a sample from the accumulated density-time function (the integral of the density-time function), it displays a double sigmoid function (with two inflections). A good candidate for the analytical description of the latter is the sum of two logistic functions. As for the dynamic description of the process, a two-dimensional system of differential equations can be obtained, where the solution's second component provides the analytical description of the original density-time function for the two-cohort population. Since the two-cohort waves apper in all three cycle stages, this reasoning applies to the subpopulations of larvae, pupae and adults. The model fitting is always performed using the SimFit package. On this basis, a mathematical model is proposed, which is sufficiently versatile to be of help in the control of this pest species in other geographical areas.

Keywords: insect population dynamics, cultural landscape, plant protection, numerical modelling, sigmoid functions.

II.1. Introduction

The red palm weevil, *Rhynchophorus ferrugineus*, hereinafter referred to as RPW (Oliver, 1790) (Col.: Dryophthoridae), is a species native to Asia and Polynesia, and is characterized as an invasive pest of great severity and economic importance worldwide [1,2]. This species has expanded from its area of origin to different continents. It has been recorded in 28 countries in Asia, six in Africa, one in North America, two in Central America and the Caribbean, 14 in

Europe and five in Oceania, causing serious problems in coconut trees, both in crop cultivation and ornamental use [1,3].

In Spain, RPW has spread widely over the Mediterranean coast, the interior of the peninsula and the islands, causing significant ecological and economic damage in areas such as the "Palmeral de Elche" (in the Valencian Community), declared a World Heritage Site by UNESCO, and in the *Phoenix canariensis* forest on the Canary Islands, which is a natural heritage site and represents the most relevant source of genetic variation for this palm tree [1,4,5]. Many aspects of RPW biology and ecology have been widely studied and are reported in several publications (e.g., [1,6-9]). However, the cold survival strategy adopted by this species, native to warm geographic areas, remains poorly known [10].

RPW infestations threaten agriculture (e.g., date plantations), affect the aesthetics of infested palm landscapes and uncontrolled urban populations (e.g., UNESCO World Heritage palm groves) and threaten natural areas (e.g., palm oases and endemic palm) [1,8,9,11,12]. In Spain during the 2005-2009 period, between 47,000 and 50,000 infested palm trees were destroyed; this involved containment/eradication cost of EUR 44.5 million, particularly in the Valencian Community where professional and family managed nurseries were affected [11,13,14]. In this regard, the FCEC [11] considered that infested (but not destroyed) palm trees might result in losses of between EUR 96 and 288 million.

Insecticide applications are, at present, the most effective method for protecting palms from attack by palm weevils [8]. Larval control, mainly by infection of insecticide into the trunk, carried out during the early stages of infestation, sometimes accompanied by application of spray for adult control, is currently the most employed (e.g., [3,8,15-19]). This is usually accompanied by monitoring and mass trapping of adults using semiochemicals (e.g., [3,9,20]). It should be noted that other control methods have been studied and developed, e.g., biological control (with entomophages and entomopathogens) and genetic methods (sterile backcrosses, sterile insect technique and chemosterilization), including two or more of the above control measures in integrated pest management programmes (e.g., [3,8,20]). The severity of *R. ferrugineus* and the problems associated with its control, including the difficulty of detecting infested palm trees early, the risk of mass captures in non-infested areas and the loss of control strategy effectiveness [9], motivate the search for new tools that increase the bioecological knowledge for dealing with RPW populations. In this regard, mathematical models can offer a holistic approach to understanding the biological behaviour of species, and they are an instrument for predicting and/or simulating complex systems that include the population dynamics of the pest and its control strategies [21,23].

In the plant protection field, mathematical models have focused on using sigmoid functions, such as Verhulst-Pearl or logistics, Gompertz, Weibull, Richards and monomolecular, as well as those models that combine them [24-27]. These functions have also been integrated to optimize the use of the resources and time spent on combating pests, through the formulation of optimal control problems aimed at keeping a pest population below the economic damage level as well as reducing the need to implement control strategies [21-23,28,29]. The existing literature on mathematical models related to the ecological aspects of *R. ferrugineus* is scarce, with only the work of Rossini et al. [30], who proposed a linear model for RPW development as a function of temperature. However, Hansen *et al.* [31] adapted a logistic model for another Curculionidae species, *Dendroctonus rufipennis* (Kirby), based on temperature as a diapause prediction factor of the insect's fourth larval stage. It should be noted that the larval diapause in Curculionidae has also been recorded for *Curculio elephans* Gyllenhal, *C. sikkimensis* (Heller) and *Smicronyx fulvus* LeConte, where the larvae were subjected to temperatures below 15 ºC [32-35].

In the *R. ferrugineus* larvae, Martín and Cabello [1] recorded a slowdown in the development of their last stages when the temperature dropped and remained in the 10-15 ºC range for a period of more than 80 days, arguing that this phenomenon was due to a larval diapause, because the delay in the larval development and metabolic activity was related to changes in temperature [36,37]. The importance of RPW within palm production systems and/or ecosystems, and the need to deepen our understanding of the population behaviour of its stages, is fundamental to the development of mathematical models that can describe the population dynamics of the larvae, pupae and adults of *R. ferrugineus*, and to propose an optimal solution to control their larvae. This was the objective of this work. The resulting modelling-methodological development also contributes to the sustainable management of the involved cultural landscape of palm groves and ornamental palm trees.

The objectives of this work were to answer two initial issues:

(1) Is it possible to fix a mathematical model for the biology of RPW in conditions of cold winter climates?

(2) Once the above model has been fixed, is it possible to use an optimal control model to simulate the optimum time for application of chemical larval control?

II.2. Materials and methods

II.2.1. Biological data used

The trial was carried out in the “Cortijo del Olivar”, Municipal District of Almuñecar, Granada, Spain, 50 m above sea level (13.748219 N, 3.690619 W). It was in a room, communicated with the outside, located inside a country building; it was carried out between July and September of the following year. The trial started with a total of 70 RPW eggs, less than 24 h old, which were obtained from the laboratory rearing populations (Entomology Laboratory, University of Almeria, Almeria, Spain). The food substrate for larvae and adults was sugar cane (cultivar: “Algarrobeña”; traditionally grown in southern Spain, Almuñecar). Specimen handling was carried out following the methodology described in [38,39]. After development, 13 pairs were used for the evaluation of adult parameters. Data recorded were number of days for egg, larval and pupal stages, as well as mortality at these stages; adult longevity and fecundity were also recorded. Temperature was recorded daily by thermograph.

II.2.2. Mathematical model

The primary data set collected is a sample from the density-time function of a two-cohort pest population. This data set suggests a bimodal analytic description. Originally, the single logistic function is:

$$\frac{K}{1+\left(\frac{K}{X_0}-1\right)e^{-rt}} \qquad (1)$$

Where x_0 is the initial population; r: the logistic growth rate or steepness of the curve; K: the curve’s saturation value; in function (4) below, I subscript (=1,2) indicate the first or second cohort of the population. Function (1) is the solution of the logistic differential equation:

$$r' = rx\left(1 - \frac{x}{K}\right)$$

with the initial condition x (0) = x_0. Function (1) is sigmoid; its derivate, with appropriate constants a_0, a_1, $a_2 > 0$, is

$$y_1(t) = \frac{a_0 e^{-a_1 t}}{(1 + a_2 e^{-a_1 t})^{2'}} \qquad (2)$$

displaying a unimodal curve. The sample from the density-time function of a two-cohort pest population suggests a bimodal analytic description, and for this, the sum of two functions of type (2) is a promising candidate [25]:

$$y_s(t) = \frac{a_0 e^{-a_1 t}}{(1 + a_2 e^{-a_1 t})^{2}} + \frac{a_3 e^{-a_4 t}}{(1 + a_5 e^{-a_4 t})^{2'}} \qquad (3)$$

where a_0, a_1, a_2, a_3, a_4, $a_5 > 0$ are the fitting parameters.

If we calculated a sample from the accumulated density-time function (the integral of the density-time function) from the data set of the density-time function, it shows a double sigmoid function (with two inflections). From the above, we conclude that a good candidate for the analytic description of the latter is the sum of two logistic functions (1):

$$\frac{K_1}{1+\left(\frac{K_1}{X_{01}}-1\right)e^{-r_1 t}} + \frac{K_2}{1+\left(\frac{K_2}{X_{02}}-1\right)e^{-r_2 t}} \qquad (4)$$

Here, K_i, r_i, $x_{oi} > 0$ (I = 1,2) are the fitting parameters.

It is worth emphasizing that, if we consider a two-cohort pest population of *Rhynchophorus*, the above reasoning can be applied to all the developmental stages: the larvae, pupae and adults.

II.2.3. Dynamic model of optimal chemical control

Considering any given stage, from functions (2) and (3), we easily obtain the following system of differential equations, where y_1 is the density-time function of a single cohort, and y_2 is the density-time function of the two-cohort population:

$$y_1' = a_1 y_1 \left(1 - \frac{2}{1 + a_2 e^{-a_1 t}}\right) \qquad (5)$$

$$y_1' = a_1 y_1 \left(1 - \frac{2}{1 + a_2 e^{-a_1 t}}\right) + a_4 (y_2 - y_1)\left(1 - \frac{2}{1 + a_5 e^{-a_4 t}}\right) \qquad (6)$$

Although the dynamic model (5)-(6) can be adapted to any stage, for the chemical control of *Rhynchophorus*, the system (5)-(6) is used for a two-cohort larval population [1]. An operational time interval is fixed [0, T], and the set of admissible controls U_ε [0,T] consisting of piecewise constant functions over a fixed uniform division of interval [0,T] with $0 \leq u(t) \leq \varepsilon$ is defined. Constants μ_1, $\mu_2 > 0$ express the efficiency of the chemical on the respective populations. Then, from the (5)-(6) dynamics, we obtain the following control system:

$$y_1' = a_1 y_1 \left(1 - \frac{2}{1 + a_2 e^{-a_1 t}}\right) - \mu_1 u y_1 \qquad (7)$$

$$y_2' = a_1 y_1 \left(1 - \frac{2}{1 + a_2 e^{-a_1 t}}\right) - \mu_1 u y_1 + a_4 (y_2 - y_1)\left(1 - \frac{2}{1 + a_5 e^{-a_4 t}}\right) - \mu_2 u y_2 \qquad (8)$$

Now, on the basis of this control system, we set up the following optimal control problem: suppose that the damage caused by larval density y_2 in unit time is αy_2; then, the total damage during the time interval [0,T] is $\int_0^T \alpha y_2(t)dt$. If this damage is remains below a boundary, *H*, for the palm tree to survive, and we wish to minimize the cost of the chemical control proportional to $\int_0^T u(t)dt$, we obtain the following constrained optimal control problem:

$$\left.\begin{array}{c} \Psi(u) = \displaystyle\int_0^T u(t)dt \to Min, \\ u \in U_\varepsilon[0,T] \\ \text{Equations (5) – (6) hold} \\ \displaystyle\int_0^T \alpha y_2(t)dt \leq H \\ y_1(0), y_1(0) \text{ are given} \end{array}\right\} \qquad (9)$$

II.2.4. Software used

The above-mentioned mathematical models were fitted to the data using the Table-Curve 2d v 5.0 [40] and SimFit v 6.9.8 [41] software packages.

II.3. Results

The results found were used to carry out the adjustment to the proposed mathematical model that explains the population dynamic of the insect's life-cycle stages (Section 3.1). Subsequently, with this model, a control variable was used that would allow us to set the time for the chemical control of the pest in order to obtain an optimal result.

II.3.1. Results of model fitting

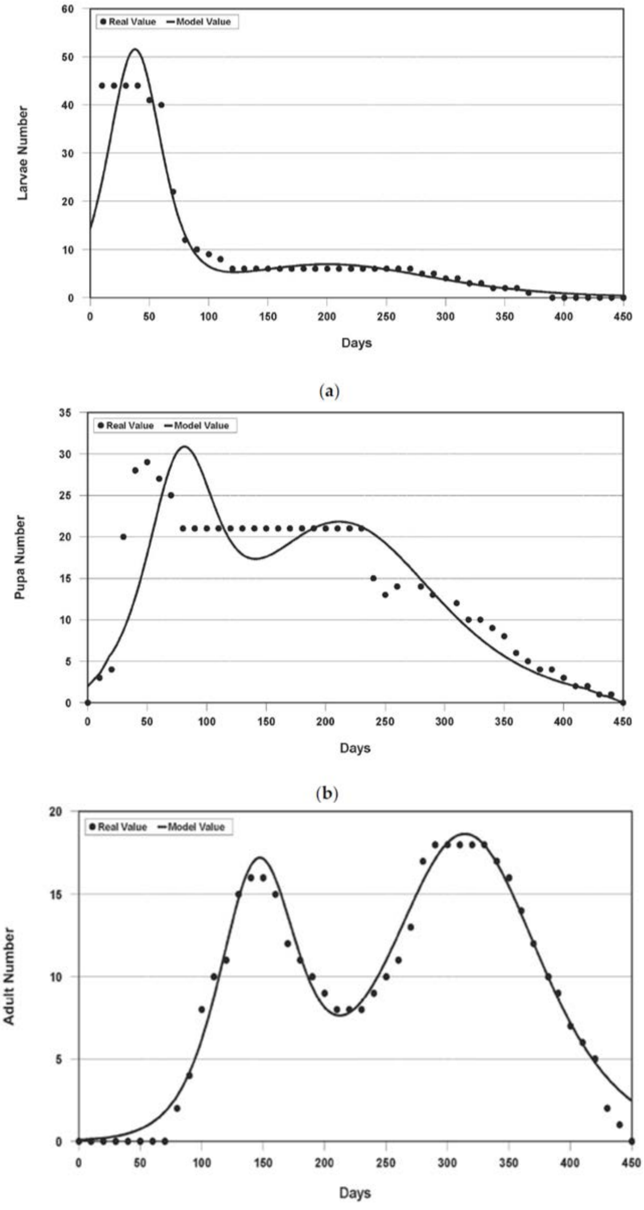
The results show that a portion of the larval population of *R. ferrugineus* developed normally (relative maximum in tp_1), while another portion of the population lengthened its development up to 370 days (**Table II.1** and **Figure II.1**). In the pupal and adult stages, two relative maxima (tp_1 and tp_3) are shown with a distance between them of approximately 160 days.

Table II.1 Values of fitting parameters of model (3) for all stages of pest population of *Rhyncophorus*

Stage	Fitting Parameters (*)						Statistical Parameters		
	a_o	a_1	a_2	a_3	a_4	a_5	d.f.	R^2 adj.	P
Larvae	673.739	0.04410	3.56691	2117.26	0.01967	80.0043	38	0.97654	< 0.01
Pupa	74980.4	0.05752	792.265	10500.9	0.02003	115.495	40	0.97744	< 0.01
Adult	28372.7	0.04231	485.713	218719	0.02546	2901.76	40	0.95999	< 0.01

$$(*)\ a_0 = \left(\frac{K_1}{x_{01}} - 1\right).K_1.r_1;\ a_1 = r_1\ ;\ a_2 = \left(\frac{K_1}{x_{01}} - 1\right)$$

$$a_3 = \left(\frac{K_2}{x_{02}} - 1\right).K_2.r_2;\ a_4 = r_2\ ;\ a_5 = \left(\frac{K_2}{x_{02}} - 1\right)$$



(a)

(b)

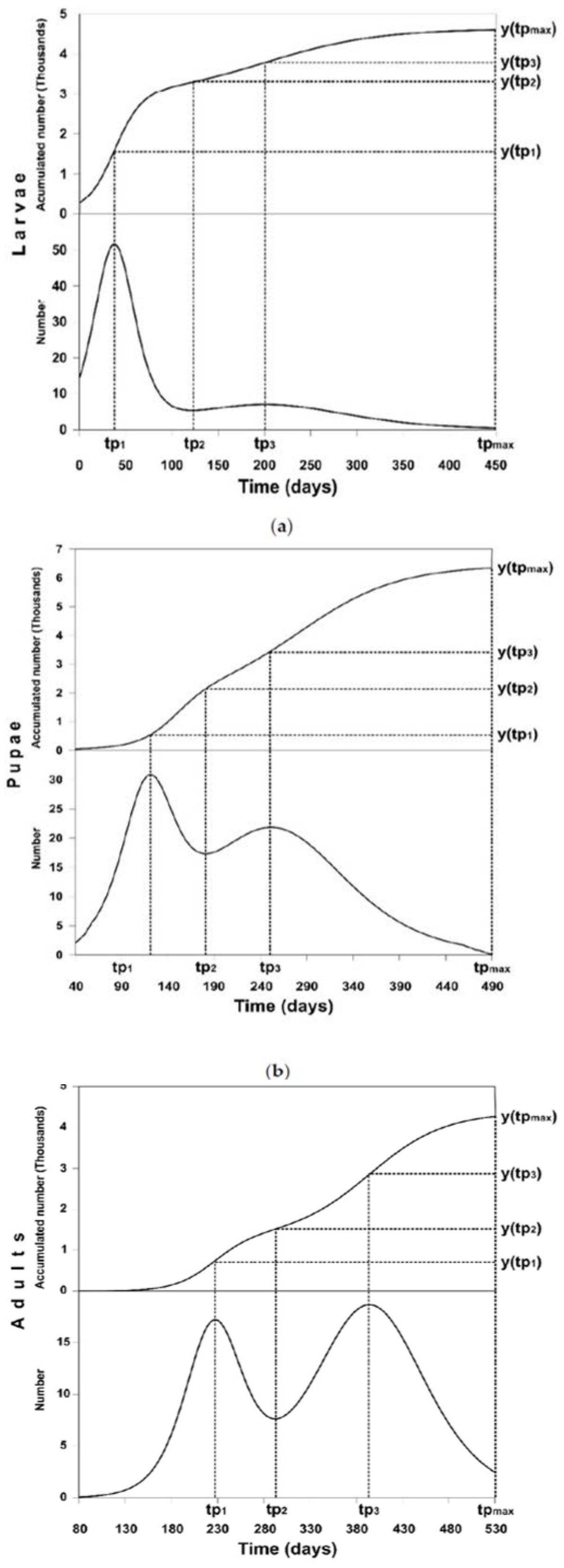
(c)

Figure II.1 Fitted curve of density-time function model (3) for larvae (**a**), pupae (**b**) and adults (**c**) of *Rhynchophorus ferrugineus*

We noted that the R^2 value indicates that the goodness of fit is better (higher) for the double logistic function fitted to the accumulated data (0.99951) than for the fitting dynamics (5)-(6) (0.90319). Similar results were obtained for the rest of the analyzed data. This observation justifies the use of a sigmoid function fitted to the accumulated data, smoother than the original density data, which displayed more random oscillations. Subsequently, double logistic curves were also fitted to the accumulated density data (see **Table II.2** and **Figure II.2**). In the latter, we also indicate the time moments t_k, were the minima and maxima of the densities are attained, according to the inflexions of the accumulated densities.

Table II.2 Values of fitting parameters of double logistic functions for pest populations of *Rhynchophorus*

Stage	Term (i)	Fitting Parameters			Statistical Parameters		
		K_i	x_{oi}	r_i	d.f.	R^2 adj.	P
Larva	1	2951.43	214.53	0.06771	37	0.99764	< 0.01
	2	1673.39	57.6115	0.01661			
Pupa	1	1810.51	4.8024	0.05484	40	0.99872	< 0.01
	2	4621.58	47.1067	0.01889			
Adult	1	1342.24	1.2492	0.04789	43	0.99951	< 0.01
	2	3031.58	1.32482	0.02461			



(a)

(b)

(c)

Figure II.2 Mathematical models (double logistic functions) fitted to the accumulated data of the palm pest species, *Rhynchophorus ferrugineus* (a) larvae, (b) pupae and (c) adult stages, also displaying the corresponding bimodal density time functions.

II.3.2. Solution of the optimal control problem

For a palm tree to survive, our objective is to keep the effect of the larval population under a boundary, *H*, at minimum cost. Using the **Table II.1** fitting parameters, we solved the optimal control problem (9) with H = 2000 units of biomass, T = 450, $\mu 1 = 0.95$, $\mu 2 = 0.5\alpha = 0.7$, $\varepsilon = 0.1$, $y1\ (0) = 13$, $y2\ (0) = 14$. We solved the optimal control problem applying the toolbox develop for MatLab by Banga *et al*. [42], the results of which are shown in **Figure II.3**.

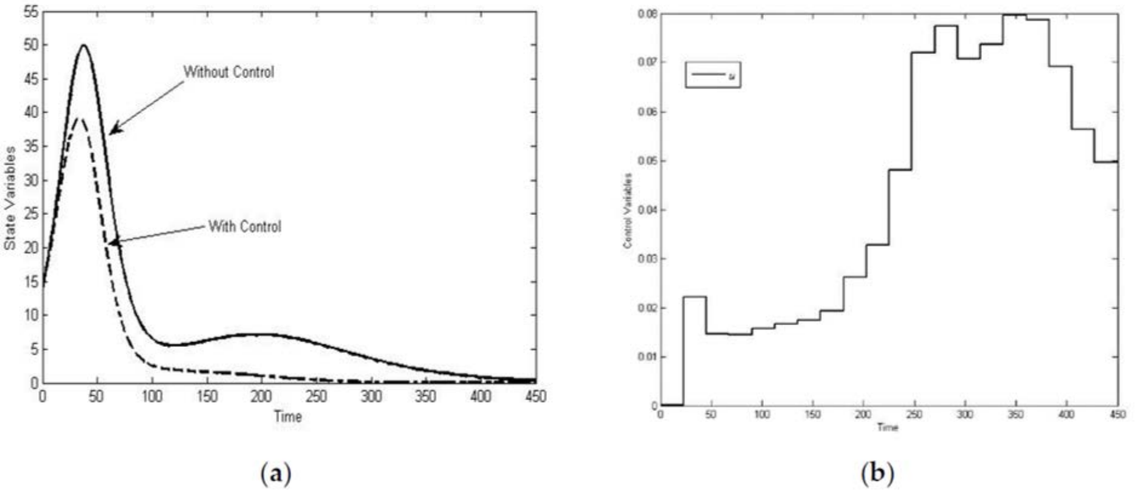


Figure II.3 Trajectories for larva stage y_2 without control in model (5)-(6) and with optimal control. (b) Optimal control to be applied and solution of problem (7)-(8).

II.4. Discussion

A plasticity is well understood when it takes the form of responding to predictive environmental signals received during the individual’s lifespan. However, the so-called mixture of intragenotypic strategies has been neglected, perhaps due to the general assumptions that it must be non-adaptive [43]. In this regard, several species of insects, especially from the Coleoptera order, may present two types of larval diapause (simple or prolonged), leading to diverse cohorts that differ in their life cycle, especially in terms of duration. The causes of what Ushatinskyaya [44] called “superdiapause” are poorly studied and not understood in detail. In our case, it seems clear from the results that RPW belongs to this group of insects.

The results for *R. ferrugineus*, showed that a portion of the larval population develop normally, but another portion could lengthen their development up to 370 days (**Figure II.1**); this is demonstrated by the two relative maxima found in the pupal and adult stages. The above statement might indicate that the diapause exists as a survival mechanism. This phenomenon was later verified in other trials [1]. The presence or lack of a diapause in this species is crucial for the early detection and control of the pest, as well as for border inspections, since detection is carried out by ultrasound – if the larvae are in diapause, they are not feeding, and, therefore, do not make a noise with their jaws that can be detected [45]. Similarly, the results can be used to develop more complex predictive models to track the dynamics and expansion of this pest species.

The results of the logistic model developed for the population dynamics of the RPW larvae show a delay of larval development that would relate to the diapause manifesting as a survival mechanism. Martín and Cabello [1] noted that the diapause of RPW larvae was stimulated by a decline in temperature, of values between 10 °C and 15 °C, during the larval growth stage. Additionally, they indicated that this phenomenon promoted the division of the larval population into two groups: the first formed before the temperature drop occurred, in which the larvae continued their normal development (such as that recorded in tp_1; **Figure II.1a**), while the second group delayed its development by 170 days (similar to that shown from tp_2 onwards; **Figure II.1a**), with the consequent delay in pupal and adult morphogenesis (as in the relative maxima, tp_1 and tp_3, in **Figure II.1b**,**c**, respectively).

The larval development behaviour of *R. ferrugineus* may represents a survival strategy that offers the maximum development opportunity in a univoltine cycle, as one group of larvae is able to accelerate their development to promote adult formation during the spring, while the other can slow down its development, influenced by the low winter temperatures, allowing adults to form that emerge during the autumn. This behaviour was also recorded by Hansen *et al.* [31] for the curculionid *D. rufipennis,* whose larval diapause was mathematically modelled in a logistic function that considers the thermal threshold as a predicting factor [31]. Other Curculionidae may also have a larval diapause in response to the drop in temperature, as in the case with *Curculio elephas* Gyllenhal [32,33], *C. sikkimensis* (Heller) [34] and *Smicronyx fulvus* LeConte [35].

Whether or not the RPW larvae are in diapause is crucial for early detection and pest control because most RPW inspection systems include equipment capable of detecting ultrasound produced by the larvae during feeding [46,47]. Therefore, detecting no noise will not mean the absence of larvae inside the palm stems, since diapause larvae do not feed [45].

The mathematical models developed for the larval, pupal and adult stages of RPW constitute a valuable tool for bioecological and/or prediction studies at the population level of this pest in various Spanish zones where the ambient temperature varies over the year and can even stay below 15 °C during the winter months. At this time, the larvae can slow their development as a survival mechanism, as recorded by Martín and Cabello [1] in the municipality of Almuñecar (Granada, Spain) from the month of November to March, in which the temperature, both of the air and inside the palm tree, remained between 10 °C and 15 °C, with fluctuations within the palm tree of around 10 °C for more than 80 days.

In this regard, mathematical models can be used in various parts of Spain where palm trees are grown, such as the Andalusian coastal zones of the Mediterranean and the coastline from the Ebro Delta to Gibraltar, the *Valle bajo* of the Guadalquivir and Guadiana, as well as in coastal areas of the *Rias Bajas* in Galicia, where the ambient temperature can range from 10 °C to 12.5 °C during the month of January [48], also having an impact on the internal temperature of the palm trees. Moreover, the optimal control model for RPW larvae reveals that the application of a chemical control measure can significantly reduce the initial larval population peak over the first 50 days. The action of chemical control also produces a significant decline in the second larval maximum, which begins to form from about day 100 in the uncontrolled population.

In turn, the rise in the chemical control trajectory seen in **Figure II.3**, which begins after day 100, corresponds to the need to increase the use of insecticide to keep the palm biomass threshold below the economic damage level, since the larval development continues in this period, having been delayed by the temperature decline experienced by the population without chemical control. Similarly, this increase in the control trajectory can be related to the formation of RPW pupae and/or adults, which require greater control.

The logistic models developed for the population dynamics of the *R. ferrugineus* stages, as well as the optimal control model proposed, are instruments that can be incorporated into integrated pest control programmes, with the aim of improving decision making and reducing the cost of managing the RPW population. Similarly, these tools can be included within more complex prediction models, allowing us to understand RPW dynamics and expansion.

In this regard, it should be noted that this mathematical model has several advantages:

(A) The model is sufficiently simple and flexible to allow its extension to this pest species in other geographical areas.

(B) It saves some of the costs of experimentation on the effectiveness of the chemical control of RPW, as simulations can be carried out before expensive field trials, providing valuable information in advance.

(C) With the introduction of optimization into the model, the model could be used to calculate the ideal timing of chemical pest control, depending on climatic conditions and/or stage of infestation, with potential savings in treatment costs.

In summary, the results from the logistic models, for the pest species as well as for the different aspects of their biology/ecology, suggest that they could be an excellent tool to use in integrated pest management (IPM) programmes. This may also corroborate the results found regarding the dynamics of crop diseases [24,25], as their use can be generalized to crop protection.

II.5. Acknowledgments

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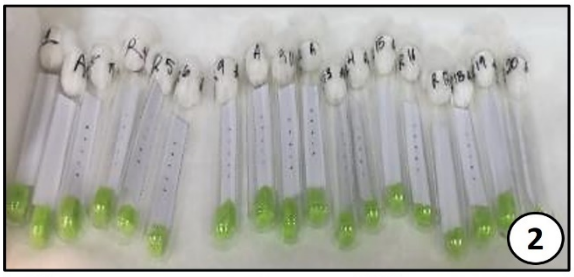
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Ilustraciones página anterior:

1. Tubos de ensayo con adultos de *Phthorimaea operculella* (Fuente: Juan R. Gallego).
2. Tubos de ensayo con adultos de *Trichogramma cacaeciae* (Fuente: Juan R. Gallego).
3. Recipientes con patatas infestadas y los tratamientos con los entomopatógenos *T. cacaeciae* y *Blattisocius mali* (Fuente: Juan R. Gallego).
4. Patatas del tratamiento control (sin entomopatógenos) (Fuente: Juan R. Gallego).
5. Patatas del tratamiento con *T. cacaeciae* (Fuente: Juan R. Gallego).
6. Patatas del tratamiento con *B. mali* (Fuente: Juan R. Gallego).
7. Patas con galerías causadas por larvas de *P. operculella* (Fuente: Juan R. Gallego).

Capítulo III

Evaluation of *Trichogramma cacaeciae* (Hymenoptera: Trichogrammatidae) and *Blattisocius mali* (Mesostigmata: Blattisociidae) in the post-harvest biological control of the potato tuber moth (Lepidoptera: Gelechiidae): Use of sigmoid functions

Y Solano-Rojas, JR Gallego, M Gámez, J Garay, J Hernandez, T Cabello. 2022. Evaluation of *Trichogramma cacaeciae* (Hymenoptera: Trichogrammatidae) and *Blattisocius mali* (Mesostigmata: Blattisociidae) in the post-harvest biological control of the potato tuber moth (Lepidoptera: Gelechiidae): Use of sigmoid functions. Agriculture 12(4): 519. DOI: https://doi.org/10.3390/agriculture12040519

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Capítulo III. Evaluation of *Trichogramma cacaeciae* (Hymenoptera: Trichogrammatidae) and *Blattisocius mali* (Mesostigmata: Blattisociidae) in the post-harvest biological control of the potato tuber moth (Lepidoptera: Gelechiidae): Use of sigmoid functions

Abstract

The potato tuber moth (PTM), *Phthorimaea operculella*, is the main potato pest in the world, having managed to adapt to a wide range of climatic conditions, and causing damage to stored tubers. The effectiveness of biological control using the parasitoid *Trichogramma cacaeciae* and the predatory mite *Blattisocius mali* was evaluated in PTM under conditions that simulated those of tubers in storage. The number of adults found at the end of the trial was significantly higher in the control (181.75 ± 11.00 adults/container) than in the treatments where *B. mali* (24.50 ± 7.22) or *T. cacaeciae* (102.00 ± 14.61) were released. The biological control's effectiveness using the Abbot formula was fitted by logistic models, and the best fit was found for the simple logistic function. Using the data generated from these functions, the control efficiencies for the mite and the parasitoid were 94.85% and 73.77%, respectively. *B. mali* showed greater potential for being incorporated into integrated PTM management programs than *T. cacaeciae*. Based on logistic functions, a novel way of estimating the effectiveness of predators and parasitoids is also presented and discussed.

Keywords: biological control; effectiveness; population dynamic; stored potato; pest.

III.1.2. Introduction

The Potato Tuber Moth (PTM) *Phthorimaea operculella* (Zeller) (Lep.: Gelechiidae) is a species native to the tropical, mountainous regions of South America, and is a pest species of potato plant (*Solanum tuberosum* L.) and other Solanaceae [1]. Its distribution is cosmopolitan (CABIhttps://www.cabi.org/isc/datasheet/40686#todistribution, accessed on 7 March 2022) and of economic importance in crop cultivations, although it is especially severe in storage [2], due to its ability to reproduce and adapt to the different environmental conditions occurring in storehouses [3-8]. Under such conditions, PTM infests potato tubers with its eggs, which are rarely detectable; later, the neonate larvae penetrate the tubers [4,8-10]. PTM damage lowers the commercial value of the potatoes and can cause losses of up to 100% when the temperature and storage period are not optimal, by favouring the development of several generations of PTM [7,9,11,12].

PTM adults can live at temperatures between 10 °C and 35 °C without being affected by the relative air humidity [7,13]; furthermore, their immature states survive for brief periods when exposed to temperatures between 4.25 °C and 13.5 °C, inducing quiescence [4]. These characteristics allow PTM to easily adapt to the storage conditions used both for seed (2 °C to 4 °C) and consumption potatoes (8 °C to 12 °C), and, of course, in unrefrigerated storehouses, as occurs in some countries [11,14-16]. For all these reasons, the control of PTM is complex, particularly in immature states.

Among the PTM management strategies used in stored are cleaning practices that prevent the entry and/or permanence of infected tubers [2,11], the use of hermetic storage conditions that prevent the entry of adult moths, the monitoring of tubers for signs of rot or larval damage [11], the use of potato varieties resistant to the moth [2], and chemical control [2], the latter being used only in tubers intended for planting. Biological control alone is recommended for potatoes destined for consumption and/or processing since there are no health risks, it does not harm the environment and it does not induce resistance in pests, as insecticides do [4,11]. In this regard, the use of entomopathogens such as viruses, bacteria and nematodes, and the use of entomophages, such as parasitoids and/or predators, may be appropriate for the control of PTM [2,4]. Among the entomophages, oophagous parasitoids of the genus *Trichogramma*, such as *T. evanescens* West., *T. cacaeciae* (Marchal), *T. achaeae* Nagaraja and Nagarkatti, *T. chilonis* Ishii and *T. principium* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae) have been evaluated in relation to PTM egg parasitism under field conditions, and also in storage [17-19]. Similarly, the mites *Blattisocius mali* (Oudemans) and *B. tarsalis* Berlese (Acari: Blattisociidae) have also been studied as potential predators of PTM eggs [16,20,21].

The techniques for evaluating the efficacy of entomophages can be carried out by direct or indirect methods, as reviewed by Mills [22]. Due to the diverse biological characteristics, direct evaluation is complicated in the case of parasitoid species (e.g., Van Driesche [23]) and even more so in the case of predatory species (e.g., Latham and Mills [24]). The question as to whether evaluation criteria can be used, and/or which of them would be most effective, has been debated at length, but is not yet clarified (van Lenteren *et al*. [25]). The effectiveness of synthetic insecticides/miticides have been evaluated in the standard way by their corrected mortalities with respect to a control (Abbott’s formula) [26] via laboratory and/or field bioassays (e.g., Robertson and Preisler [27], Matthews [28]). This has also been extended to evaluating entomopathogens (e.g., Navon and Ascher [29]). The Abbott’s correction (or

Effective Kill Rate) is based on a multiple (double) decay life table over a single time interval (age) [30]. It should be noted that, firstly, in the case of insecticides/acaricides, such evaluation is problematic, and can lead to biased values with overestimations and/or underestimations of effectiveness (e.g., Fleming and Retnakaran [31], Rosenheim and Hoy [32]). Secondly, in the case of entomophages (predators and parasitoids) that do not act promptly and whose response is density dependent, the effectiveness evaluation seems inadequate, especially over the long term.

Within this context, it may be necessary to evaluate the effectiveness of an entomophage (predator or parasitoid) with the help of mathematical models. Thus, Berryman [33] considers that there are two basic trends in the modelling of interactions between predators and prey. One arises from logistic equations, which describe the growth of a consumer population living in a finite environment, and the other, from the Lotka-Volterra equations, describes the physics of interacting predators and prey populations – thus, different conclusions can be drawn about the biological control practice depending on the approach. Based on the above, the objectives of this work have been twofold – firstly, to try to improve the effectiveness evaluation of entomophages by fitting it to mathematical models, and secondly, to study the effectiveness of PTM control that uses two biological control agents, *T. cacaeciae* and *B. mali*, to combat PTM under storage conditions.

III.1.3. Materials y methods

III.1.3.1. Evaluation of the effectiveness of *T. cacaeciae* and *B. mali* used in PTM control under storage conditions

A completely randomized univariate experimental design was employed with a single factor or treatment. This factor presented 3 levels: (1) the release of the predatory mite *B. mali*, (2) the release of the parasitoid *T. cacaeciae*, and (3) the control with no release of entomophages. There were 8 repetitions for each treatment. The trial was carried out following the methodology described by Saour [17] and Arthurs et al. [34]: 15 PTM adults (8 females + 7 males, 2 days old) were released into each cylindrical plastic container (diameter: 28 cm, height: 23 cm, volume: 12 L) and covered with a fine mesh (30 x 30 threads per cm^2), into which 13- 14 potato tubers (≈2 kg) (Variety: Melody, category 1, size 45/80 mm) were placed onto a layer of vermiculite (1 cm) as a substrate for the pupae. At 48 hours after the release of the PTM adults, the *T. cacaeciae* and *B. mali* were released, at the following doses: 20 female parasitoids (2 days old) or 216 adult predator mites (2 days old) per container, according to the treatment; except for the control containers (the check) into which the entomophages were not released. The containers were left to evolve for 49 days (1-2 PTM generations) under controlled conditions (22.7 ± 4.0 °C and 52.2 ± 14% RH) and natural diffused light.

During the test, 4 containers from the 3 treatments were left to evolve until the end of the test; and another 4 containers were sampled twice a week (13 samples). In each sampling event, 4 potatoes (1 per container) were randomly selected and frozen (at -20°C). At the end of the test, the contents of each container (that had not been sampled during the test) were also frozen. Subsequently, all the frozen samples were inspected, the tubers dissected, and the PTM individuals counted (eggs, larvae, pupae, and adults). In addition, the potatoes damaged by the infestation, and the number of galleries found per tuber, were estimated. During the trial, the ambient temperature and relative humidity values were recorded daily, every 15 minutes, using a Data logger (model EBI 20-TH, Ebro® Xylem Analytics Germany Sales GmbH and Co. KG ebro, Ingolstadt, Germany).

The values of PTM's new adults obtained at the end of the trial were statistically analyzed by means of generalized linear models (GZLM) with the normal distribution and the identify link function using IBM SPSS version 26 software; the mean values were compared in pairs using the Wald test, performed with the same software. The effectiveness of the PTM control using both biological control agents, compared to the control, was calculated using the Abbott formula [26,30]:

$$EKR = \frac{P_{nat} - P_{trt}}{P_{nat}}$$

where EKR = effective kill rate, P_{nat} = the proportion alive in the control, P_{trt} = the proportion alive in the treatment, and ($P_{nat} - P_{trt}$) giving the proportion dead owing to the treatment alone.

III.1.3.2. Mathematical model: logistic functions

The data obtained from the PTM (eggs, larvae, pupae, and adults) in the 13 samplings carried out during the trial were transformed into accumulated insect-days (AID) according to Ruppel [35]. This index has already been used to evaluate biological control under field conditions (e.g., Cabello *et al.* [36]). The above data were fitted to the Verhulst-Pearl logistic function (single, double, and triple). In this case, the logistic differential equation is

$$\frac{dx}{dt} = rx\left(1 - \frac{x}{K}\right)$$

the solution curve of which is

$$x(t) = \frac{K}{1 + \left(\frac{K}{x_0} - 1\right) e^{-rt}}$$

where x = the population density, x_0 = the initial population density, r = the instantaneous growth rate, and K = the system support capacity. In the case of double and triple functions, the following adjustments were made to the sums of the previous equation:

$$x(t) = \frac{K_1}{1 + \left(\frac{K_1}{x_{01}} - 1\right). e^{-r1t}} + \frac{K_2}{1 + \left(\frac{K_2}{x_{02}} - 1\right). e^{-r2t}}$$

$$x(t) = \frac{K_1}{1 + \left(\frac{K_1}{x_{01}} - 1\right). e^{-r1t}} + \frac{K_2}{1 + \left(\frac{K_2}{x_{02}} - 1\right). e^{-r2t}} + \frac{K_3}{1 + \left(\frac{K_3}{x_{03}} - 1\right). e^{-r3t}}$$

The parameters are the same as before but considering the double (2 sections) or triple (3 sections) logistic functions in each section. TableCurve 2D version 5.01 software was used for the logistic adjustments. The best fit to the model was evaluated by the lowest value of the corrected Akaike information criterion (AIC_c). The effectiveness of each entomophagous was calculated using the adjusted surfaces under the logistic curve for the predator and the parasitoid, with respect to the control, corrected for the estimated initial values (X_0) (Carreño *et al.* [37]).

III.1.4. Results

III.1.4.1. Effectiveness of *T. cacaeciae* and *B. mali* used in PTM control under storage conditions

The number of adults that completed their development under tuber storage conditions are shown in **Figure III.1** In the statistical analysis, the model was significantly adjusted to the data according to the Omnibus test (X^2 of the likelihood ratio = 29.478, d.f. = 2, P < 0.01). and a high statistical significance was found in the pairwise comparison of the predator and parasitoid effect with respect to the control (Wald $X^2 = 127.972$, d.f. = 2, $P < 0.01$). The mean values for specimens that were able to complete their development were 181.75 ± 11.00 PTM adults in the control compared to 24.50 ± 7.22 and 102.00 ± 14.61 adults in the containers where the mites or parasitoids were released, respectively.

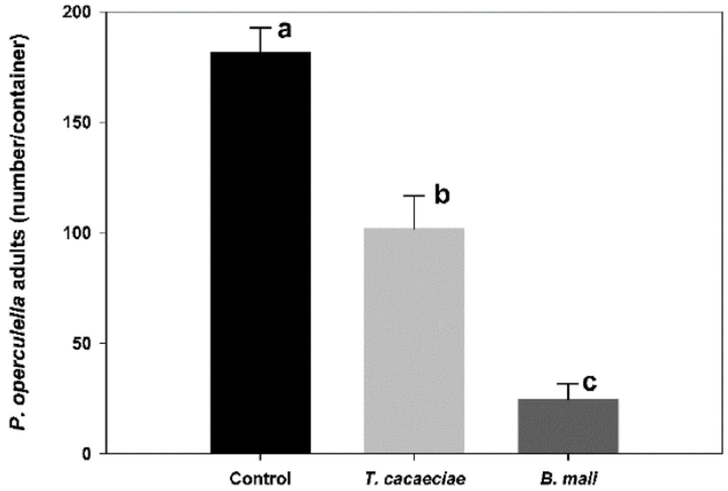


Figure III.1 Mean number of *P. operculella* adults (PTM) (± SE) after 49 days when an initial release of *B. mali* or *T. cacaeciae* was carried out, compared to the control, under storage conditions (values with different letters indicate significant differences at $P = 0.01$).

The average number of galleries per tuber was significantly adjusted to the model according to the Omnibus test (X^2 = of the likelihood ratio = 33.407, d.f. = 2, $P < 0.01$) (**Figure III.2**), and these values were significantly affected by the predators and the parasitoids with respect to the control (Wald's $X^2 = 14.586$, d.f. = 2, $P < 0.01$). The effectiveness or "effective kill rate" (*EKR*), according to the Abbott formula, was 86.52% for *B. mali* and 43.88% for *T. cacaeciae*.

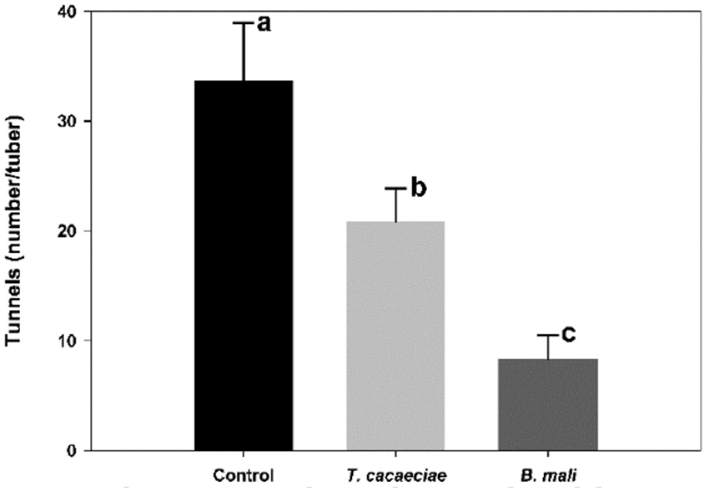
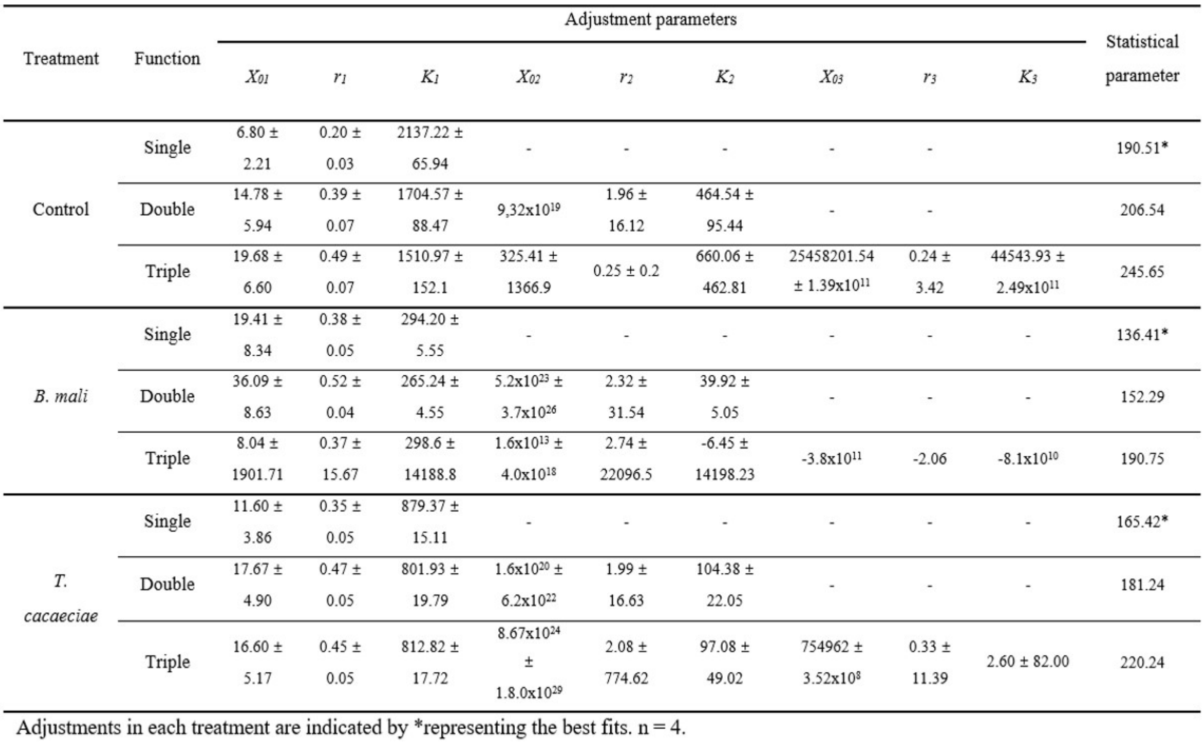


Figure III.2 Damage by *P. operculella*: mean number of tunnels (± SE) per potato after 49 days, when an initial release of the predatory mite *B. mali* or the parasitoid *T. cacaeciae* was carried out, with respect to the control, under storage conditions (values with different letters indicate significant differences at $P = 0.01$).

Table III.1 shows the adjusted AID values for the PTM in the three treatments performed. One can observe that, in each case, the best fit is the simple sigmoidal function, as it presented the lowest *AICc* values. This shows that only one PTM generation was present during the bioassay period (49 days) under the climatic conditions previously described. **Figure III.3A** shows the simple logistic adjustments carried out in each treatment while, in turn, **Figure III.3B** shows the functions derived from said adjustments. Confirming the values found in the previous section, one can see that the predatory mite was able to limit the PTM populations to a greater extent than the parasitoid (**Figure III.3**). Using the result found in the previous logistic adjustments, we can estimate the effectiveness values for the predatory mite (94.85%) and for the parasitoid (73.77%) in both cases, compared to the control.

Table III.1 Parameters and statistical significance for the logistic adjustments (single, double, or triple) of the total number of *P. operculella* individuals when an initial release of the predatory mite *B. mali* or the parasitoid *T. cacaeciae* was carried out, with respect to the control, under storage conditions.



Treatment	Function	Adjustment parameters									Statistical parameter
		X_{01}	r_1	K_1	X_{02}	r_2	K_2	X_{03}	r_3	K_3	
Control	Single	6.80 ± 2.21	0.20 ± 0.03	2137.22 ± 65.94	-	-	-	-	-		190.51*
	Double	14.78 ± 5.94	0.39 ± 0.07	1704.57 ± 88.47	9,32x10^{19}	1.96 ± 16.12	464.54 ± 95.44	-	-		206.54
	Triple	19.68 ± 6.60	0.49 ± 0.07	1510.97 ± 152.1	325.41 ± 1366.9	0.25 ± 0.2	660.06 ± 462.81	25458201.54 ± 1.39x10^{11}	0.24 ± 3.42	44543.93 ± 2.49x10^{11}	245.65
B. mali	Single	19.41 ± 8.34	0.38 ± 0.05	294.20 ± 5.55	-	-	-	-	-	-	136.41*
	Double	36.09 ± 8.63	0.52 ± 0.04	265.24 ± 4.55	5.2x10^{23} ± 3.7x10^{26}	2.32 ± 31.54	39.92 ± 5.05	-	-	-	152.29
	Triple	8.04 ± 1901.71	0.37 ± 15.67	298.6 ± 14188.8	1.6x10^{13} ± 4.0x10^{18}	2.74 ± 22096.5	-6.45 ± 14198.23	-3.8x10^{11}	-2.06	-8.1x10^{10}	190.75
T. cacaeciae	Single	11.60 ± 3.86	0.35 ± 0.05	879.37 ± 15.11	-	-	-	-	-	-	165.42*
	Double	17.67 ± 4.90	0.47 ± 0.05	801.93 ± 19.79	1.6x10^{20} ± 6.2x10^{22}	1.99 ± 16.63	104.38 ± 22.05	-	-	-	181.24
	Triple	16.60 ± 5.17	0.45 ± 0.05	812.82 ± 17.72	8.67x10^{24} ± 1.8.0x10^{29}	2.08 ± 774.62	97.08 ± 49.02	754962 ± 3.52x10^{8}	0.33 ± 11.39	2.60 ± 82.00	220.24

Adjustments in each treatment are indicated by *representing the best fits. n = 4.

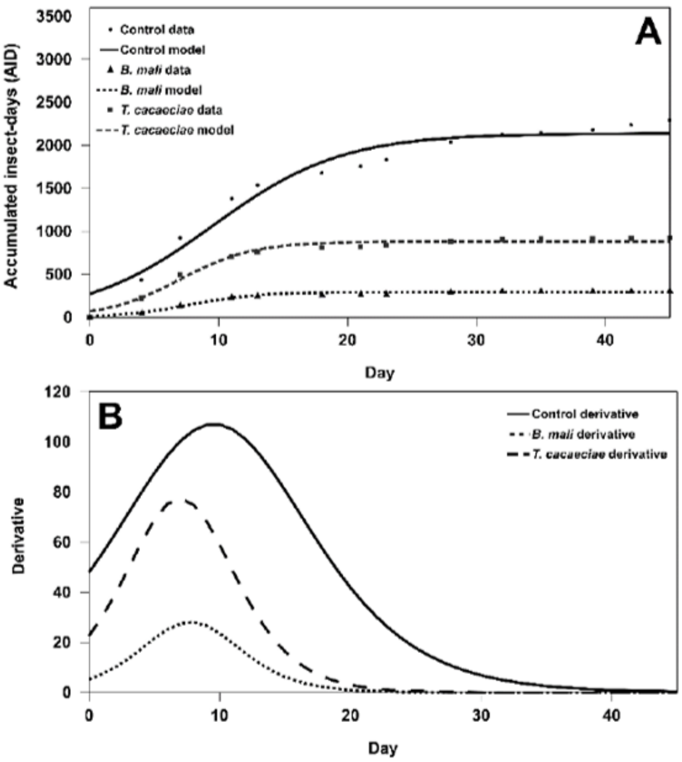


Figure III.3 (**A**) Adjustments to simple logistic functions and (**B**) their derived functions from the accumulated insect-days (AID) of *P. operculella*/container when an initial release of the predatory mite *B. mali* or the parasitoid *T. cacaeciae* was carried out, with respect to the control, under storage conditions (whiskers represent the confidence limits at *P* = 0.05). n = 4.

III.1.5. Discussion

The conducted trial has made it possible to evaluate the effectiveness of two biological control agents under simulated potato tuber storage conditions, and to propose a new way to estimate said effectiveness of the entomophages (predators and parasitoids) as control agents in a more reliable way, which does not require overly complex calculations – this now enables us to consider their potential application under real conditions in stored tubers. Of the two ways to address the dynamics of the predatory – prey or host – parasitoid population using mathematical models (as indicated in the Introduction), the use of logistic functions was chosen (**Table III.1**). In this regard, it should be noted that the Lotka – Volterra equation has been applied more frequently, both theoretically and experimentally, in the biological control of pests compared to logistic equations (e.g., Gurr *et al*. [38], Barlow [39]).

The logistic functions were fitted using the AID values (**Figure III.3A**). These were proposed by Rupper [35] to evaluate the efficacy of insecticides and allow the magnitude of the number of insects, and their duration over time to be combined in a single index. This index has been used for biological control evaluation under field conditions (e.g., Sanchez and Lacasa

[40], Cabello *et al*. [36]) as well as in integrated pest management programmes [41]. In addition, these accumulated values allow for easy adjustment to single or multiple logistic functions. This has been done in the case of insect population dynamics (e.g., Carreño et al. [42]; Cabello & Carreño [43], Solano-Rojas et al. [44]) as well as in the epidemiology of phytopathogens [45-48]. Such an approach has been carried out in the present work (**Figure III.3A**) allowing us also to obtain its derivate function (Figure 3B). In addition, these adjustments allow one to obtain an estimate of the instantaneous growth rate of the pest population (r) in the three treatments (**Table III.1**). As Birch [49] pointed out, in a logistically growing population, the value of “r” can be determined indirectly by calculating the appropriate logistic curve.

The best adjustment to simple logistic functions indicates that there was only one generation of the pest population during the test period in the three treatments (**Figure III.3B**). Double or triple adjustments within the trial period could have indicated that either there were different cohorts developing at different rates (e.g., Solano-Rojas *et al*. [44]) or that there were two or more generations (e.g., Carreño et al. [42]), apart from overlapping populations. It should be noted that a way to address the efficiency of entomophages has also been proposed using “the pest kill rate” (K_m) [50,51] based on the calculation of life tables. Thus, K_m = Ln (K_0/T_k), where T_k (the generation time) is the period during a generation that allows the predator to kill the prey and K_0 is “the net consumption rate”. This has been validated by evaluating different entomophagous species of the pest species *Tuta absoluta* (Lep.: Gelechiidae) [25,52]. We consider that this way of addressing the efficacy evaluation problem is highly suitable and quite well-founded. Regarding our proposal, both approaches for evaluating the effectiveness of entomophages are similar: both are based on life tables. “The net consumption rate”, according to the classification by Mills [22], is a direct method of evaluating entomophagous effectiveness. Conversely, in our case, it is an indirect method based on only assessing the effects on the phytophagous population.

As mentioned in the introduction, the predation/parasitism rates present calculation problems, especially under field conditions. Coupled to this is the fact that life table calculations under field conditions present greater practical difficulty than under laboratory conditions (e.g., Bellows and Van Driesche [53], Southwood and Henderson [54]). Therefore, our proposal for evaluating entomophages may be less complex to carry out, especially under field conditions.

Returning to the effectiveness values found for *B. mali*, these were 86.52% using the Abbott formula and 94.85% using the new formula; for *T. cacaeciae*, the values were lower, 43.88% and 73.77%, respectively. In our view, the differences between the two methods of evaluating the effectiveness of both entomophages occur mainly because, when applying the Abbott formula, it only considers the initial and final values of the phytophagous populations in the trial. In contrast, the new calculation method considers all the intermediate values of the pest population, according to the number of samplings carried out over the trial in the different treatments. The initial application of this novel approach was proposed by Carreño et al. [37], although without applying logistic adjustments, and its validity was verified by evaluating the effectiveness of arthropod pests, phytopathogens, and weeds [55].

In the results obtained, *B. mali* presented an effectiveness of 94.85% in reducing the adult potato tuber moth population. This value is like that found by Gallego et al. [21], who reported that this mite species had a maximum PTM-control effectiveness of 92.51% (at the highest dose) under laboratory conditions and over the short term. In turn, for the species *B. tarsalis*, similar efficiencies of 98.86% have been cited [20] and 92.31% [16] in short-term trials under laboratory conditions.

In contrast, the parasitoid species *T. cacaeciae* presented and effectiveness of 73.77%, lower than that of the predatory mite. This value is in line with Gallego et al. [19] for the same species, with a parasitism percentage of 70.88% under laboratory conditions. These values were higher than those found for this species, as well as for *T. evanescens* and *T. principium* by Saour [17], which never presented parasitization percentages of PTM eggs higher than 35%. Furthermore, under experimental conditions such as ours, Mandour et al. [18] only achieved reductions of 25.9% in the PTM infestation percentage, compared to the control, when they used the species *T. evanescens*. Conversely, Gallego et al. [19] reported parasitization percentage values for *T. achaeae* of 92.78%, which may indicate a greater effectiveness of this species, although the test was carried out in the laboratory.

III.1.6. Conclusions

We can conclude that, firstly, the use of PTM biological control agents in stored potatoes has proven a practical alternative, and that they can potentially adjust to the warehouse conditions, particularly those that are not refrigerated, as has been indicated by other authors

[16-18,21]. Secondly, we believe that the Abbott formula, even with the aforementioned drawbacks, is still useful in evaluating the effectiveness of pest control agents over the short term, especially in laboratory tests, with equal initial numbers of phytophagous between treatments, and for evaluations over hours or a few days. Finally, we consider that the proposed way of evaluating efficiencies, using logistic models, may be more appropriate for biological control agents (predators and parasitoids) than the standard methods (e.g., the Abbott formula). In addition, we believe it is especially appropriate when biological control is carried out by augmentation via inoculative releases. Accordingly, it is expected that the entomophagous populations released at low doses will increase over time and can thus control the pest population – this can be achieved after several generations of the species involved (e.g., Vila and Cabello [56]).

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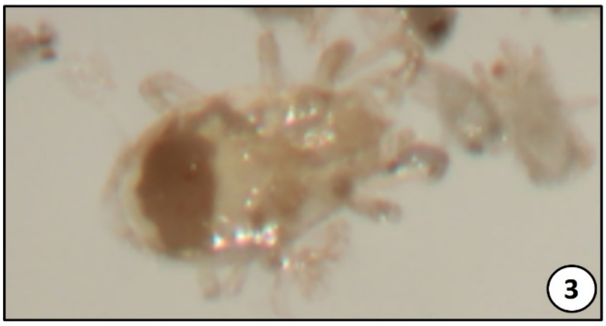
Capítulo IV



Ilustraciones página anterior:

1. Sobre de liberación lenta del ácaro depredador *Amblyseius swirskii* (Fuente: Tomás Cabello).
2. Sustrato interno del sobre de liberación lenta (Fuente: Juan R. Gallego).
3. Adulto de *A. swirskii*.
4. Larvas, ninfas y adultos de *Carpoglyphus lactis*.

Population dynamics of the predator *Amblyseius swirskii* and its prey *Carpoglyphus lactis* in slow-release sachets

Capítulo IV. Population dynamics of the predator *Amblyseius swirskii* and its prey *Carpoglyphus lactis* in slow-release sachets

IV.1.1. Introducción

Los ácaros depredadores son uno de los grupos de entomófagos más utilizados en los programas de manejo integrado de plagas debido a su factibilidad para criarlos comercialmente a gran escala y utilizarlos en el control de ácaros, trips, moscas blancas y otros insectos plaga en cultivos bajo invernadero (van Lenteren 2012, Knapp et al. 2018). Entre los ácaros depredadores mayormente producidos por las industrias de biocontrol se encuentran *Neoseiulus californicus* (McGregor), *N. cucumeris* Oudemans, *Amblyseius swirskii* (Athias-Henriot) y *Phytoseiulus persimilis* (Athias-Henriot), los cuales son criados sobre presas alternativas como *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) y *Tyrophagus putrescentiae* (Schrank) (Gerson et al. 2003, Bolckmans y Van Houten 2006, Calvo et al. 2015), y pueden ser liberados en campo a través de aplicaciones directas utilizando sopladores mecánicos o por medio de sobres de liberación lenta (Vila y Cabello 2014, Calvo et al. 2015).

Los sobres de liberación de ácaros están diseñados con papel repelente al agua y un pequeño orificio que permite la salida de los depredadores (Jacobson et al. 2001, Midthassel et al. 2014a), e internamente contienen salvado de trigo, serrín o vermiculita y alimento para la presa que será consumida por el ácaro depredador (Bolckmans y Van Houten 2006, Bolckmans et al. 2013). El control de calidad de estos sobres está basado en la determinación de los parámetros biológicos del depredador, particularmente en su crecimiento poblacional en condiciones de confinamiento y en su capacidad de dispersión y búsqueda de presas cuando sale del sobre, pero también en la densidad poblacional inicial del depredador y su presa (van Lenteren et al. 2003, Midthassel 2015), lo cual es crucial para garantizar un control exitoso en campo, ya que el número de presas debe ser suficiente para mantener al depredador, cuya densidad inicial también debe ser óptima para que su población crezca y paulatinamente salgan del sobre hacia el cultivo (Midthassel 2015). En este sentido, las metodologías de extracción de ácaros para su posterior cuantificación juegan un rol importante dentro del control de calidad de los sobres, pero también en el estudio del crecimiento poblacional del depredador y su presa.

La dinámica poblacional de los ácaros en el interior de los sobres depende de diversos factores entre los que se encuentran la variación de las condiciones ambientales y los desbalances en la interacción depredador – presa a causa de la complejidad espacial del sustrato y su capacidad de retención de humedad, la densidad de los ácaros, la interacción entre coespecíficos, y la disponibilidad, condición y calidad del alimento de la presa, entre otros (Colloff 2009, San et al. 2012, Midthassel et al. 2014a, Ghazy et al. 2016, Shimoda et al. 2017). En el campo, los sobres permanecen expuestos durante varias semanas a las condiciones climáticas (p. ej. temperatura, humedad relativa (HR), lluvia, otros) y a las prácticas de manejo de los cultivos (p. ej. riego, aplicación de pesticidas, otros), las cuales pueden favorecer o restringir el crecimiento poblacional, o incrementar o reducir la capacidad de dispersión del depredador en el cultivo (Bakker et al. 1993, Jacobson et al. 2001, Ferrero et al. 2010, Addesso et al. 2018), particularmente cuando ocurren desequilibrios generados por el gradiente de humedad en el interior del sobre y la HR, que conllevan a la deshidratación de los huevos o de los inmaduros y adultos que pierden agua por difusión (Ferrero et al. 2010, Midthassel et al. 2014b, Ghazy et al. 2016, San et al. 2021).

En este contexto, el estudio del efecto de la HR sobre la dinámica poblacional de los ácaros en el interior de los sobres es imperativo para definir estrategias de manejo que garanticen su eficiencia en el control de plagas y alarguen su vida útil en campo (Buitenhuis et al. 2014, Midthassel 2015, Shimoda et al. 2017). Estos estudios de ecología poblacional deben complementarse con modelos matemáticos que representen la tasa de liberación de los ácaros depredadores frente a variaciones de la HR, con el propósito de simular o predecir situaciones futuras en campo, como las curvas sigmoides que han usado diversos autores para explicar las fluctuaciones de ácaros fitoseidos en función de cambios en las variables ambientales, mientras que otros han construido modelos teóricos para entender la dinámica poblacional del sistema depredador - presa (Bakker et al. 1993, Nakai et al. 2021, Kuang et al. 2017, Téllez et al. 2020).

El siguiente capítulo esta divido en dos secciones, la primera se enfocó en desarrollar una metodología de contabilización de ácaros basada en flotación en hexano con el propósito de optimizar el control de calidad de las formulaciones comerciales de sobres de liberación lenta, y utilizar este método para estudiar la dinámica poblacional de *A. swirskii* y *C. lactis* en el interior de sobres. En la segunda sección se investigó el efecto de tres regímenes de humedad relativa sobre el crecimiento poblacional del depredador y su presa dentro de los sobres y en la tasa de liberación del depredador, la cual se ajustó a un modelo logístico representar la dinámica y periodo de liberación de *A. swirskii*.

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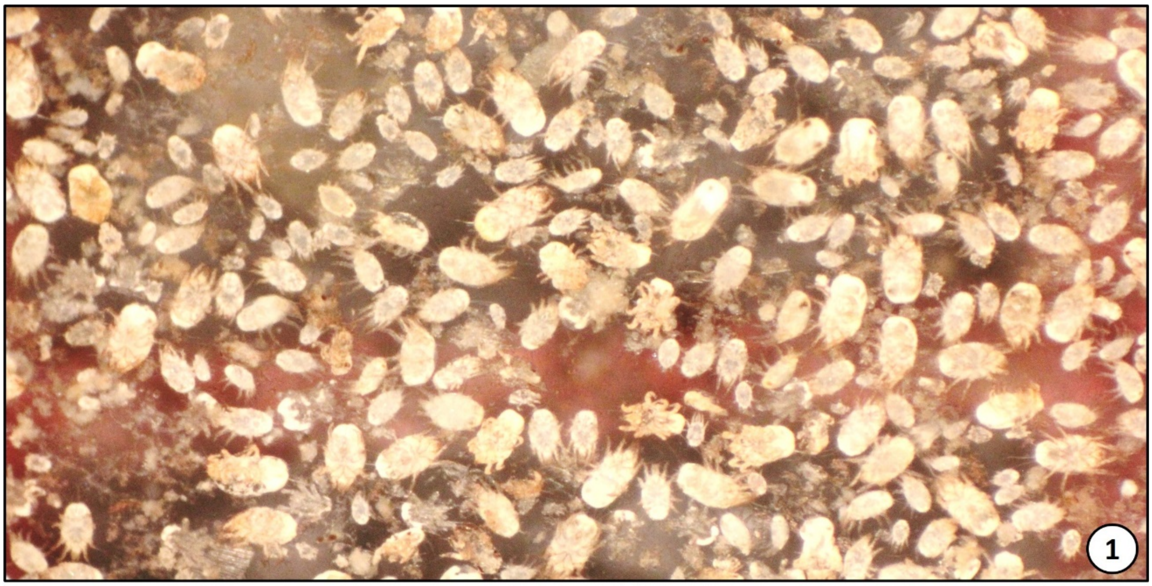
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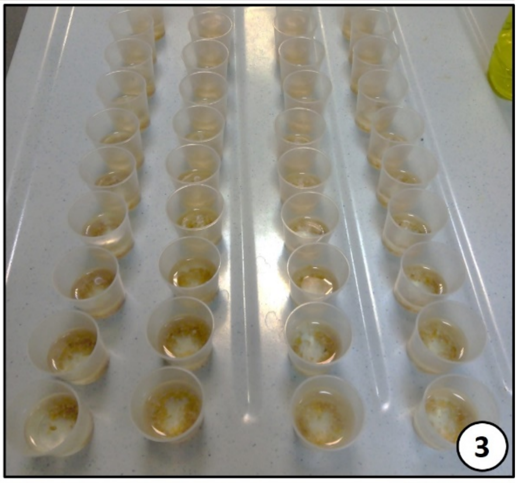
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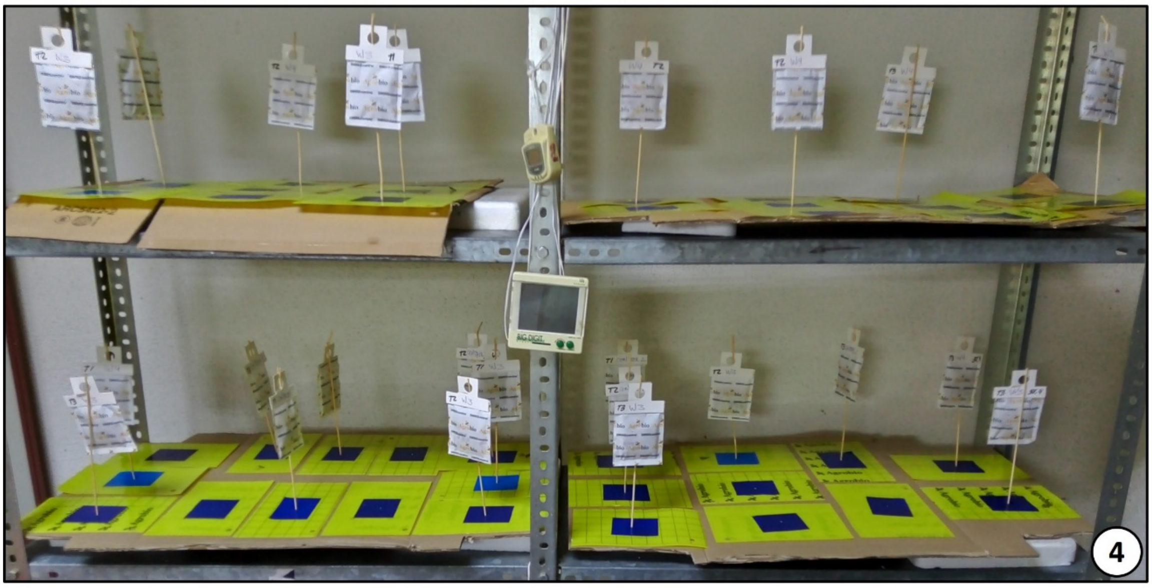
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Ilustraciones página anterior:

1. Larvas, ninfas y adultos de *Carpoglyphus lactis* extraídos por flotación en hexano.
2. Adultos de *Amblyseius swirskii* señalados en círculo rojo.
3. Submuestras para la contabilización de ácaros en sobres de liberación lenta, con el método de extracción por flotación en hexano.
4. Métodología de evaluación en el ensayo de evaluación de la tasa de liberación del depredador desde los sobres.

Population dynamics of mites in slow-release sachets used in biological control: a new study methodology

JR Gallego, Y Solano-Rojas, B Tiseyra, M Gámez, T Cabello. 2022. Population dynamics of mites in slow-release sachets used in biological control: a new study methodology. Experimental and Applied Acarology. DOI: https://doi.org/10.1007/s10493-022-00739-2

Quality Index:

Impact Factor (JCR) = 2.380, Q2 Entomology

Impact Factor (SJR) = 0.576, Q2 Environmental Science/Ecology

Sección IV.1. Population dynamics of mites in slow-release sachets used in biological control: a new study methodology

Abstract

Predatory mite species (Acari: Phytoseiidae) are essential tools in the biological control of greenhouses pests. The natural enemies can be released directly into a crop. A better, partly preventive system is to place slow-release sachets on the plants. Inside such sachets is a factitious prey's food substrate – which also acts as refuge – and the predator. The objective of this study was to develop a new methodology to evaluate the population dynamics of this sachet system, based on the factitious prey *Carpoglyphus lactis* and the predatory mite *Amblyseius swirskii*. Through two tests carried out under laboratory conditions, the sachets were first compared to the traditional extraction method that uses Berlese-Tullgren funnels and an extraction method using flotation in hexane. The latter method proved more effective at sampling the motile states (larvae, nymphs, and adults), both for the predatory species and for the factitious prey, extracting up to 3.7× more mites than the Berlese-Tullgren funnel. Second, the population dynamics of both mite species was studied in a laboratory test, both inside and outside the sachets. In this way, a positive correlation was demonstrated between the number of predatory mites and the number of prey mites inside the sachets. Conversely, no correlation was found between the interior population of predatory mites and the number that venture outside. We can conclude that hexane extraction is very useful both in quality control of predatory mites and in studying how the sachets behave when faced with various factors.

Keywords: Phytoseiidae, Carpoglyphidae, *Amblyseius swirskii*, *Carpoglyphus lactis*, Hexane flotation, quality control.

IV.1.1.2. Introduction

The worldwide use of predatory mites (mainly the family Phytoseiidae) constitutes one of the basic pillars of integrated pest management in greenhouse crops (Knapp et al. 2018). There are several commercial formulations for manipulating and releasing them in crops, all of which have food substrate for factitious prey (usually astigmatid mites) and/or carrier substances (Vila and Cabello 2014).

One of the factors determining the success of a biological control programme is the quality of the predators supplied by the manufacturers (Vila and Cabello 2014). In addition to biological parameters, such as, among others, fecundity, sex ratio, and search capacity, the number of predatory mites and prey present in the substrate is very important to the success of the control used in the crop (van Lenteren et al. 2003). Releases made with low-quality material, or with fewer individuals than necessary, lead to failures and uncertainty in the augmentative biological control (O'Neil et al. 1998; van Lenteren et al. 2003).

Detailed guidelines for various species of natural enemies have been developed by the International Organization for Biological Control and are used worldwide for the quality control of phytoseiid mites (van Lenteren et al. 2003). Thresholds for quantity, longevity, sex ratio and fecundity are included in these guidelines to set the requisite quality standards in certain mite species of the family Phytoseiidae. Regarding the quantification of mites present in commercial substrate, three methodologies are detailed for four species: *Neoseiulus cucumeris* (Oudemans), *Iphiseius degenerans* (Berlese), *Phytoseiulus persimilis* Athias-Henriot, and *Neoseiulus californicus* (McGregor). Quantifications are performed on the living material by hot water sieving (*N. californicus*), direct counting (*I. degenerans* and *P. persimilis*) and the Berlese-Tullgren funnel technique (*N. cucumeris*).

On the other hand, since 2005, the marketing of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and its implementation in biological control programmes has represented a great advance in the treatment of protected crops in Europe (Calvo et al. 2015). The application of mites on greenhouse crops is performed using mechanical blowers or slow-release sachets (Midthassel et al. 2014; Vila and Cabello 2014; Calvo et al. 2015). These sachets mainly contain a wheat bran and other food sources are present, such as yeast and wheat germ. The wheat bran acts mainly as carrier material (Vila and Cabello 2014; Calvo et al. 2015).

These types of sachets allow the phased dispersion of the predator mites from the sachets to the crop, especially important when there is no alternative food source (usually pollen) other than the target pest available for crop colonization by predator. However, the predator-prey dynamics inside the sachets can be affected by various factors that might impact the success of the biological control. Intrinsic factors include the spatial complexity between the substrate, the quality of the prey's feed substrate, the initial predator-prey density, and the intra- and inter-specific interactions (Midthassel et al. 2014). Extrinsically, the mite populations can be affected by environmental conditions, such as temperature, humidity, water availability and phytosanitary treatments (Ghazy et al. 2016; Shimoda et al. 2017; San et al. 2021).

The aim of the present work was to develop a system that could be more adequate and timesaving to assess the populations of the prey mite *Carpoglyphus lactis* (Acari: Carpoglyphidae) and the predatory mite *A. swirskii* in the formulations currently available in slow-release sachets.

IV.1.1.3. Materials and methods

IV.1.1.3.1. Biological material

Swirski ulti-mite© (Koppert, Almeria, La Mojonera, Spain) slow-release sachets with a 1.85 ± 0.15 g net weight were used, containing *A. swirskii* and *C. lactis* mites, in all life stages, and wheat bran as the main dispersion and feeding element of the prey (*C. lactis*). The material was used within 24 h of reception and following the handling instructions given by the company, to avoid decrease of quality. Two motile mite extraction methods were used, as described below.

IV.1.1.3.2. Extraction methods

a. Extraction based on the Berlese-Tullgren funnel

The methodology proposed by Kim et al. (2001) and van Lenteren et al. (2003) was followed for *N. cucumeris*, and that proposed by Lopez et al. (2016) for *A. swirskii*. To do this, the contents of the sachets were homogenized carefully with a spoon for 1 min and then 0.5 g samples were taken. The samples were put on a 6-cm-diameter sieve with a 750-µm mesh and successively placed at two heights under a 60-W incandescent bulb to achieve an ascending temperature ramp. The initial moisture content of the sachets was 23.07 ± 0.01%; hence, prior to the tests, the times, and the optimal distance at which the sieve was placed with respect to the bulb were determined to achieve the greatest possible extraction, as sachet moisture contents greater than 16.5-19% require more exposure time (van Lenteren et al. 2003). The sieve was set at a distance of 20 cm from the bulb for 10 min, then it was placed at 10 cm for 5 min. The motile mites (predators and prey) that fell through the sieve were collected on a plate containing a liquid soap film and counted under a binocular magnifying glass (larvae, nymphs, and adults). The number of replications was four sachets, taken at random from the same commercial batch.

b. Extraction by flotation in hexane

Hexane extraction was performed by modifying the methodology of Geurs et al. (1987) proposed for the extraction of microarthropods in soil samples using heptane flotation. To do this, after homogenizing the sachet, a 0.5-g sample was taken and divided into 4 sub-samples of 0.125 g each. Separation by hexane (Hexanux Nazza, Industrias Químicas Eurotex, El Viso del Alcor, Seville, Spain) was carried out in three phases. In phase I, the material was fixed with 20 ml of 96º alcohol; then, 20 ml distilled water was added to create an aqueous phase along with a drop of Triton X-100 (A4975, Panreac Quimica, Barcelona, Spain) to decrease the surface tension and facilitate the bran deposition at the bottom of the beaker, as well as to prevent the formation of fat drops from the bran. In phase II, 20 ml hexane was added and stirred with a spoon to facilitate the disconnecting of mites that are trapped in the bran fragments. In phase III, the mites were extracted at the interface between the hexane and the alcohol and water using a glass pipette, after which the motile *A. swirskii* and *C. lactis* mites were counted under a binocular magnifying glass. Specimens that showed symptoms of dehydration and/or collapsed were not counted. The number of replications was four sachets, taken at random from the same commercial batch.

IV.1.1.3.3. Population dynamics of mites inside the sachet and their release to the outside

Variation in the motile forms of the mites was evaluated by sampling the individuals present both outside and inside the sachets. For the external evaluation, the methodology of Shipp and Wang (2003) was modified by placing the sachets on wooden sticks (8 cm high) punctured into the centre of gummed plates (20 × 24 cm) that had a rectangle of ungummed dark paper (5 × 7 cm) and a Vaseline edge around the plate to prevent possible escape of the arena. The plates were changed on days 3, 7, 13 and 21, and the moving mites present on the plate were counted using a binocular magnifying glass. These represented the releases from the sachets. To count the population inside the sachets, destructive sampling was performed after positioning the sachets (T = 0 days) and on the same days as the external population was determined. To do this, the number of mites present inside four sachets for each day of sampling was quantified following the hexane method. The test was conducted in a ICP 600 climate chamber (Memmert, Schwabach, Germany) at 25±1 ºC, 60-70%RH and L16:D8 photoperiod.

IV.1.1.3.4. Statistical analysis

In the extraction method assay, the experimental design was univariate totally randomized with only one factor or treatment (with sub-sampling per sachets). This factor had two levels: extraction by Berlese-Tullgren and extraction by flotation in hexane. The total number of motile mites within the sachets, extracted using the two methods, was analysed using Generalized Linear Models with the gamma distribution and the logarithmic link function, since these have been reported as the best approach for describing the population size (Dennis and Costantino 1998; Benton et al. 2002). The mean values were compared using the Wald test. In the population dynamics assay, the relationships between the motile forms of the predator and the prey, inside and outside the sachets, were determined using Pearson’s correlation coefficient. In both cases, the analyses were carried out with IBM SPSS v.26 statistical software.

IV.1.1.4. Results

IV.1.1.4.1. Extraction methods

Figure IV.1.1 shows the number of motile mites extracted, their different states and species, using the two methods. In the statistical analyses, the models were significantly adjusted to the data according to the Omnibus tests in all the categories analysed and highly significant statistical differences were found between the two methods (**Table IV.1.1**). More motile individuals, both adult and immature of both species, were extracted by hexane flotation than by the Berlese-Tullgren funnel (**Fig. IV.1.1**)

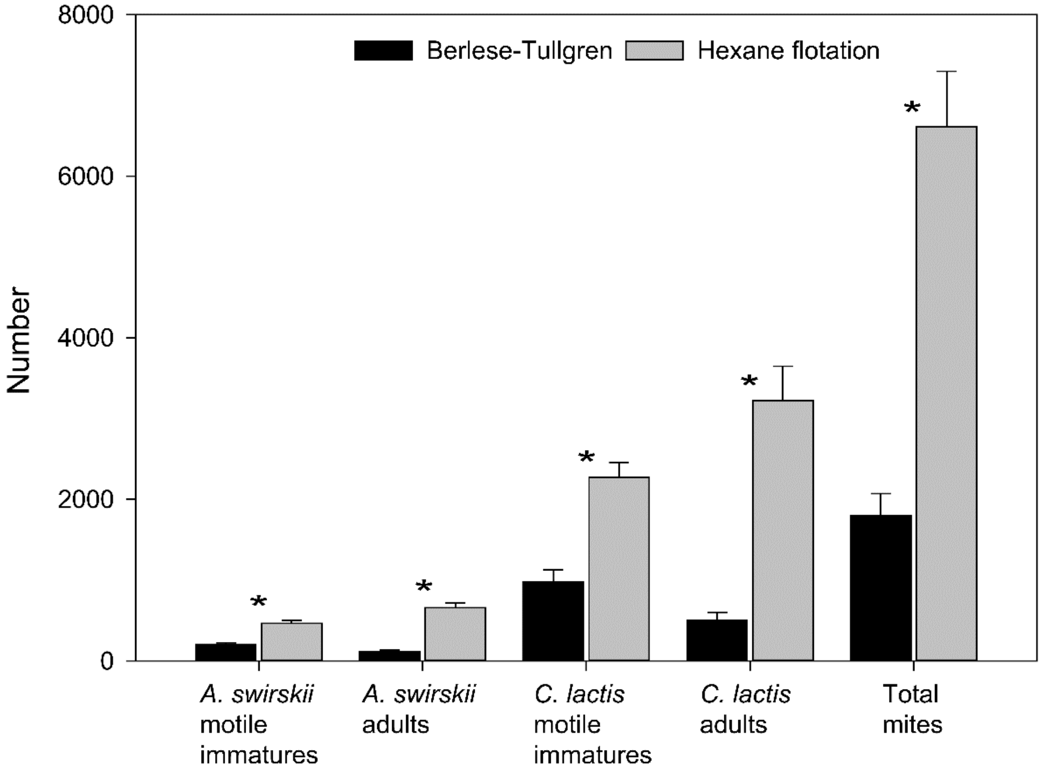

Figure IV.1.1 Mean (+ SE; n = 4) number of motile immature and adult *Amblyseius swirskii* and *Carpoglyphus lactis* mites per sachet according to the extraction method (Berlese-Tullgren or flotation in hexane). Asterisks denote significant differences between extraction methods (Wald's test: $P < 0.05$)

Table IV.1.1 Statistical analysis of the motile immature and adult *Amblyseius swirskii* and *Carpoglyphus lactis* mites extracted by the Berlese-Tullgren method or by flotation

Category	Model fit (Omnibus test)		Pairwise comparison (Wald test)	
	χ^2 (likelihood ratio; df = 1)	*P*	Wald χ^2 (df = 1)	*P*
A. swirskii motile immature	16.928	< 0.001	45.602	< 0.001
A. swirskii adult	21.189	< 0.001	46.067	< 0.001
C. lactis motile immature	11.269	< 0.001	19.024	< 0.001
C. lactis adult	17.992	< 0.001	27.715	< 0.001
Total	16.580	< 0.001	32.456	< 0.001

IV.1.1.4.2. Population dynamics of mites inside the sachet and their release to the outside

Figure IV.1.2 shows the number of motile immature and adult *A. swirskii* and *C. lactis* present inside the sachets throughout the sampling period. The maximum number of motile forms of *A. swirskii* inside the sachets was reached after 7 days (**Fig. IV.1.2A**), whereas the maximum population of *C. lactis* was recorded on the 3rd day (**Fig. IV.1.2B**). In both cases, the number of immature forms was higher than that of adults. Compared to the start of the test, a gradual decrease was recorded in the number of adults of both species present inside the sachets (**Fig. IV.1.2**). Inside the sachet, the initial predator-prey ratio was 1:4.9 for the first 3 days, after which it gradually decreased until day 13, when they were practically equal (1:1.2). At 21 days, the populations of both species inside the sachet were close to 0 under the conditions tested.

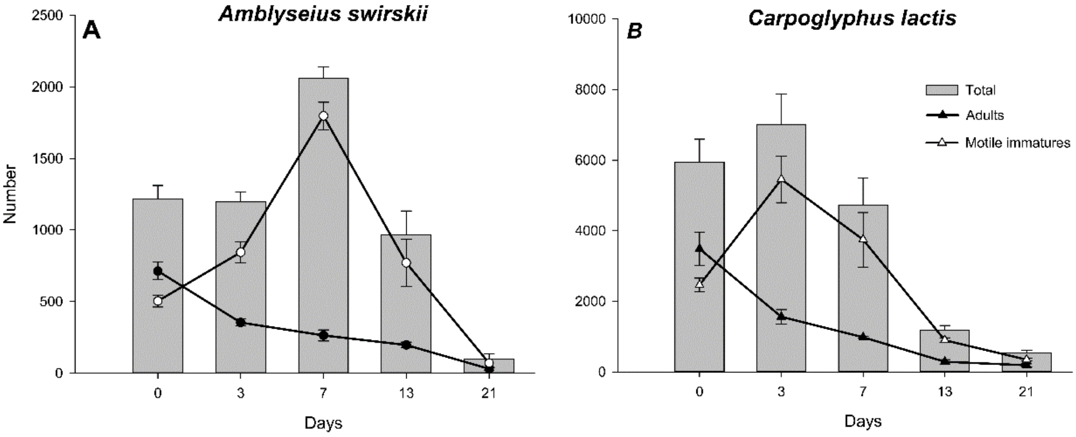

Figure IV.1.2 Mean (± SE; n = 4 for each sampling day) number of motile immature, adult and total (**A**) *Amblyseius swirskii* and (**B**) *Carpoglyphus lactis* mites present inside the slow-release sachets under controlled environmental conditions as a function of time since start of the sampling. Note the differences in scale on the vertical axes

The number of *A. swirskii* and *C. lactis* individuals coming out of the sachets are shown in **Fig. IV.1.3**. It has been established that the cumulative number of predators over the test period exceeded the product specifications for use in crops (325.25 ± 50.76 vs. 250 predators/sachet). The maximum number of predators leaving the sachets occurred between days 7 and 13 (approximately 33 predators per day and per sachet) (**Fig. IV.1.3A**). With regard to *C. lactis*, it was observed that it is mostly the motile immature states leaving the sachets, and this occurs during the first 7 days, decreasing practically to 0 over the following days (**Fig. IV.1.3B**).

Regarding the interior contents of the sachets, Pearson's correlation coefficients revealed that the number of *A. swirskii* motile immature was positively correlated with the number of immature prey (*C. lactis*) (**Table IV.1.2**). The Pearson's correlation coefficient was highly significant regarding the number of *A. swirskii* adults to adult prey (*C. lactis*) inside the sachets. On the other hand, the number of motile immature predators that left the sachets was negatively correlated with the interior content of *C. lactis* adults, and positively correlated with the number of motile immature predators present inside the sachets (**Table IV.1.2**). The number of adult predators that left the sachets was negatively correlated with the number of *C. lactis* immature motile and adult contents inside the sachet. Conversely, this correlation was positive with respect to the number of immature co-specifics that came out of the sachets (**Table IV.1.2**).

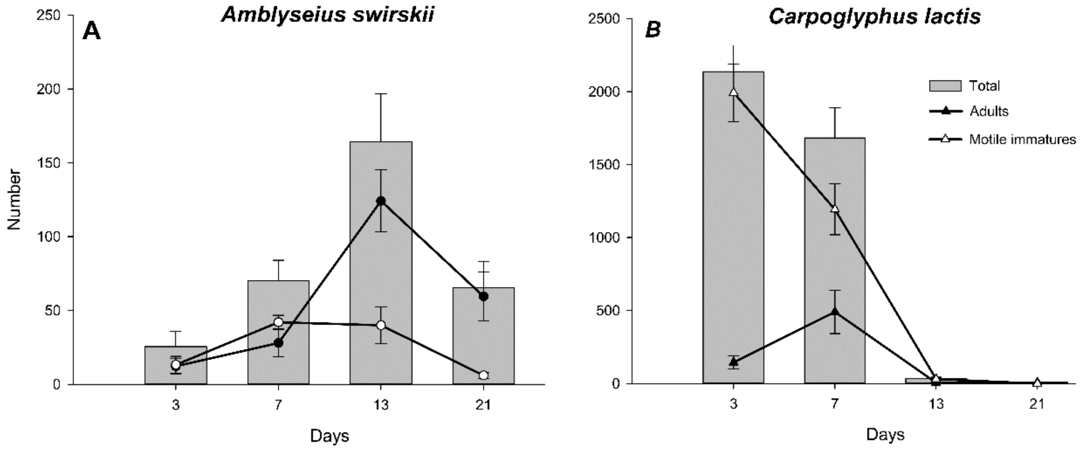

Figure IV.1.3 Mean (± SE; n = 4) number of motile immature, adult, and total (**A**) *Amblyseius swirskii* and (**B**) *Carpoglyphus lactis* mites exiting the slow-release sachets under controlled environmental conditions, accumulated at the time intervals indicated on the x-axis. Note the differences in scale on the vertical axes

Table IV.1.2 Pearson's correlation matrix for the mites presents inside the sachets and those accounted for outside (Pearson's coefficient/bilateral significance)

		Inside				Outside			
		A. swirskii (motile immature)	*A. swirskii* (adult)	*C. lactis* (motile immature)	*C. lactis* (adult)	*A. swirskii* (motile immature)	*A. swirskii* (adult)	*C. lactis* (motile immature)	*C. lactis* (adult)
Inside	*A. swirskii* (motile immature)	1							
	A. swirskii (adult)	0.060/0.802	1						
	C. lactis (motile immature)	0.502/0.024*	0.345/0.136	1					
	C. lactis (adult)	-0.001/0.996	0.950/0.001**	0.382/0.097	1				
Outside	*A. swirskii* (motile immature)	0.528/0.017*	-0.303/0.194	0.038/0.874	-0.451/0.046*	1			
	A. swirskii (adult)	-0.161/0.497	-0.532/0.016*	-0.538/0.014*	-0.630/0.003**	0.598/0.005**	1		
	C. lactis (motile immature)	0.446/0.049*	0.035/0.883	0.836/0.000**	0.013/0.958	0.123/0.606	-0.406/0.076	1	
	C. lactis (adult)	0.687/0.001**	-0.078/0.743	0.502/0.024*	-0.113/0.635	0.381/0.098	-0.268/0.253	0.512/0.021*	1

sterisks denote the significance of the Pearson's coefficients: * $0.01 < P < 0.05$ and ** $P < 0.01$)

IV.1.1.5. Discussion

The hexane flotation method extracted a higher number of both motile immature and adult *A. swirskii* and *C. lactis* mites than the method based on the Berlese-Tullgren funnel proposed for *N. cucumeris* (Kim et al. 2001; van Lenteren et al. 2003). Moreover, this new application has allowed us to verify the extraction far more rapidly and to dissociate the sampling over time with the extraction of the mites following their fixation. This enables mite populations to be studied in detail when a large number of sachets need to be analysed simultaneously, as is the case in biofactory quality control and/or in field tests. However, it should be noted that this technique does not allow the extraction of mite eggs.

This is not the first time that the affinity between arthropod cuticles and petroleum derivatives has been used to extract them from different media; indeed, it has been successfully used in diverse substrates such as soils, debris, stream substrates, moss and leaves (Barmuta 1984; Walters et al. 1987; Geurs et al. 1991; Kethley 1991; Belascoain et al. 1998; Andrew and Rodgerson 1999; Proctor 2001; Faraji et al. 2004; Rolland and Laroque 2006; Harris et al. 2017). In our case, hexane flotation extracted 3.7× the total number of motile mites in a totally organic medium composed almost entirely of wheat bran (**Fig. IV.1.1**) compared to the conventional method based on the Berlese-Tullgren funnel. Similar values (up to 4.1× more individuals) were obtained by Walter et al. (1987) in soil samples employing the same principle but using heptane flotation versus a Macfadyen-type funnel with a temperature gradient. A greater quantity and diversity of mites and other arthropods was obtained in stream substrates and debris samples when comparing flotation in kerosene versus direct extraction under a dissection microscope (Barmuta 1984; Proctor 2001) and even in cephalic capsules of chironomid dipterans (Rolland and Laroque 2006). Extraction has also been higher in other arthropods, such as springtails (Collembola) and Diptera, when this technique is compared to sugar flotation (based on density differences) (Andrew and Rodgerson 1999). Only Harris et al. (2017) reported a lower rate of mite recovery from a sample when using paraffin to extract *Tetranychus urticae* Koch (Acari: Tetranychidae) from apple and cherry leaves compared to other methods, including the Berlese-Tullgren funnel.

From the biological control standpoint, the dispersion rate of *A. swirskii* from the inside of the sachet to the crop is the reason for using this release technique. In predator-prey mite systems, two types of dispersal strategies can be distinguished: the ‘killer-strategy’ in which the predators remain in the ‘patch’ while prey are available, and the ‘milker-strategy’ in which predators disperse from the patch at a constant rate while the interaction lasts (Pels and Sabelis 1999). Furthermore, it is assumed that the predator emigration rate decreases as a function of prey density and is also influenced by the increased density of predators in the patch (Eveleigh and Chant 1982; Bernstein 1984). In the context of slow-release sachets in controlled conditions, if one compares the predator and prey populations, the maximum number of prey (motile forms) inside the sachet was recorded on day 3, from which point the population of *C. lactis* gradually decreased (**Fig. IV.1.2B**), whereas the maximum predator population inside the sachet was recorded on day 7 (**Fig. IV.1.2A**). With regard to the predator population leaving the sachet, the largest number of *A swirskii* exits was recorded within 5 days of recording the maximum number inside the sachet, most being adult forms (**Fig. IV.1.2**). Based on these results*, A. swirskii* might use a strategy similar to the 'killer' type, as supported by the negative correlation found between the density of adult predators present in the slow-release sachets and the proportion of the population that went outside (**Table IV.1.2**).

However, this should be viewed with caution as there may be differences among populations within a species (Pels and Sabelis 1999; Revynthi et al. 2018). Despite the above, it should be noted that a sachet such as the one we used, cannot exactly be considered a 'patch' due to the dispersion limitations that the sachet presents, having a single exit hole. On the other hand, the predator-prey relationship is not the only dispersion factor; at the individual level, there are numerous local conditioners and interactions that influence dispersion, as documented by Revynthi et al. (2018), all of which might influence the predator dispersion rate. Likewise, the sachets do not provide a watertight system; they will suffer variations in temperature, moisture content and relative humidity depending on the external environmental conditions. These physical factors are the most important influencing the development of mite populations (Collins 2012), affecting the dispersion rate from inside the sachet.

Regardless of the population fluctuations that occur inside the sachet, it was observed that the initial number of predators packaged in the biofactory was much higher than the total number leaving the sachet during the trial (1216.67±94.50 *A. swirskii* individuals packaged in the biofactory compared to a total of 325.25±50.76 predators leaving the sachet under the test conditions) and the amount of *A. swirskii* indicated by the manufacturer. These data suggest that the sachet performance may be improved. Given the complexity of comparing the inner content of the sachets and the number of predators that emerge, future research is required that investigates the most influential variables and that can model these interactions in a way that allows their behaviour to be explained.

In general, it has been shown that the extraction methods must be effective in recovering most of the population, be efficient in extracting all the species and states present and be capable of rapidly producing clean samples that allow arthropod identification (McSorley and Walter 1991). Another desirable feature is that they allow the indefinite storage of the samples prior to counting, as with this new proposed methodology. Considering all that is mentioned above, we believe that the hexane flotation extraction methodology proposed in this work offers an important advantage over dynamic and direct extraction methods, especially in the context of slow-release sachets used in biological control programmes.

IV.1.1.6. Acknowledgements

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IV.1.1.8. References

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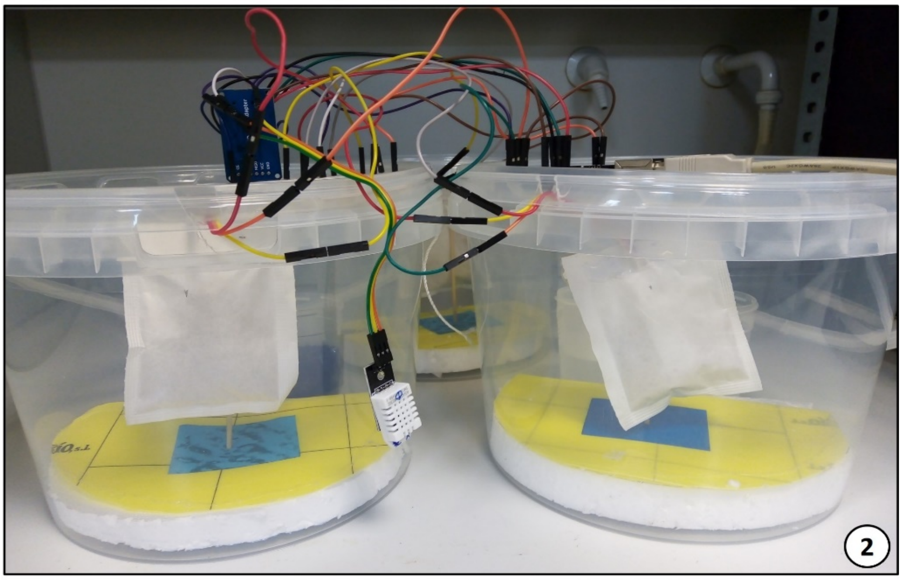
Sección IV.2



Ilustraciones página anterior:

1. Ensayo de dinámica poblacional del depredador *A. swirskii* y su presa *C. lactis* en el interior de sobres de liberación lenta, y tasa de liberación del depredador, bajo tres regímenes de humedad relativa ambiental.
2. Sistema Arduino® para monitorización de la humedad en el interior de los sobres de liberación lenta.
3. Muestras en estufa a 100 °C para determinar el porcentaje de agua en el sustrato de los sobres de liberación lenta.

Effect of relative humidity on the population dynamics of the predator *Amblyseius swirskii* and its prey *Carpoglyphus lactis* in the context of slow-release sachets for use in biological control in greenhouses

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Sección IV.2. Effect of relative humidity on the population dynamics of the predator *Amblyseius swirskii* and its prey *Carpoglyphus lactis* in the context of slow-release sachets for use in biological control in greenhouses

Abstract

Amblyseius swirskii is a predatory mite that is widely used for biological control in greenhouses. One way this predator is released is in a formulation in slow-release sachets. These sachets are prepared with the predatory mite, the factitious prey mite *Carpoglyphus lactis*, and a food substrate for the latter. The objective of the present study was to study the effects of microclimatic conditions in this type of formulation on the population dynamics of mites inside the sachets and on the release of predatory mites. These experiments were under laboratory conditions in two trials. The ambient relative humidity affected the water content of the food substrate of the prey mite inside the sachets, with an initial value of 21.49 ± 0.42%, which was reduced to values of 4.7±0.25%, 10.87±1.03% and 17.27±0.82% after 21 days of trials when they were exposed to low, medium and high ambient relative humidity levels, respectively. Relative humidity significantly altered the dynamics of the populations of both species inside the sachets and the exits of the predator from the sachets to the external environment.

Keywords: horticultural crops; microclimatic conditions; inoculative releases; prey-predator; mathematical model

IV.2.1.2. Introduction

The predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) is widely used worldwide for the control of small insects and mites that cause damage to crops [1,2]. This organism is successful because some species are omnivorous and can complete their biological cycle by feeding on pollen, which allows them to establish on the crop in a preventive manner and even develop in the absence or scarcity of pests [3,4]. Information on its origin, distribution and biological characteristics in relation to its use in biological control of this species is reviewed by Calvo et al. [5].

Environmental conditions are more stable and controlled for protected crops than for other outdoor crops. However, parameters such as temperature, relative humidity (RH) and light intensity vary over time. The main factors that influence these fluctuations are climate, season, geographic location, type of crop and the crop's plant structure [6]. The establishment of a population of predatory mites and their effectiveness in pest control will depend on the environmental conditions of the crop under field conditions [6]. Microclimatic conditions such as temperature and RH are crucial factors contributing to the survival of these phytoseiids [2] and influence the increase in their populations. Specifically, RH determines the viability of eggs and the developmental success of adults [6]. The two typical methods for the release of phytoseiid mites are direct release on the plant, through inoculative or inundative release when pest species are present, and the more recently developed novel method of slow-release sachets [7].

The slow-release sachets ensure better colonization and installation of the predator in the crop, which is key for successful biological control [8, 9]. These sachets release a large number of predatory mites over a sustained period [10] over several weeks [6]. They consist of a sachet with water-repellent walls and an outlet opening. The interior contains wheat bran along with sawdust or vermiculite as a carrier medium and food for the factitious prey, which is the food source for *A. swirskii*, ensuring its development until its spontaneous exit to the crop [8, 9, 11 - 13]. An alternative prey used as food for *A. swirskii* in slow-release sachets is *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) and *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) [5, 8, 14].

The commercial production of predatory mites is easy if the biological requirements of these arthropods are met. However, in the agroecosystems, large populations of phytoseiids are not frequently observed, suggesting that some natural factors affect their abundance in the field crops [1]. Therefore, an understanding of the effects of the parameters involved on the life cycle is necessary to evaluate the potential growth of the population of the species [2].

RH is one of the most important microclimatic factors that influences the biological parameters of mite populations [1, 2, 10, 15], as it is crucial for their survival [1, 2]. It directly affects the net reproduction rate and the intrinsic rate of natural increase for both *A. swirskii* and *C. lactis* along with other parameters that determine the population dynamics of predators [2]. In most phytoseiid mites, the egg stage is the most sensitive to dry conditions [1, 10]. Thus, an RH $\leq$ 70% reduces the viability of eggs [10, 16]. The motile forms (larvae, nymphs and adults) of the mites are capable of restoring their water content through food, consuming free water, seeking favorable places, etc. [1]. In this sense, a high RH may alleviate extreme conditions such as extremely hot temperatures and water scarcity [1, 2, 15]; in contrast, low RH may limit population growth at optimal temperatures, especially if the mite uses energy to balance hydration at the expense of reproduction [1, 2].

Based on the information described above, the environmental conditions inside the slow-release sachets seem to determine both the proliferation of predatory mites and prey mites. Therefore, the objective of work were twofold – firstly, to try to improve the evaluation of the slow-release sachet system by fitting it to mathematical models, and secondly, to evaluate the effect of RH on this biological control system and to discuss its repercussions on greenhouse crops.

IV.2.1.3. Results

The results obtained in the trial are described below in four sections. The first (IV.2.1.3.1) shows the variation in the RH of the environment (three treatments) and its effect on the humidity inside the sachets along with the water content in the substrate at the beginning and end of the trial. And the results of the population dynamics of both mite species inside the sachets at each ambient humidity regime are provided (IV.2.1.3.2). Subsequently, in section (IV.2.1.3.3), the effects of these RH levels on the exits of the predatory mite from the sachets are shown. Finally, in the fourth and closing section (IV.2.1.3.4), the results of the fit to the mathematical model of these mite release data are presented.

IV.2.1.3.1. Trial 1: Dynamic changes in the populations of *A. swirskii* and *C. lactis* inside the sachets according to RH

Figure IV.2.1 shows the variation throughout the RH trial inside the sachets (solid lines) as a function of the RH (dotted lines). Thus, the internal RH of the sachets having a 22.5% RH in the environment decreased substantially during the first 7 days from 72.80% to 20%, at which point the internal RH of the sachet fluctuated within the range of 14-16% until the end of the trial. In turn, the RH curve inside the sachets from the 52.5% RH regime progressively decreased from 70.8% to 43.1%, remaining above 60% during the first ten days. Finally, the internal RH of the sachets exposed to 87.5% ambient RH increased from 73.1% to 85% on Day 8 and then remained within the range of 84-86.48%. By comparison, the water content of the substrate varied from the initial value of 21.49 ± 0.42% to values of 4.78% ± 0.25%, 10.87 ±

1.03% and 17.27 ± 0.82 after 21 days of exposure to the low, medium and high RH values, respectively.

Figure IV.2.2A and **B** show the temporal variation in the populations of the prey mite *C. lactis* and the predatory mite *A. swirskii* (motile mites, in both cases) inside the slow-release sachets when exposed to three different relative humidity levels outside the sachets. The statistical analyses performed using generalized linear models (GZLM) for the data on the total number of *C. lactis* prey mites per sachet showed the high statistical significance of the model used (omnibus test: Chi-square test of the likelihood ratio = 104.610; degrees of freedom d.f. = 11; P < 0.01). Highly significant effects of the factors analyzed were observed, namely, RH (Chi-square likelihood ratio = 25.950; d.f. = 2; P < 0.01), trial time (days) (likelihood ratio Chi-square = 96.399; d.f. = 3; P < 0.01) and their interaction (Chi-square likelihood ratio = 21.715; d.f. = 6; P < 0.01), on motile factitious prey mites *C. lactis* inside the sachets.

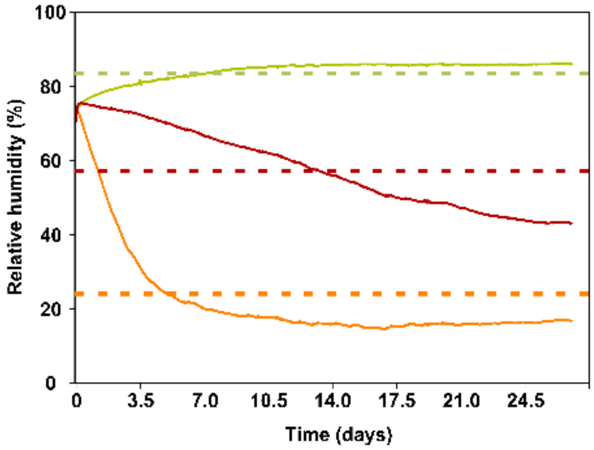

Figure IV.2.1 Temporal variation in the RH inside the slow-release mite sachets (solid lines) when they were maintained at three values of ambient RH, namely, low (22.5 ± 2.5%) (orange), medium (52.5 ± 2.5%) (red) and high (87.5 ± 2.5%) (green) (dotted lines), and at a constant temperature (25 ± 1 °C) under laboratory conditions.

According to the results (**Figure IV.2.2A**), the population of the prey mite exhibited a progressive decrease in all treatments, except for the high RH treatment (87.5%), where the population increased slightly from its initial density of 4235 ± 154.73 mites/sachet to 4352.5 ± 409.46 mites/sachet on Day 4. However, this density was not significantly different from that recorded at medium ambient RH, even until Day 7, because on Day 15, the population observed at this RH was significantly higher than that of the other treatment groups. At low ambient RH (22.5%), the *C. lactis* population remained consistent from Day 4 to Day 15, with values lower than those recorded in the other two RH groups. Finally, on Day 21, the three treatments did not differ significantly in the mite population.

Regarding the data for the motile predator mites *A. swirskii* inside the slow-release sachets, the statistical analysis using GZLM indicated the high significance of the model used (omnibus test: Chi-square likelihood ratio = 69.421; d.f. = 11; P < 0.01). The factors analyzed, namely, ambient RH, time (day) and their interaction, exerted highly significant effects on the total number of motile mites of this species (Chi-square likelihood ratio = 18.896; d.f. = 2; P < 0.01; Chi-square likelihood ratio = 53.746; d.f. = 3; P < 0.01 and Chi-square likelihood ratio = 20.377; d.f. = 6; P < 0.01; respectively).

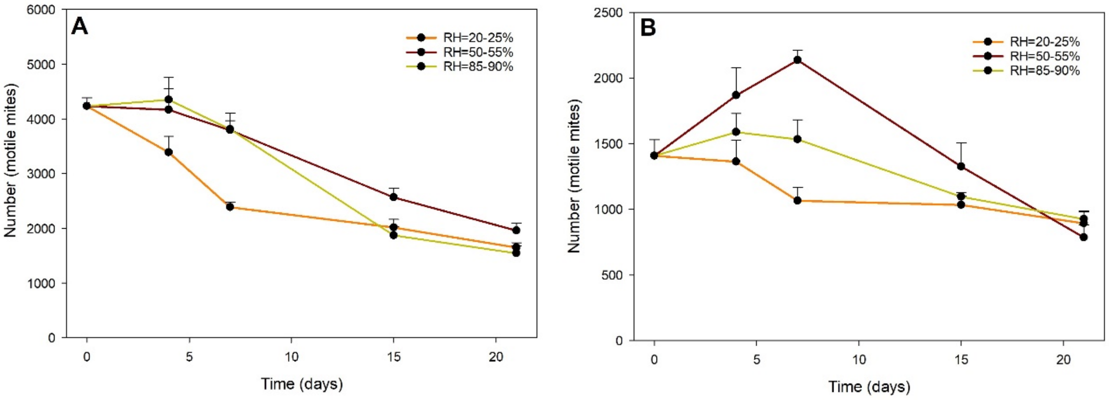

Figure IV.2.2 Total numbers of motile mites/sachet (means ± SE) of the prey mite *Carpoglyphus lactis* **(A)** and the predatory mite *Amblyseius swirskii* **(B)** inside slow-release sachets subjected to three levels of RH, namely, low (22.5%), medium (52.5%) and high (87.5%), in laboratory trials under controlled conditions (temperature = 25 ± 1 °C) (whiskers show the SE values).

As shown in **Figure IV.2.2B**, on Days 4 and 7, a significantly greater number of motile *A. swirskii* mites was observed in the sachets exposed to a medium RH of 52.5% (1867.5 ± 210.85 and 2135 ± 74.44 mites/sachet), than in sachets exposed to 87.5% RH (1587.5 ± 142.50 and 1532.5 ± 150.08 mites/sachet) and 22.5% RH (1362.5 ± 165.04 and 1065 ± 103.16 mites/sachet), respectively, while on Days 15 and 21, this value did not show significant differences between treatments. In general, the population of *A. swirskii* treated with low or high ambient RH progressively decreased from the initial value (Day 0), while at medium RH, the population peaked on Day 7.

IV.2.1.3.2. Trial 2: Evaluation of the release of *A. swirskii* from the sachets, according to RH

Figure IV.2.3 shows the temporal variation in the total motile mites of the predator *A. swirskii* that hatched from each sachet after exposure to the three levels of RH. The values for treatment and day represent the number of total mites accumulated during the intervals of days between sampling (0-4, 5-7, 8-15 and 15-21, respectively).

In the statistical analysis of the previous data, which was also performed using GZLM, a highly significant effect of the model used was identified (omnibus test: Chi-square likelihood ratio = 65.376; d.f. = 11; $P < 0.01$). Highly significant effects of the RH factor (Chi-square likelihood ratio = 20.096; d.f. = 2; $P < 0.01$), the time factor (day) (Chi-square likelihood ratio = 51.199; d.f. = 3; $P < 0.01$), and the interaction of both factors (Chi-square likelihood ratio = 58.813; d.f. = 6; $P < 0.01$) were observed.

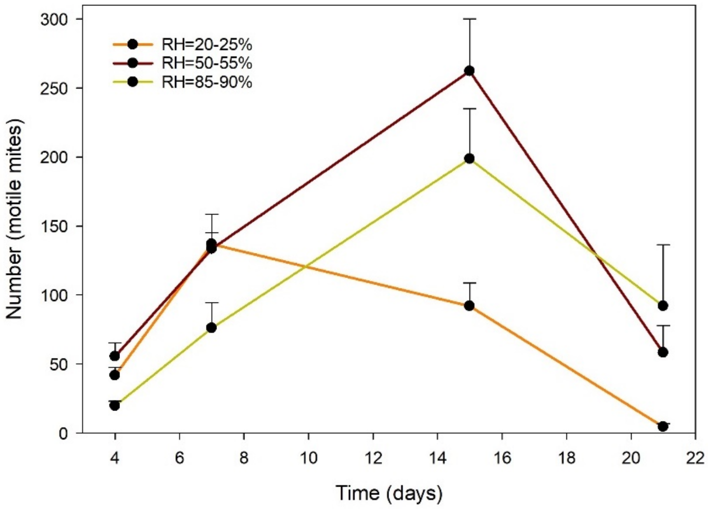

Figure IV.2.3 Total numbers of motile mites/sachet (means ± SE) of the predatory mite *Amblyseius swirskii* (immature motile and adult) that hatched from the slow-release sachets in periods of 0-4, 5-7, 8-15 and 15-21 days at the following RH levels in a laboratory trial conducted under controlled conditions (temperature = 25 ± 1 °C): low (22.5 ± 2.5%), medium (52.5 ± 2.5%) and high (87.5 ± 2.5%) (whiskers show the SE values).

In the period of 0-4 days, the release of *A. swirskii* did not differ significantly be-tween the three treatment groups, while in the period of 5-7 days, the number of predators that exited the sachets at low and medium RH did not differ significantly from each other. Both numbers were significantly higher than the value obtained at a low RH. The latter value corresponds to the maximum of this treatment (137 ± 21.69 mites/sachet). Subsequently, the release of predatory mites at low RH always remained significantly lower than that at the other two humidity levels. In turn, at medium and high RH, the values increased in the samples to maximum values in the interval of 8-15 days, when they reached their maximum values (262.25 ± 37.88 mites/sachet and 198.75 ± 36.24 mites/sachet, respectively). Subsequently, the exit of predators gradually de-creased until the end of the trial.

IV.2.1.3.3. Fit of the mathematical model to the number of predatory mites released from the sachets: rate and period of mite release, according to the RH regime

The previous data for the exit of motile mites of the predator *A. swirskii* (Trial 2) (Section IV.2.1.3.2) may be better interpreted by the mathematical model used. Thus, **Figure IV.2.4** shows the Verhulst-Pearl logistic models fitted to the total cumulative population (motile mites) that hatched from the sachets at the three values of ambient RH evaluated and their derivative functions. These fits were highly significant (**Table IV.2.1**). Although it is simpler, this mathematical model seems to better represent the actual conditions observed when establishing the exit speed of the predatory mites from the sachets (derivative functions) and their release window (period) (**Figure IV.2.4**).

The total number of predatory mites released (accumulative values) from the sachets at low RH was less than the value of 300 motile mites/sachet (**Figure IV.2.4C**). This value is the standard number for these formulations established by the International Organization for Biological Control (IOBC) [17]. In addition, the release period was noticeably short at 2-3 days. In contrast, at medium and high RH levels, the accumulative number of the total mites released was higher at approximately 500, covering periods of 10-15 days (**Figure IV.2.4A, B**).

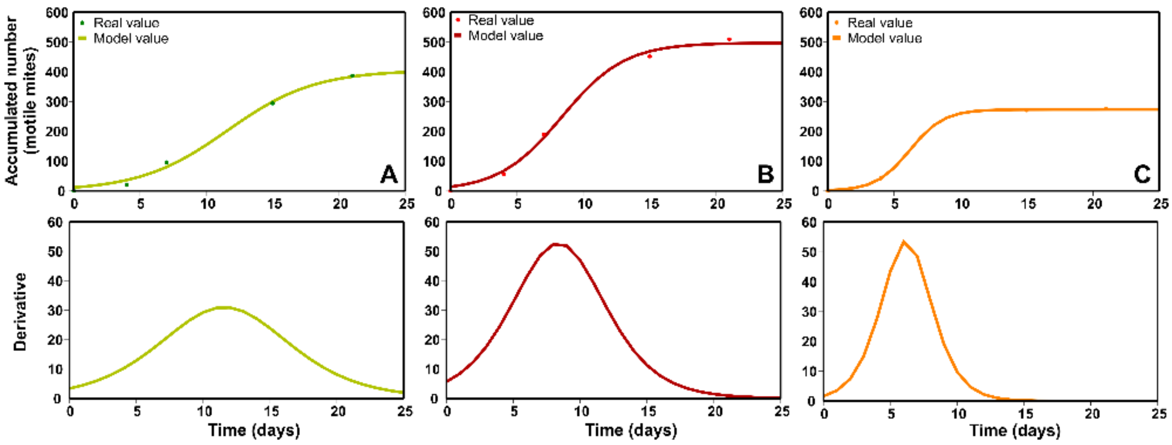

Figure IV.2.4 Mathematical models fits (upper panels) to the temporal variation in the total cumulative number of motile mites of the predator *Amblyseius swirskii* that hatched from the slow-release sachets at the following three humidity regimes under laboratory conditions (constant temperature = 25 ± 1 °C) and their derivative functions (lower panels): **(A)** high (87.5 ± 2.5%), **(B)** medium (52.5 ± 2.5%) and **(C)** low (22.5 ± 2.5%). The derivate describes the non – cumulative population size.

Table IV.2.1 Parameters and statistical significance for Verhulst-Pearl logistic functions fit to the total cumulative number of predatory mites *Amblyseius swirskii* (motile mites) that hatched from the slow-release sachets in the ambient RH treatment groups (22.5, 52.5 and 87.5%) under laboratory conditions (at a constant temperature = 25 ± 1 °C).

RH (%)	Fitting Parameters			Statistical Parameters		
	K	x_0	r	d.f.	R^2adj.	P
22.5	273.378	2.096	0.7857	3	0.9991	< 0.01
52.5	497.206	13.805	0.4243	3	0.9815	< 0.01
87.5	405.467	11.250	0.3059	3	0.9769	< 0.01

IV.2.1.4. Discussion

The importance of the microclimate (usually RH) in the performance of beneficial arthropods (predators and parasitoids) was established more than 80 years ago by Taylor [18] and is caused by the possible effects of stress under nonoptimal conditions on the development and survival of these entomophages (e.g., [19, 20]). The survival and other biological parameters of predatory mites are particularly affected by a lack of water and food (e.g., [21]). This finding is important, especially in greenhouses, when predatory mites are released directly on the crop. However, the use of controlled re-leases from mite sachets may change the microclimatic conditions, especially the availability of water, rather than food, as discussed below.

According to the results obtained here, the RH (actual RH measured inside the sachets, as well as the water content of the substrate) influenced the population dynamics of *A. swirskii* and *C. lactis* inside the slow-release sachets along with the number of predators that were released. Similarly, the moisture content inside these sachets was influenced by the RH. At 22.5% RH, the predator population did not increase, and the maximum release occurred during the first week. However, higher values of ambient RH caused higher population densities of predatory mites inside the sachet and in the number of *A. swirskii* released, although not proportionally, since these values were higher at 52.5% RH than at 87.5% RH. The internal *C. lactis* population did not increase in any humidity regime and decreased gradually, but the decrease was faster at low RH than at medium or high humidity.

The RH inside the sachets decreased during the first seven days of exposure to low RH, coinciding with the decrease in the populations of the predatory mite and its factitious prey. Subsequently, both populations remained stable (**Figure IV.2.1**). This finding may be because environments with low RH influence the microclimate inside the sachets, causing dehydration of the carrier substrate and/or reducing the survival of mite populations, as previously indicated [1, 12, 22]. At the end of trial 1, the humidity values inside the sachets subjected to this 22.5% RH level were very low, leaving the substrate practically dry (substrate water content, initial = 21.49 ± 0.42% and final = 4.78% ± 0.25%), while those that remained at a high ambient RH had lost very little water in the substrate (substrate water content, initial = 21.49 ± 0.42% and final = 17.27 ± 0.82%) at the end of the trial. In contrast, fungi induced a decrease in the general fitness of the phytoseiid mite [1]. In turn, the sachets subjected to 52.5% RH recorded values higher than 40% internal RH at the end of the experiment without the appearance of fungi. In addition, with this regime of medium RH, the humidity of the sachet remained above 60% until Day 10, which favored better biological parameters of both species and the release of predators (the water content of the substrate was reduced by half at the end of the trial), as will be described in more detail below. In summary, the increase in RH inside the controlled-release sachets improves water retention in the carrier media, resulting in an optimal microclimate for the biological development of mites, as previously indicated [6, 10, 23].

In mites, the egg is the stage of development most sensitive to low RH, since they cannot feed or move to obtain water from prey or the environment [1, 2, 16, 21, 24, 25], whereas motile mites tend to lose water by excretion or diffusion at low RH [24 - 26]. Therefore, at 22.5% RH, decreases in both the hatching and survival of the immature stages of the predatory mite and its factitious prey were observed, coinciding with the results for *A. swirskii* reported by San et al. [2], who showed that the eggs of this species do not hatch at 33% RH. Our findings are also consistent with the results reported by Midthassel [22], who indicated that the mortality of the motile stages increases in the range of 10 to 20% RH. Thus, Ferrero et al. [16] indicated that *A. swirskii* has a hygro-preference for oviposition and/or hatching of eggs such that the fecundity and/or hatching of larvae are reduced with the decrease in RH, while the duration of its motile stages is extended [2]. Thus, researchers concluded that the reduction in the population of this mite occurred as a result of the desiccation that caused the low RH near the eggs, larvae and protonymphs of the predator, and the immature stages of *C. lactis*, which induced eventual cannibalism in the females of *A. swirskii*, which feed on conspecific protonymphs due to the scarcity of prey, causing a decrease in longevity and fecundity [27]. On the other hand, the immature stages of *C. lactis* were affected by exposure to this low RH of 22.5% because the eggs of this species need a minimum value of 58.5% RH to hatch [28].

The population growth of *A. swirskii* inside the sachets exposed to medium RH (52.5%) differs from that indicated by other authors, who found that an RH less than 50-60% limits the development of predatory mites [24, 25, 29]. Although the population of *C. lactis* did not experience growth, it remained higher than that of the predatory mite due to the predator-prey relationship that characterizes commercial slow-release sachets. On the other hand, as mentioned above, the hatch rate of *C. lactis* eggs at 58.5% RH is 95%; however, the larvae do not reach the adult stage at this humidity level [28]. This result would explain why the treatment at medium RH did not increase the prey population but did increase oviposition, with the eggs of this prey mite maintaining and supplying the necessary moisture to the predatory mite *A. swirskii*, as cited by Midthassel et al. [13]. The increase in populations of the predatory mite with this medium RH occurred during the first 10 days, coinciding with a moisture content inside the sachets > 60%, as already indicated. In addition, it is also consistent with the fact that the hatching of the eggs from the biofactory and/or deposited inside the sachet occurs, which will reach the adult stage in approximately 7 days and subsequently consumes prey at 53% RH during this period, producing this increase in the population of *A. swirskii*.

In general, the increase in relative humidity induces the growth in mite populations and increases their survival [2, 30, 31]. However, the results from the present study indicate that the growth of *A. swirskii* populations inside the sachets exposed to 87.5% RH was higher than that obtained at low humidity but did not exceed the treatment with medium humidity. The slight increase in growth of the populations of *A. swirskii* and *C. lactis* during the first four days at high ambient RH might be promoted by the increase in the internal humidity of the sachets, thus allowing the population to reach the adult stage, which was observed for *A. swirskii* at 6 days when feeding on *C. lactis* at an RH of 70% [14, 32] and at 8 or 9 days for *C. lactis* when the RH was 84% [28]. After the seventh day at high RH, a decrease in the population of *A. swirskii* was observed, which corresponded to the decrease in available prey mites. This fact agrees with the study by Okamoto [28], who states that only between 49.1% and 66% of *C. lactis* larvae reach the adult stage in conditions of 84 and 94% RH, while the remaining larvae will be consumed by the predator, which shows a great preference for the youngest stages of its prey [13, 33, 34]. Thus, a lower availability of prey together with a decrease in the feeding of the predatory mite associated with the conditions of ambient RH [35] have influenced the functional response of *A. swirskii* [13], causing a reduction in the population.

The release of *A. swirskii* from the sachets also varied as a function of the RH regime to which they were exposed (**Figures IV.2.4A - C**). The shortest release interval (2-3 days) was recorded at low RH, a treatment at which the maximum release rate of *A. swirskii* was reached faster (Day 7) than in the other treatments (Day 15). This result is consistent with the study by Midthassel et al. [6], in which they indicated that *A. swirskii* reached its maximum release at 60% RH on Day 12, faster than with an RH of 75% (Day 17). Additionally, the results reported by Midthassel [22] seem to confirm our results by indicating that the greatest exit of *A. swirskii* from sachets occurs at 10% RH compared to others exposed to 20 or 30% RH. After this maximum release value and particularly beginning on Day 7 (**Figure IV.2.4C**), relatively few predators exited from the sachets, suggesting that this behavior might be motivated by the low population density of C. lactis or a decrease in the prey population caused by the increase in predation of *A. swirskii* due to low humidity conditions, as indicated by Mori and Chant [35] for *Phytoseiulus persimilis* (Athias-Henriot) and by Doker et al. [36] for *Neoseiulus californicus* (McGregor), in both cases at low RH.

The lowest total number of predatory mites that hatched from the sachets was recorded at low RH (22.5%), < 300, the standard value for formulations established by the IOBC [17]. In contrast, at medium and high RH, the total number of releases was higher at approximately 500. The treatment at medium RH (52.5%) resulted in the re-lease of the highest accumulative number of *A. swirskii*. Maximum release was recorded one week after the increase in its population inside the sachets (Day 15). Therefore, the production capacity of these rearing formulations was confirmed under these medium humidity conditions, where the presence of the prey *C. lactis* seems to dampen the effect of the ambient RH on the value of the internal RH in the sachet and the water content of the substrate. Shimoda et al. [10] indicated that the water supply under low humidity conditions promotes the release of *A. swirskii* from sachets exposed to 31.5-36.0% or 69.0-81.6% RH. Similarly, San et al. [2] indicated that *A. swirskii* shows a reproduction rate at 53% RH with a water supply, which is like that obtained at 92% RH with or without and extra water supply.

At high RH, the release of *A. swirskii* during the first ten days was lower than that recorded at 22.5% RH; its release profile was also lower than that recorded at 52.5% RH. These differences might be explained by the rapid exit of *A. swirskii* under low humidity conditions and by the population dynamics of the predator and prey at medium humidity, as previously described. Several authors have noted that the release of *A. swirskii* and other phytoseiid increases with the increase in RH, and they attribute this effect to the adequate development and reproduction of the predator inside the sachets [6, 10]. However, the predator-prey relationship inside the sachets determines the release of predators since the predation rate is reduced if this ratio is high [13]. A potential explanation for this finding is that phytoseiid decrease the consumption of prey when they have high population densities [13, 37] and/or in environments with high humidity [35], generating a crowded environment inside the sachets that accelerate the exit of the predator [22].

RH is one of the factors with the greatest effect on the population dynamics of predatory mites and their prey inside the breeding sachets [2, 22, 24]. Hence, some studies show that the increase in RH in the environment promotes a greater release of predatory mites from slow-release sachets [6, 10, 23]. However, our results suggest that the fluctuation of the microclimate inside these confined spaces differently affects the species of mites coexisting inside them, which have different biological and/or reproductive responses to the variation in humidity. Notably, the complex dynamics of the predator-prey system inside sachets depend on the interaction of moisture with other variables, such as the quantity and quality of the prey food, the moisture retention capacity of the substrate, the spatial complexity of the substrate, the interference of congeners [12, 13, 34], the porosity of the paper and other materials that constitute the walls of the sachet, the development of fungi in the carrier substrate, and the location of the sachets in the field [38, 39].

Greenhouses in the Mediterranean area, on the one hand, do not normally have heating and/or cooling systems; nonetheless, a need to meet market demands with products throughout the year exists in these greenhouses; thus, growers must extend the crop cycles toward summer seasons, when temperatures reached inside the greenhouses are well above the optimal temperatures (T > 35 °C) and ambient RH is very low with a high differential vapor pressure (DVP > 3 kPa) [40]. Based on the results we obtained, we must note that the effectiveness of the mites formulated in release sachets is potentially affected by the microclimate of these greenhouses. This effect might be mitigated with possible water misting when the RH conditions of the greenhouses are low. This solution should be evaluated under commercial greenhouse conditions.

IV.2.1.5. Materials and Methods

IV.2.1.5.1. Biological material

SWIRScontrol© slow-release sachets (Agrobio S.L., La Mojonera, Almeria, Spain) with a net weight of 4.83 ± 0.12 g were used and contained *A. swirskii* and *C. lactis* mites in all life stages, wheat bran as the main dispersal element of the prey (*C. lactis*) and other food substances for the latter. The material was used within 24 hours of receipt according to the handling instructions provided by the company to avoid a decrease in quality.

IV.2.1.5.2. Trials conditions

Two trials were conducted in climatic chambers at 25 ± 1 °C, with 52.5 ± 2.5% relative humidity and a light: dark (L:D) photoperiod of 16:8 hours. The treatments consisted of exposing the sachets to three levels of RH: high (87.5 ± 2.5%), medium (52.5 ± 2.5%) and low (22.5 ± 2.5%). These treatments were performed in sealed plastic containers with a 4.6 l capacity, in which silica gel (SiO_2) was introduced to adjust the low and medium RH; in turn, a saturated solution of potassium chloride (KCl) was used to achieve high RH [41].

The RH levels in each treatment group were recorded daily (every 15 minutes) with thermohygrometers placed inside the containers (Data logger, model EBI 20-TH, Ebro® Xylem Analytics Germany Sales GmbH and Co. KG Ebro, Ingolstadt, Germany). The RH was also monitored inside the sachets exposed to the three regimes using chromatic plates (chromotropic trap, medium size, Agrobio S.L., La Mojonera, Almeria, Spain) and humidity sensors provided with free software connected to configured DHT-22 humidity sensors (Sensor-Th, HAIGU)

(Arduino®, Somerville, MA, US) to record the RH every 10 minutes (Arduino® ArduinoHome, [42]. These sensors were introduced inside the sachets. All the recorded data were stored in an SD memory card for subsequent analysis. The final water content of the substrate inside the sachets was determined by oven-drying at 100 °C until the dry weight was constant [43].

IV.2.1.5.3. Trial 1: Evaluation of the population of *A. swirskii* and *C. lactis* inside the sachets

The study of the population dynamics of the predatory mite and the prey mite in-side the sachet exposed to the three levels of RH was performed by placing sachets in-side the plastic containers and, in turn, the silica gel or the KCl solution according to the corresponding treatment. The numbers of motile mites (larvae, nymphs and adults) of *A. swirskii* and *C. lactis* inside the breeding sachets were counted at the beginning of the trial (Day 0). Samples were collected at 4, 7, 15 and 21 days at each RH level. The treatments were replicated four times, and one sachet was needed for each day of re-cording and repetition, for a total of 16 sachets per treatment. The extraction of the motile stages of *A. swirskii* and *C. lactis* from the interior of the sachets to count the populations was performed using the hexane flotation extraction method described by Gallego et al. [44].

IV.2.1.5.4. Evaluation of the release of *A. swirskii* from the sachets

The exit of the predatory mites from the slow-release sachets was determined using the standard methodology of biological control companies [44], which consists of installing yellow chromatic plates to capture the specimens. In this experiment, each sachet was placed at the upper end of an 8-cm-long wooden rod. This rod was then inserted into the center of a yellow plate with entomological glue (20 x 20 cm), and a 3 x 2-cm fragment of dark blue shiny wrapping paper was placed in the center of this plate to achieve good dispersion of the mites and subsequent immobilization in the plate. Finally, the trap with the sachet installed was placed on an expanded polystyrene (EPS) support. The chromatic plates were replaced at 4, 7, 15 and 21 days after exposure to the three treatments. Immediately after completing the aforementioned sampling procedures, the number of *A. swirskii* motile mites present on the yellow plates and the dark shiny wrapping paper were counted using a stereo microscope. After obtaining the counts, the same sachet was placed on a new yellow plate with its respective sheet of dark shiny wrapping paper until the next sampling procedure.

IV.2.1.5.5. Statistical analysis

In trial 1, the values corresponding to the total number of motile mites (larvae, nymphs and adults) of the predator *A. swirskii* and of the prey mite *C. lactis* inside the sachets and those that exited from them were subjected to statistical analysis using GZLM and the gamma function and the logarithm link function as the distribution of the dependent variable. Two factors were considered: RH (at three levels), time (at four levels) and their interaction. The mean values were compared in pairs using the Wald test. For this analysis, the statistical software IBM SPSS version 26 [45] was used. In trial 2, only the values corresponding to the motile mites of the predator *A. swirskii* that hatched from the slow-release sachets were analyzed; the same statistical procedure mentioned above was used for this analysis.

IV.2.1.5.6. Mathematical model

The cumulative values of the total number of motile mites (larvae, nymphs and adults) of the predator that hatched from the sachets in each ambient RH treatment group over time were fit to a logistic-type or Verhulst-Pearl mathematical model using the following equation [46]:

$$x = \frac{K}{1+(\frac{K}{x_0}-1)\cdot e^{-r_m \cdot t}} \qquad (1)$$

$$\frac{dx}{dt} = r_m \cdot x \cdot (1 - \frac{x}{K}) \qquad (2)$$

where x_0 is the initial population of the mite (*A. swirskii*); x = number of accumulative mites hatching from the sachet; r_m = intrinsic rate of population growth; K = maximum capacity of the system; and t = time (in days). The models were fitted using TableCurve 2D software version 5.0 [47].

IV.2.1.6. Conclusions

(1) RH affects the population dynamics of the predatory mite *A. swirskii* and the factitious prey mite *C. lactis* when they are formulated in slow-release sachets.

(2) These effects of ambient RH alter the release of the predatory mite from the sachet to the external environment.

(3) The low ambient RH (22.5%) decreases populations of both the factitious prey and the predatory mites inside the sachet, which are significantly lower than the medium (52.5%) and high (87.5%) humidity levels.

(4) Verhulst-Pearl logistic models show the release dynamics of the predatory mite *A. swirskii* in the context of slow-release sachets.

(5) These models show that at low RH (22.5%), the total number of predatory mites released is < 300 mites per sachet, with this value representing the standard for this type of formulation established by the IOBC. In addition, the release period of these mites is noticeably short at 2-3 days.

(6) In contrast, the maximum release of the predatory mite was achieved at an intermediate RH (52.5%) with total values of approximately 500 mites released per sachet during an interval of 15 days.

(7) Finally, at high RH (87.5%), the values were slightly lower than the values obtained at intermediate RH (52.5%).

IV.2.1.7. Acknowledgments

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IV.2.1.8. References

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Capítulo V

Conclusiones / Conclusions

Capítulo V. Conclusiones / Conclusions

Capítulo II: Estrategia de conservación de los palmerales: Modelo de control químico óptimo del picudo rojo, *Rhynchophorus ferrugineus*

1. El modelo logístico ha permitido comprobar la existencia de dos cohortes en la población de *R. ferrugineus* causada por la diapausa larvaria que ocurre en climas con temperatura menor a 15 °C durante, aproximadamente, 3 meses. El modelo propuesto está compuesto por una doble función logística en la que cada una representó un tiempo de desarrollo diferente, y es suficientemente versátil para ser usado en zonas geográficas con condiciones climáticas similares.

2. El modelo de control óptimo puede ser usado para calcular el momento ideal de aplicación del control químico, dependiendo de las condiciones climáticas y/o el estado de infestación de *R. ferrugineus*.

3. La estrategia de control químico de larvas de *R. ferrugineus* es efectiva cuando se sincroniza con el máximo poblacional larvario, que, de acuerdo con el modelo óptimo de control químico, ocurre antes de los 50 días desde el inicio de la infestación. En dicho periodo, el coste del control químico será menor a la inversión que habría que hacer para controlar las pupas y/o adultos del picudo, según la curva solución del modelo.

Capítulo III: Evaluación de *Trichogramma cacaeciae* (Hymenoptera: Trichogrammatidae) y *Blattisocius mali* (Mesostigmata: Blattisociidae) en el control biológico de la polilla del tubérculo de la patata (Lepidoptera: Gelechiidae): Uso de funciones sigmoides

4. La efectividad depredadora de *B. mali* evaluada tanto por la eficacia de Abbot: 86,53%; como por el modelo logístico: 94,85%, así como del parasitismo de *T. cacaeciae* (eficacia de Abbot: 43,88% y modelo logístico: 73,77%), en los huevos de *P. operculella* muestran el potencial uso de estos agentes de control biológico en condiciones de almacenamiento.

5. La ecuación de Abbot es poco práctica, en este caso, ya que expresa únicamente la eficacia del control con relación a los individuos de la plaga supervivientes, de forma puntual y al final del ensayo, sin considerar las posibles reinfestaciones y el hecho de que los

entomófagos actúan de forma no instantánea y su actividad de depredación o parasitismo depende de la densidad de la presa/huésped, entre otros factores.

6. El modelo logístico propuesto es más exacto, en nuestro caso, en la evaluación de la efectividad de control de entomófagos por considerar todos los valores intermedios de la dinámica poblacional de la plaga, convertidos al índice de insectos acumulados por día en función del tiempo (AID) y bajo la regulación del depredador y/o el parasitoide.

Capítulo IV: Dinámica poblacional del depredador *Amblyseius swirskii* y su presa *Carpoglyphus lactis* en sobres de liberación lenta

Sección IV.1: Dinámica poblacional de ácaros en sobres de liberación lenta usados en control biológico: una nueva metodología de estudio

7. El método de flotación en hexano permitió extraer 3,7 más formas móviles (larvas, ninfas y adultos) de *A. swirskii* y *C. lactis*, y en un tiempo más corto que la extracción con el embudo de Berlese – Tullgren. Adicionalmente, este método permitió analizar muestras extraídas y almacenadas en refrigeración y, de esta forma, obtener una muestra limpia para identificar y contabilizar los ácaros.

8. La correlación positiva entre el número de *A. swirskii* y *C. lactis* en el interior del sobre y el hecho de que *C. lactis* registrara su máximo poblacional el día 3 mientras que *A. swirskii* lo alcanzara el día 7, así como la disminución consecutiva de la proporción depredador – presa durante el ensayo, parece indicar que la dinámica poblacional de los ácaros fue óptima para mantener la cría del depredador.

9. El número acumulado de depredadores liberados fue superior (325,25 ± 50,76 *A. swirskii*/sobre) a las especificaciones del producto para uso en cultivo (250 *A. swirskii*/sobre, según normativa IOBC), y la máxima tasa de liberación ocurrió entre los días 7 y 13, cuando la población de la presa comenzó a disminuir en el interior del sobre, reflejando una correlación negativa entre la salida de *A. swirskii* y la población interna de *C. lactis*.

Sección IV.2: Efecto de la humedad relativa sobre la dinámica poblacional del depredador *Amblyseius swirskii* y su presa *Carpoglyphus lactis* en el contexto de sobres de liberación lenta para uso en control biológico en invernaderos

10. La dinámica poblacional del depredador *A. swirskii* y su presa *C. lactis* en el interior de los sobres de liberación lenta, y la tasa de liberación del depredador, fueron afectados por la humedad relativa (HR) ambiental baja la cual indujo cambios en la humedad interna de los sobres.

11. No se registró crecimiento poblacional de *C. lactis* en ningún régimen de HR, y esto influyo en la tasa de crecimiento de la población de *A. swirskii*, particularmente en HR baja. Sin embargo, el depredador mostró un incremento poblacional en los regímenes de HR media y alta, en donde el nivel de presa fue suficiente para promover su reproducción, supervivencia y mantener la hidratación de los estados móviles en el interior de los sobres.

12. El modelo logístico utilizado representó de forma adecuada el periodo y la velocidad de liberación de *A. swirskii* en cada régimen de HR. De manera que esta metodología de evaluación pudiera incorporarse en los protocolos de control de calidad de los sobres (adaptándola a los estándares de la Organización de Control Biológico (IOBC, por sus siglas en inglés).

Conclusions

Chapter II: Conservation strategy for palm groves: Optimal Chemical Control Model for Red Palm Weevil, *Rhynchophorus ferrugineus*

1. The logistic model has allowed us to verify the existence of two cohorts in the population of *R. ferrugineus* caused by the larval diapause that occurs in climates with temperatures below 15 °C for approximately 3 months. The proposed model is composed of a double logistic function in which each one represented a different development time and is versatile enough to be used in geographical areas with similar climatic conditions.

2. The optimal control model can be used to calculate the ideal time of application of chemical control, depending on the climatic conditions and/or the infestation status of *R. ferrugineus*.

3. The chemical control strategy for *R. ferrugineus* larvae is effective when synchronized with the maximum larval population, which, according to the optimal model of chemical control, occurs before 50 days of the beginning of the infestation. In this period, the cost of chemical control will be less than the investment that would have to be made to control weevil pupae and/or adults, according to the solution curve of the model.

Chapter III: Evaluation of *Trichogramma cacaeciae* (Hymenoptera: Trichogrammatidae) and *Blattisocius mali* (Mesostigmata: Blattisociidae) in the post-harvest biological control of the potato tuber moth (Lepidoptera: Gelechiidae): Use of sigmoid functions

4. The predatory effectiveness of *B. mali* evaluated by both Abbot’s efficacy: 86,53% and logistic model: 94,85%, as well as parasitism of *T. cacaeciae* (Abbot efficacy: 43,88% and logistic model: 73,77%), on *P. operculella* eggs show the potential use of these biological control agents under storage conditions.

5. Abbot’s equation is impractical, in this case, since it expresses only the control efficacy in relation to the surviving pest individuals, in a timely manner and at the end of the trial, without considering possible reinfestations and the fact that entomophages act non-instantaneously and their predation or parasitism activity depends on the density of prey/host, among other factors.

6. The proposed logistic model is more accurate, in our case, in the evaluation of the effectiveness of the entomophagous control because it considers all the intermediate values of the population dynamics of the pest converted to the index of accumulated insect per day (AID) as a function of time and under the regulation of the predator and/or parasitoid.

Chapter IV: Population dynamics of *Amblyseius swirskii* and its prey *Carpoglyphus lactis* in slow-release sachets

Section IV.1: Population dynamics of mites in slow-release sachets used in biological control: a new study methodology

7. The hexane flotation method allowed the extraction of 3.7 more motile forms (larvae, nymphs, and adults) of *A. swirskii* and *C. lactis*, and in a shorter time than extraction with the Berlese – Tullgren funnel. In addition, this method made it possible to analyze samples extracted and stored under refrigeration and, thus, to obtain a clean sample to identify and count the mites.

8. The positive correlation between the number of *A. swirskii* and *C. lactis* inside the sachets and the fact that *C. lactis* registered its population maximum on day 3 while *A. swirskii* reached it on day 7, as well as the consecutive decrease in the predator-prey ratio during the trial, seems to indicate that the population dynamics of the mites was optimal to maintain the predator's breeding.

9. The cumulative number of predators released was (325.25 ± 50.76 *A. swirskii*/sachet) than the product specifications for use in culture (250 *A. swirskii*/sachet), and the maximum release rate occurred between days 7 and 13, when the prey population began to decrease inside the sachet, reflecting a negative correlation between the output of *A. swirskii* and the internal population of *C. lactis*.

Section IV.2: Effect of relative humidity on the population dynamics of the predator *Amblyseius swirskii* and its prey *Carpoglyphus lactis* in the context of slow-release sachets for use in biological control in greenhouses

10. The population dynamics of the predator *A. swirskii* and its prey *C. lactis* inside the slow-release sachets, and the predator release rate, were affected by low environmental relative humidity (RH) which induced changes in the internal humidity of the sachets.

11. No population growth of *C. lactis* was recorded in any RH regime, and this influenced the growth rate of *A. swirskii*, particularly at low RH. However, the predator showed a population increase in the medium and high RH regimes, where the prey level was sufficient to promote reproduction, survival and maintain hydration of the mobile stage inside the sachets.

12. The logistic model used adequately represented the period and rate of release of *A. swirskii* in each RH regime. In this way this evaluation methodology could be incorporated into the quality control protocols of the sachets (adapting it to the standards of the Biological Control Organization. IOBC).


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