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Santa Cruz de Tenerife
September 2019

Advances in the Integrated Pest Management of the banana pest Chrysodeixis chalcites (Esper) in the Canary Islands

TESIS DOCTORAL

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Universidad de La Laguna

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TAYLAN ÇAKMAK para optar al grado de Doctor en el programa de Doctorado
en Biodiversidad y Conservación por la Universidad de La Laguna

Advances in the Integrated Pest Management of the banana pest *Chrysodeixis chalcites* (Esper) in the Canary Islands

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INFORMAN:

que la presente memoria de Tesis Doctoral titulada "Advances in the Integrated Pest Management of the banana pest *Chrysodeixis chalcites* (Esper) in the Canary Islands" elaborada por D. TAYLAN ÇAKMAK ha sido realizada bajo nuestra dirección, y que cumple las condiciones exigidas por la legislación vigente para optar al grado de Doctor.

Y para que así conste, firman la presente en Santa Cruz de Tenerife a 16 de septiembre de 2019,

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Far from home, close to everyone.

All my best regards

Thank you everyone

“Verily, with hardship there is relief”.

With love

Taylan

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RESUMEN

Los problemas generados por la utilización de plaguicidas químicos (residuos, resistencias, etc.) y los riesgos que representan para la salud humana y el medio ambiente han propiciado que la Unión Europea desarrolle medidas legislativas que obligan a los productores de los estados miembros a adoptar la Gestión Integrada de Plagas (GIP) como estrategia de control en los cultivos.

Chrysodeixis chalcites (Esper, 1789) es una plaga polífaga que en España origina pérdidas económicas en cultivos de gran importancia (platanera, tomate, etc.), y para la cual actualmente no se dispone de métodos de control no químicos cuya efectividad sea satisfactoria. En un proyecto anterior (RTA2010-00016-C02) se desarrolló un nuevo bioinsecticida basado en el Alphabaculovirus de *C. chalcites* (ChchNPV-TF1) que es muy efectivo y selectivo para las larvas de este insecto.

En esta contribución se han llevado a cabo una serie de estudios en condiciones de campo y laboratorio dirigidos a: (i) Conocer la distribución de la oviposición de *C. chalcites* en la platanera; (ii) Mejorar la persistencia y la transmisión del baculovirus en las poblaciones del insecto, para desarrollar una formulación efectiva; (iii) Determinar la eficacia de las aplicaciones de insecticidas biorracionales en racimos de platanera para el control de *C. chalcites*.

En el estudio de distribución de la puesta de *C. chalcites* se encontró una diferencia significativa en el comportamiento oviposicional entre las tres etapas de crecimiento de la platanera. La plaga pone más huevos en las plantas jóvenes que en las plantas desarrolladas con racimo y sin racimo, probablemente debido a que las hojas son más tiernas en estas plantas jóvenes. Además, se observó que el número de huevos y larvas de *C. chalcites* era más abundante en el envés que en el haz de las hojas de platanera. En base a estos datos, y a la distribución de las puestas según la posición de la hoja en la planta, se sugiere que en los muestreos para detectar *C. chalcites* en cultivos de platanera se debería mirar la 5ª hoja de las plantas jóvenes, o la 8ª hoja en plantas desarrolladas con racimo, o la 8ª hoja en plantas desarrolladas sin racimo. En todos los casos, primero se debe revisar el envés de las hojas.

Para el desarrollo de una formulación efectiva de ChchNPV-TF1, que mejore su persistencia en la superficie de la hoja, se probaron como sustancias fotoprotectoras el carbón vegetal (charcoal) al 1% y el cacao al 1%. Se encontró que la aplicación del virus

con la adición de carbón vegetal o cacao como pantallas UV en condiciones de campo permite que se produzca mortalidad larval por períodos más largos y se prolongue la actividad del virus, incluso en zonas de alta radiación UV. El período sensible es los primeros tres días después de la aplicación. Para dosis letales altas en los primeros días del experimento, la adición de carbón vegetal o cacao protege más al ChchNPV-TF1, especialmente al comienzo del experimento (1-3 días). En el trabajo de campo realizado en 2017, la aplicación de 1×10^8 OB / ml resultó en una infección letal de 54 ± 10% (20.85 ± 10.42) en larvas recolectadas 1 día después de la aplicación. Por otro lado, la aplicación de 1×10^9 OBs / ml resultaron en una infección letal del 86 ± 7% en las larvas recolectadas 1 día después de la aplicación a una infección letal del 92 ± 5% en las larvas recolectadas a los 3 días después de la aplicación, lo que muestra un pico de mortalidad crucial en esas dosis.

En los ensayos para determinar la eficacia de productos biorracionales para el control de *C. chalcites* en platanera, se evaluaron preparados comerciales en base a extractos de plantas y/o complejos bioenzimáticos: Intruder®, Avenger®, BioKnock®, Cinamite®, Garlitrol-Forte®, Prevam® e Indasol®, en condiciones de laboratorio e invernadero de malla. El ensayo de elección (prueba de % de repelencia) se basó en el consumo evitado de las hojas tratadas. Se agregaron dos larvas de *C. chalcites* en el segundo estadio en cada placa de Petri y se dejaron alimentar en las hojas durante 24 h, para elegir entre discos tratados o no tratados. Las hojas tratadas con Prevam® (85.19 ± 1.7%) diferían significativamente ($P < 0.05$) de los otros productos. En la segunda prueba de laboratorio (prueba sin elección), se agregaron dos larvas de *C. chalcites* en el segundo estadio en cada placa de Petri y se dejaron alimentar en las hojas durante 24 h, lo que obligó a las larvas a comer en las hojas tratadas. Prevam® (0.92 ± 0.4%) e Indasol® (0.98 ± 0.33%) tuvieron el área de menor consumo 24 h después de la aplicación. Y en la prueba de toxicidad por contacto, para evaluar el efecto directo de cada producto en las larvas de *C. chalcites* de segundo estadio, un disco de hoja de plátano infestado con 10 larvas de *C. chalcites* de segundo estadio. Se observaron larvas a 1 día, 3 días y 7 días después de la aplicación, y se registró el tiempo hasta la muerte (si ese fuera el caso). Siete días después de la aplicación, se detectó la mayor mortalidad en las hojas tratadas con Intruder® (77.77 ± 5.7%), seguidas de las hojas tratadas con Indasol® (76 ± 9.27%) y Prevam® (58 ± 11.13%). Luego, se realizó una prueba en invernadero para comparar la

eficacia del producto en el nivel de daño por *C. chalcites* de segundo estadio en racimos de banano. Para la infestación artificial se utilizaron 40 larvas de *C. chalcites* del segundo estadio por racimo de banano. Una semana después de los tratamientos, se evaluó la mortalidad de *C. chalcites* y el nivel de daño a los frutos. Se discute la reducción del daño de la fruta. Con respecto a la eficacia de los productos siete días después de la aplicación, los racimos tratados con Prevam® mostraron (100,0 ± 0) eficacia en *C. chalcites* de segunda etapa. Indasol® (42.53 ± 21.47), Intruder® (36.25 ± 23.75) y Ripelser® (36.74 ± 12.74) estaban en el grupo intermedio. Bioknock® (11.16 ± 6.47), Avenger® (9.88 ± 5.71) y Cinamite® (15.59 ± 9.03) siguieron siendo el grupo menos efectivo en el séptimo día posterior a la aplicación. Los experimentos en invernaderos mostraron que Prevam®, Intruder® e Indasol® redujeron el número de larvas y el nivel de daño en el racimo de plátano, y tenían alrededor del 70% de racimos clasificados en la Categoría 1. En este ensayo, la aplicación de estos productos duplicó los ingresos de la fruta con respecto al control. En la ultima contribución, el objetivo era; "Determinar la eficacia de los productos biológicos o naturales utilizados actualmente en platanera en el control de *C. chalcites*". BioKnock®, Avenger®, Cinamite®, Garlitrol® y Ripelser® tenían una protección intermedia contra las larvas de *C. chalcites* en racimos de plátano después de 7 días de aplicación, el nivel de daño era alto y más del 50% de racimos se consideraron como fruta no comercializable en este tratamiento. Los resultados de pérdidas económicas evitadas son paralelos a los obtenidos de las pruebas de eficacia de laboratorio e invernadero. Como resultado, un análisis completo de laboratorio, experimento en invernadero y pérdidas económicas evitadas mostró que Prevam®, Intruder® e Indasol® fueron los tres productos más exitosos contra las larvas de *C. chalcites* en el cultivo de platanera.

Los resultados obtenidos en este estudio proporcionan información original y prometedora que contribuyen a los programas de desarrollo de la Gestión Integrada de *C. chalcites* en el cultivo de la platanera en las Islas Canarias, incluyendo el baculovirus ChchNPV-TF1 y los productos biorracionales. En futuros trabajos sería interesante comparar los resultados obtenidos en estos ensayos con estudios en cultivos de platanera al aire libre, y ampliando la evaluación a otros productos biorracionales.

SUMMARY

The problems generated by the use of chemical pesticides (waste, resistance, etc.), and the risks they pose to human health and the environment, have led the European Union to develop legislative measures that oblige the producers of the member states to adopt Integrated Pest Management (GIP) as a control strategy in the main crops. *Chrysodeixis chalcites* (Esper, 1789) is a polyphagous pest, which in Spain causes economic losses in large crops (banana, tomato, etc.), for which there are currently no non-chemical control methods whose effectiveness is satisfactory. In a previous project (RTA2010-00016 C02), a new bioinsecticide based on the *C. chalcites* Alphabaculovirus (ChchNPV-TF1) has been developed that is very effective and selective for the larvae of this insect. The objective of this project is to optimize the use of ChchNPV-TF1 in GIP programs that are effective and sustainable.

In this contribution, a series of studies have been carried out, in field and laboratory conditions, aimed at: (i) The distribution of *C. chalcites* in the plant, (ii) The addition of natural subsances to ChchNPV-TF1 baculovirus to improve persistence of baculovirus on open air conditions against UV light. (iii) Better understanding on the criteria of efficacy of several bioinsecticides.

In the **Chapter 2**, we discussed the matter under the title "*Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) oviposition preferences on different growing stages of banana (*Musa acuminate* Colla, Musaceae) plants". Significant difference in ovipositional behaviour was found between the three growing stages of banana plants. Significant differences on the preferences of C. chalcites adult preferences on banana plantas were detected. As a result, in order to detect *C. chalcites* populations on a banana plantation, it can be suggested to look firstly to the 5th leaf on young plants, secondly to the 8th leaf on pants with bunch, and finally plants without bunch. In all cases, more eggs and first instar larvae were found on young plants and on the underside of the leaves.
When the number of eggs and larvae of *C. chalcites* counted in different combinations of plant stages of banana plants, *C. chalcites* lays more eggs on “young plants” than plants “without bunch”, this is likely due to the fact that leaves are more tender in young plants.

Secondly, in the **Chapter 3**, we discussed "Effects of several UV protected substances on the persistence of the insecticidal activity of the Alphabaculovirus of *C. chalcites* (ChchNPV-TF1) under laboratory and open field conditions on young banana (*Musa acuminata*, Musaceae, Colla) plant" on the matter of "Development of an effective ChchNPV-TF1 formulation, improving its persistence on the leaf surface aganist UV light." In the field experiment with cacao (1 %) and charcoal (1 %) additives for UV protection, larvae from the leaves collected at up to 7 days post-treatment continued to show mortalities caused by lethal virus disease suggests that the application of the virus with addition of cacao or charcoal as UV screens longers the virus activity in the field conditions. Also, addition of 1% charcoal or 1% cacao to ChchNPV-TF1 10^9 OBs/l solution may extend the period of pest control, protecting OBs from UV degradation by producing larval mortality for longer periods and the duration of activity of ChchNPV-TF1 virus protected with these natural substances may increase the performance of virus application even in high UV radiation zones.

Thirdly in the **Chapter 4**, Efficacy of biorational insecticides to prevent damage of *C. chalcites* (Esper) (Lepidoptera: Noctuidae) on banana fruit was presented as an article. In this contribution the objectve was to; "Determine the efficacy of bio-rational or natural products currently used on banana plantations on the control of *C. chalcites.".* Laboratory and greenhouse experiments were conducted during the summer seaason in 2017-2018. The idea of the choice assay (% repellence test) was based on the avoided consumption of the treated leafs. Two 2nd instar larvae of *C. chalcites* were added in each per Petri dish and were let to feed on leaves for 24 h, in order to choose between treated or untreated discs. Prevam® treated leaves (85.19±1.7 %) significantly differed (*P< 0.05*) from the other products. In the second laboratory test (no choise test), Two 2nd instar larvae of *C. chalcites* were added in each per petri dish and were let to feed on leaves for 24 h, forcing the larvae to eat on the treated leaves. Prevam® (0.92±0.4 %) and Indasol® (0.98±0.33 %) had the lowest consumed area 24 h post application. And in the contact toxicity test, in order to evaluate the direct effect of each product on *C. chalcites* 2nd instar larvae, one disc of banana leaf infested with 10 2nd instar larvae of *C. chalcites*. Larvae were observed at 1 day, 3 days, and 7 days post-application, and time to death (if that was the case) was recorded. Seven days post application, the highest mortality was detected on Intruder® treated leaves (77.77±5.7%), followed by

Indasol® (76±9.27%) and Prevam® (58±11.13%) treated leaves. Then, a greenhouse trial was conducted to compare the efficacy of the product on damage level by 2nd instar *C. chalcites* on banana bunches. Forty 2nd instar *C. chalcites* larvae per banana bunch were used for artificial infestation. One week after the treatments, *C. chalcites* mortality and fruit damage level were evaluated. Fruit damage reduction is discussed. Regarding the efficacy of the products seven days post application, Prevam® treated bunches showed (100.0 ±0) efficacy on *C. chalcites* 2nd stage instars. Indasol® (42.53 ± 21.47), Intruder® (36.25 ± 23.75) and Ripelser® (36.74 ± 12.74) were in the intermediate group. Bioknock® (11.16 ± 6.47), Avenger® (9.88 ± 5.71) and Cinamite® (15.59 ± 9.03) remained the less effective group at the 7th day post application. Greenhouse experiments showed that Prevam®, Intruder® and Indasol® reduced number of larvae and damage level on banana fruit, and had around 70% bunches classified into Category 1 (High quality). As a result, a complete analysis of laboratory, greenhouse experiment and avoided economic losses showed that Prevam®, Intruder® and Indasol® was those three succesful products against 2nd stage larvae of *C. chalcites*. The avoided economic losses results are paralel to those obtained from laboratory and greenhouse efficacy tests. As a result, a complete analysis of laboratory, greenhouse experiment and avoided economic losses showed that Prevam®, Intruder® and Indasol® were the three most successful products against 2nd stage larvae of *C. chalcites*. Overall, obtained results in this study is providing promising information that can be a useful tool for the future of biological control of *C. chalcites* with ChchNPV-TF1 formulation. This study is providing additional information on the control of *C. chalcites* on banana plantations in Canary Islands by its original content. It may support the IPM development programs and may serve farmers as practical information. However, it is also necessary to compare greenhouses and open-field trials and obtain results with different substances and conditions to develop a better formulation for ChchNPV-TF1 for future studies.

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CHAPTER 1

1. GENERAL INTRODUCTION

Chrysodeixis chalcites is a polyphagous insect, native to the Mediterranean basin, which currently has a wide geographical distribution that includes Europe, Africa, Oceania and Asia (Engle et al. 2008; Fuentes et al., 2018). The larvae of *C. chalcites* are polyphagous, feed on many cultivated plants (fruit, horticultural and ornamental), belonging to more than thirty plant families (Cabi, 2007, 2013, Cabello et al., 1996), where they usually cause significant economic losses (Napiorkowska-Kowalik & Gawlowska, 2006; Broza & Sneh, 1994). In Spain it causes damage to various crops both outdoors and in the greenhouse. In recent years, in the Canary Islands, *C. chalcites* has become an occasional pest by causing economically important damage on banana plants (Del Pino et al., 2011b) where it produces losses of up to 30% by weight of the crop (Fuentes et al., 2017). In the peninsular territory, *C. chalcites* causes damages of different consideration in various horticultural crops (tomato, pepper, etc.) although the most important losses occur in tomato both outdoors and under cover (Cabello & Belda, 2004; Torres-Vila, 2010).

Currently, the control of *C. chalcites* in the Canary Islands is based mainly on the continuous application of synthetic chemical insecticides and to a lesser extent on other bio-rational or natural products (azadirachtin, Spinosad, etc.) and entomopathogenic bacteria *Bacillus thuringiensis* var. *kurstaki.* However, the effective control of this pest with chemical insecticides requires repeated applications, increasing production costs, serious environmental risks, and the accumulation of chemical residues that hinder the commercialization of products. In addition, the EU has established a strategy on the sustainable use of phytosanitary products (Directive 2009/128 / EC) whose main objective is to adopt a series of measures that mitigate the risk posed by the use of pesticides and enhance the implementation of Integrated Pest Management (GIP); This community strategy has resulted in a National Action Plan (NAP) that is mandatory since January 1, 2014, which establishes, among its general principles, that biological methods or other sustainable non-chemical methods should be preferred to chemical methods, provided they allow satisfactory control of pests.

There have been already two national research projects on *C. chalcites* funded by INIA. The first project was named "Incidence of *Chrysodeixis chalcites* in the Canary Islands and its control with a bioinsecticide based on a baculovirus" (RTA2010-00016-C02). In this project the molecular and insecticidal characterization of the *C. chalcites* Alphabaculovirus (ChchNPV-TF1) has been carried out (Bernal et al., 2013a, 2013b, 2013c), a mass production method was optimized (Bernal et al., 2014a) and it was demonstrated that simple formulations of this virus were equally efficient to the control agents currently used (Bernal et al., 2014b). The conclusions of this project was based on several contributions that presents fundamental information. On tomato and banana plants, the efficiency of the ChchTF1 was tested in greenhouse trials. The $1x10^9$ OBs/l treatment was found 3 to 4-fold more effective in reducing larval infestations than the chemical or Bt treatments. Given that pesticides are usually applied to banana crops at volumes of 1600-2000 l/ha, depending on plant phenology, the rate of $1x10^9$ OBs/l would be equivalent to approximately $1.6\text{-}2.0x10^{12}$ OBs/ha. Taking into account that $8.07x10^{13}$ OBs are obtained with 150-infected larvae between 40 and 50 ha would be efficiently tretaed. ChchTF1 appears to have a remarkab*le potential* as a biological control agents against *C. chalcites* larvae on tomato or banana plants, which merits its registration as a crop protection product for use in banana or tomato crops in the Canary Islands. The results of this thesis have contributed as a Spanish patent application (P201330487). The second project was named "*Chrysodeixis chalcites* Alphabaculovirus, a new biological control agent: its integration into Integrated Pest Management (IPM) programs". In this project, the efficacy of the most prevalent isolate of the *C. chalcites* nucleopolyhedrovirus was studied as a biological insecticide. The prevalence of ChchSNPV infection in *C. chalcites* populations was 2.3% (103 infected larvae out of 4,438 sampled), but varied from 0±4.8% on Tenerife and was usually low (0±2%) on the other islands. Application of $1.0x10^9$ viral occlusion bodies (OBs)/l of ChchNPV-TF1 significantly reduced *C. chalcites* foliar damage in young banana plants as did commonly used pesticides, both in greenhouse and open-field sites. The insecticidal efficacy of ChchNPV-TF1 was similar to that of indoxacarb and a *Bacillus thuringiensis* (Bt)- based insecticide in one year of trials and similar to Bt in the following year of trails in greenhouse and field crops. However, larvae collected at different time intervals following virus treatments and reared in the laboratory experienced 2±7 fold more

mortality than insects from conventional insecticide treatments. This suggests that the acquisition of lethal dose occurred over an extended period (up to 7 days) compared to a brief peak in larvae on plants treated with conventional insecticides. (Fuentes et al., 2017).

This contribution brings several advances on the control of *C. chalcites* on banana plantations of Canary Islands such as: (i) improving the information on spatial distribution of the insect oviposition on banana plants, (ii) persistence of baculovirus on banana plantations by using natural substances as UV protectants in virus formulation and (iii) improving the knowledge on use of bioinsecticides and their efficacy on banana plants.

2. *Chrysodeixis chalcites*

2.1. Taxonomy and morphology

Chrysodeixis chalcites (Esper) (Lepidoptera: Noctuidea) in Spain is known with different names such as “lagarta”, “bicho camello” and “oruga medidora”. In English there are several denominations, such as "twin golden spot" (Zhang, 1994), "tomato looper" (Harakly & Farag, 1975) and "green garden looper" (Zimmerman, 1958) (Table 1.1.).

Table 1.1. Taxonomical classification of *C. chalcites*.

Kingdom	**Animalia –** Animal
Phylum	**Arthropoda** – Arthropods
Class	**Insecta** – insects
Order	**Lepidoptera** – butterflies
Family	**Noctuidae Latreille** – noctuid moths
Subfamily	**Plusiinae (Boisduval)**
Tribe	**Argyrogrammatini (Eichlin and Cunningham)**
Genus	**Chrysodeixis (Hübner)**
Species	***Chrysodeixis chalcites* (Esper)**

2.2. Geographical distribution

Chrysodeixis chalcites is found mainly between 45° N and 35° S latitude in the area that ranges from South Europe, including the Mediterranean and Middle East countries, to South Africa (Fig. 1.1.) (Balachowsky, 1963; Murillo et al., 2000; Crop Protection Compendium, 2007).

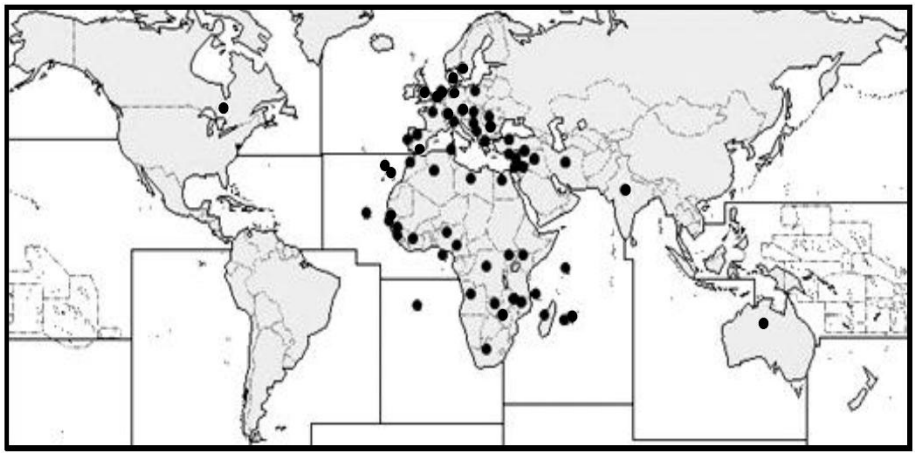

Figure 1.1. Worldwide geographical distribution of *Chrysodeixis chalcites* (black spots) (CABI, 2013).

There is nevertheless a little confusion between this species and *Chrysodeixis eriosoma*, as referred by Zhang (1994). In his paper he points out that the distribution of *C. chalcites* in South and East Asia and Oceania usually referred by literature corresponds indeed to *C. eriosoma*. It is likely that all the distribution records of the vicariate species *C. eriosoma* reported for the Afrotropical and Mediterranean area, including also some of the Canary Islands, are incorrect (Spitzer & Jaros, 2004). In the Spanish Peninsula *C. chalcites* is acknowledged for being a pest of orchards, ornamental and industrial crops. On the Canary Islands it was first described in 1904, after being detected on Tenerife Island (Cie, 1977). At present, it is present on all the islands of the archipelago (Bacallado A., 1972; García et al., 1992; Hernández, 2007). For a long time, *C. chalcites* was known for being a pest of banana plants, even though Perera & Molina (2002) consider it as a sporadic pest on La Palma and Tenerife islands since 2000. At present it is among the most severe pests for this crop, without exception for those in open air or in greenhouse (Garcia, 2003, Del Pino et al., 2011a).

2.3. Biology, ecology and life stages

Biology: The first studies on *C. chalcites* originated from the need to face the serious economic impact caused by this pest: nearly fifty years ago Gaumont & Moreau (1961) studied the biology of the species in a number of vegetable hosts. The most recent works are those by Goodey (1991) and Amate et al. (1998). These studies provide the knowledge about *C. chalcites* biology, explaining that, upon hatching, larvae - place themselves on the underside of the leaf and eat the parenchyma, thus becoming hard to be detected. Only during the second and third stage the larvae start to eat the leaf edges (Rashid et al., 1971), and in the following stages they become even more voracious: they bite, sting and eat up all the leaves, but the central veins. During the night, the adults lay eggs on the underside of the leaf (Cayrol, 1972), either isolated or in small groups (Harakly & Farag, 1975), so that they are scattered in the crop (Linden, 1996). In some crops the larvae may eat the fruits, as it was observed in bananas by Vilardebo & Guèrout (1964) who witnessed the larger larvae placing themselves in the cigar leaf (the unopened leaf), the softer part of the plant, where their formation is then completed. If efflorescence appears, the larvae can eat the inner tissues of bracts and of the fruits in formation, causing the skin to be wounded to different degrees and rapidly turning darker, only seldom damaging the pulp.

In the first stages the larvae have nocturne behavior, and after reaching the maximum development at their sixth stage, they stop eating and create a silk white cocoon on the underside or on the axils of the leaves, along the central vein, where metamorphosis takes place in the state of pupa or chrysalis (Passoa, 1995; Cabello et al., 1996). Occasionally, cocoons can be found on the ground (Harakly & Farag, 1975). Adults emerge, and start immediately to fly and mate: males mate as soon as they emerge out of the pupa, while females mate within 1 to 4 days after (Amate, 1998; Mau, 1999). Adults are semi-nocturnal and generally avoid strong sunlight (De Liñán, 1998).

The life cycle duration varies according to temperature, food composition and climate conditions. The full cycle is usually completed within 45 days at 20° C, while at temperature lower than 25° C the larvae cycle can be completed in 44 to 50 days (Del Pino 2011a). The activity and the development can be influenced to some extent by the photoperiod. According to Del Pino et al. (2011a) The complete biological cycle lasts

about 4-5 weeks at 25 ° C. The adults (moths) of this pest usually make egg laying in different parts of the banana plant, especially in the leaves. Therefore, the larvae are mostly found on the leaves, where they are able to complete their biological cycle. The passage from larva to pupa on *C. chalcites* is usually performed on the underside of the leaves, along the central nerve.

Ecology:

The cycle of *C. chalcites* is bivoltine. Despite being most probably a species with optional diapause, in some years it can reach over numbering generations, and also that is what occurs in populations associated to protected crops (De Liñán, 1998). The adult can fly impressive distances, as it migrates from North Africa or Southern Europe, especially during spring or autumn (García et al., 1998). For Spain, the ecology of *C. chalcites* is not much studied. However, in the south-Eastern part of the Country a sedentary behavior is observed (Cabello, 1988; Cabello & Belda, 1994; Cabello et al., 1996). On the Canary Islands, this pest is frequently observed at low and medium altitudes all year round, with several generations per year, as it occurs in the populations of the Peninsula (Garcia et al., 1992). The spatial distribution of the eggs, on different stages of banana plants was a missing point on GIP programme of *C. chalcites.* One of the focus point was that issue on this contribution.

Life Stages:

Chrysodeixis chalcites presents a holometabolic postembryonic development, namely a complete metamorphosis with four stages of development: egg, larva, pupa and adult. Eggs are translucent, bright greenish-white, domed and decorated with 28-32 longitudinal stripes, from the micropyle to the base. Its color may vary depending on the age (Goodey, 1991).

The mature larva is green, with a cylindrical body, soft and flexible, except for the head capsule that is strongly chitinized; the anterior part is significantly thin; the insect has a small head. The head capsule is green, depressed, with lateral black spots at the height of the ocelli (Sannino et al., 1988). The mandibulates mouthparts are located at the bottom of the head. The thorax is composed by three segments, located below the head, each

one with a pair of legs, composed by five knuckles. In the first segment there is a stigma on each side, but in the other two segments there is no entrance to the tracheas.

The abdomen has ten segments, generally the last three (8, 9 and 10) appear as a single unit. In sections 5, 6 and 10, there are three pairs of false legs, instead of the usual five. Therefore, its peculiar way of moving by arching the body makes it look like it is "measuring the earth", just like the insects of the Geometridae family. Its common names in Spanish are derived from this characteristic. The meaty paws are completed with a series of tiny and sharp hooks arranged in rows or crown that are used by the caterpillar to grasp branches and leaves. The segments 1-8 are provided with abdominal stigma on either side, while 9 and 10 have none. In the last segment there is the anus. Usually, *C. chalcites* presents six larval stages, followed by a period of prepupa (Amate et al., 1998); the stages are described below (Rashid et al., 1971; Goodey, 1991). The first larval stage (L-I) has translucent color. The head capsule is dark gray. After the first food intake, the larva turns bright green and a series of prominent black spots appear on the chest and abdomen. The appearance of the second stage (L-II) is similar to the final stages; the most notable change is observed in the head that changes to the body color. The prothoracic segment is barely visible, giving to the body a uniform appearance, pale green, with two pale lines that run along the back. At this stage the thoracic legs are black and the abdominal legs are pale green.

In later stages (L-III and L-V) the number of dorsal body lines increases, up to a total of six, with a brighter appearance. Towards the end of the third stage (L-III) there is a visible thin dark line above the spiracles; in this moment the black spots of the body become less visible. The fourth and fifth stage (L-IV and L-V) are similar in color, shape and structure to the one described above, however the larva is larger.

In the sixth stage (L-VI) on each side of the body, above the spiracles, a thin dark green or black line extending from the head appears, reaching in length the seventh abdominal segment; below there is a thicker white line that starts from the head, passing through each spiracles until the tip of the anal extension. The spiracles are black. The ventral region is speckled with white dots (Haggett, 1980; Bretherton, 1983; Passoa, 1995; Porter, 1997).

The pupa has a fusiform shape, and is wrapped in a cocoon of white silk made by the larva when it reaches its maximum stage of development. Its color is pale green when is

recent formed; when it reaches the maturity it becomes black with hazelnut-ivory areas on the ventral and lateral abdomen (Harakly & Farag, 1975; Bretherton, 1983).

The head in adults males has a yellow-red color, the forehead is flat and covered with flakes, with short palps and a well-defined proboscis. The antennae are filiform. The thorax is robust, reddish-yellow color. The dorsal planes of the forewings have a general brown color mixed with metallic gold color. The dorsal planes of the back wings are yellowish-brown with a broad dark band on the outer margin and a marked venation of the same color. Ventrally, both pairs of wings have ocher color with a dark strip, near the outer margin (Garcia et al., 1992). The abdomen is pale-ocher color, crested in the first and second segments, with two groups of yellowish hairs along the sides (Rivers Mesa, 1989). The back wings are yellowish-brown, darker towards the edges (Baez, 1998).

Females are similar to the male; the difference is the lack of the two plumes of yellow hairs arranged laterally on the abdomen (Ríos Mesa, 1989).

2.4. Injury and damage

The larvae feed on the leaves and fruits of vegetables, fruit and ornamental crops. (Fig. 1.2., Fig, 1.3.) It is considered one of the most serious pests of Lepidoptera in many countries, although there are no data to quantify the extent of damage that occurs (Crop Protection Compendium, 2007). It used to be considered an exceptional or secondary pest of banana plants on Canary Islands, because its damage was limited to the most tender leaves of young seedlings (Perera & Molina, 2002). As soon as the plant grows, the damage decreased and pest could be easily kept under control. On open-air traditional plantation damage was less serious and sporadic, while it can be more severe in plastic or mesh greenhouses, especially on young plants from *in vitro* culture, delaying their development and production by affecting the youngest leaves of the plant (Perera & Molina, 2007; Cabello, 2009). The larvae prefer to eat young, unopened leaves – called "cigar" leaves (Perera & Molina, 2007). The presence of small larvae in a fully developed plantation does not entail any appreciable damage, while in a newly transplanted plantation, they may eat the apical bud affecting the plant. However, when *C. chalcites* attacks a plant, it destroys a relatively small area of the total foliage surface, rarely surpassing the critical threshold of 10% (Vilardebo & Guérout, 1964). As the crop develops, the levels of leaf damage decrease dramatically. Nevertheless, since 2000 this

pest has become one of the most serious of banana crops in greenhouses of El Hierro, the Southern part of Tenerife and La Palma islands, and most recently also in the Southern part of Gran Canaria (Garcia, 2003; Del Pino et al., 2011b). Previous studies showed that complete defoliation of the plant crop at the 5-leaf stage had no effect on the weight of bunches but delayed harvest, whereas complete defoliation when the plant had 35 leaves reduced the production of fruit by about 30% (Turner and Hunt, 1987). Similarly, *C. chalcites* feeding damage to the leaves of mature plants was negligible and was unlikely to have had a detectable effect on fruit production, whereas direct damage to fruit had a clear and important influence on the commercial value of fruit. Particularly, damage on the fruit in the spring is particularly important as it coincides with the development of the fruit after flowering which is the most susceptible period for determining the quantity and quality of the harvested fruit (Del Pino et al., 2011; Galán-Saúco, 1992; Robinson & Galán-Saúco, 2010; Fuentes et al., 2018).

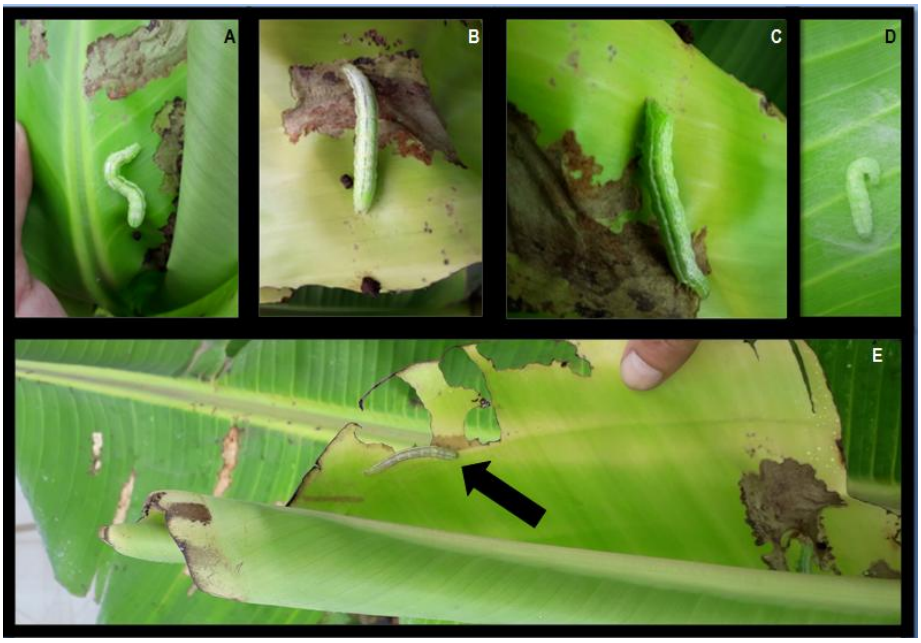

Figure 1.2. Injuries caused by *Chrysodeixis chalcites* in crops: A) late-stage larvae feeding on a leaf surface; B) C) E) defoliation caused by final instars; D) Early pupa stage of *C. chalcites* on banana leaf.

2.5. Control methods

2.5.1. Cultural practices and monitoring

Traditionally, insects of the family Noctuidae are controlled by applying synthetic chemical insecticides (Cabello & Belda, 1994). In order to minimize the negative impacts of the continued use of chemical insecticides, the search for other control methods is fostered, including use of predators, parasitoids and entomopathogenic microorganisms (Murillo et al., 2000).

In principle, the control of *C. chalcites* seems to be relatively easy, since its whole life is spent outside the plant exposed to the action of different control agents (chemical or biological). The problem arises inside the greenhouses, or when chemicals are employed to reach the elimination of the different growing stages of the pest. It seems that *C. chalcites* populations are somehow resistant to these compounds, making the problem even more difficult to solve (Cabrera et al., 2007).

Figure 1.3. A)Surface damage of early stages of *C. chalcites*; B) Recently hatching larvae on the beam side; C) Damage on the cigar leaf of banana plant by *C. chalcites*.

The simplest control measure consists in monitoring for tears in the cover of the greenhouse in order to avoid the entry of adults. Removing weeds inside and outside the greenhouse also helps, because adult females prefer to place the eggs upon them. Another method is to monitor the early stages of crop development, as attacks might then be very serious and bring irreversible effect on buds and stems (Perera & Molina, 2002). Wrapping the fruits in fruit covers and cutting the leaves near the bunch is also recommended (Camacho, 2006). These covers prevent individuals to enter the bunch, and cutting the leaves ensures the phytosanitary treatments to cover most of the surface. Successful results were obtained by using trap-plants, such as cabbages or other species of Brassicae as intercrops or along its edges, to attract *C. chalcites* and prevent

them from colonizing banana plants. In this way, phytosanitary treatments can be made delayed (Fuentes et al., 2018).

Here, it has been not easy to control the pest, because of the limited number of chemical or biological insecticides authorized for this crop. Also correct application is difficult and, due to the lack of commercial biological control agents, the development and implementation of Integrated Pest Management has been hindered (Martin, 2007).

2.5.2. Chemical control

The larvae of Noctuideae pest species have been widely battled by means of synthetic organic insecticides. Currently, to control *C. chalcites* in banana plantation, chemical synthetic insecticides are used as preventive and curative applications during major risk periods (spring and summer). Treatments are made as soon as the first adults of the pest appear, or as soon as plants show the first damage; it is then recommended that applications reach the entire leaf underside and all plant organs, where larvae may conceal (Cabrera et al., 2007).

These kind of control caused agronomic problems as well as other relevant complications (Cabrera et al., 2010; Del Pino et al., 2011b), such as the rise of resistance (with consequent low effectiveness of the product used) (Castañé, 2002; Torres-Vila et al., 2002, b; Van Der Blom, 2002) (Fig 1.4). The level of resistance seems to be higher in greenhouse rather than in open-air plantation (Vanlaecke et al., 1995). Also, had appeared new pests (due to the elimination of natural enemies), and has been noted the risk of intoxication both for the farmer and the consumer (chemical products persist in the environment for generations). The reduction of the authorized products that can be used in the crop according to the 91/414/CEE directive. Moreno I. P. (2000) suggests that they should be used with the utmost care: it is recommended to rotate the products and use them as ultimate solution, just when agronomic and economic reasons are necessary (Del Pino et al., 2014). According to Agricultural Services of Canary Islands, the list of allowed active materials aganist lepidoptera species was recently updated by the date of 3 of September 2019, these active materials are: piretroide: lambda cihalotrin 2.5% (WG); Oxadiacina: indoxacarb 30% (WG) and: Spinosad 48 % (SC)

(Government of Canary Islands, 2019).

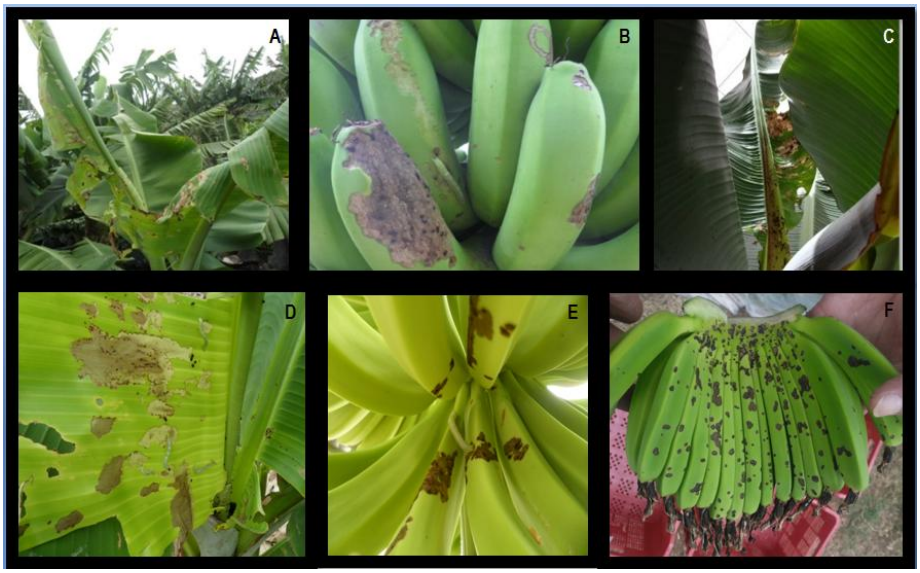

Figure 1.4. Damage caused by *Chrysodeixis chalcites* on banana plants: A) early-stage larvae feeding on a leaf underside surface and cigars; B) defoliation caused by final instars on late fruit stage; C) Damage on the early stages of the plant (mainly on the central leaf); E) Early damage on banana fruit; F) Late stage damage on banana fruits.

2.5.3. Natural enemies

C. chalcites is a host for a diverse group of natural enemies that regulate naturally the pest populations in protected conditions. However, commercial availability cannot cover the grower's demands so far (Del Pino et al., 2011a).

Alcippe brunnea (Passeriformes: Pellorneidae), a bird found in dense forest in India, is successfully used to control *C. chalcites* on sweet peppers grown in glasshouses in the Netherlands (van der Linden, 2000).

Many predators have been used extensively in different regions. In Italian glasshouses, the predatory *Podisus maculiventris* and *Podisus nigrispinus* (both Heteroptera: Pentatomidae), from North America, have been tested as good control agents (Vacante et al., 1996). Several parasitoids have also been described from different regions. In the UK, the endoparasitoid *Meteorus gyrator* (Hymenoptera: Braconidae) showed considerable potential as a biocontrol agent against *C. chalcites* under controlled conditions (Bell et al., 2000; Smethurst et al., 2004). In the Cape Verde Islands, the

solitary endoparasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae) was introduced with some success for the control of *C. chalcites* in the field (Lima & van Harten, 1985).

Specifically, in the banana crops in the Canary Islands, *Chrysoperla carnea* (Neuroptera: Chrysopidae) has been recognized as an important predator. Larval parasitoids like *Cotesia* sp. have also been described in this region, but the most common species is the egg-endoparasitoid *Trichogramma achaeae* (Nagaraja & Nagarkatti, 1973) (Hymenoptera: Trichogrammatidae), which can parasitize up to 87% *C. chalcites* populations (Del Pino et al., 2011b; Nagaraja & Nagarkatti, 1973). In the Palaearctic region, several parasitoids and predators of *C. chalcites* have been recorded (Alcázar et al., 2002; Cabello, 1989; Garzia et al., 2003; Linden, 1996; Vilardebo & Guerout 1964) and evaluated as biological control agents (Bell et al., 2000; Bolckmans & Teteeroo 2002; de Clercq et al., 1998; Messelink, 2002; Pizzol, et al., 1997; Vacante, et al., 1996; Zimmermann, 2004), although they are not yet commercially available (Cabello, 2009). High levels of egg parasitism have been detected during these surveys with a significant impact on the pest populations (Del Pino et al., 2011a). Prospecting for potential natural enemies of the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) and *C. chalcites,* Polaszek et al., (2012) and Del Pino et al., (2013) reported the discovery of five species of *Trichogramma* on the Canary Islands archipelago: four are relatively widespread in between the islands, which are; *T. achaeae* (Nagaraja & Nagarkatti, 1973), *T. bourarachae* (Pintureau & Babault, 1988) *T. euproctidis* (Girault, 1911) and *T. evanescens* Westwood; and, a fifth species close to *T. brassica* (Bezdenko, 1962).

2.5.4. Microbiological control

The microsporidian *Nosema manierae* (Protozoa: Microspora) is known to kill *C. chalcites* larvae in a few days (Toguebaye & Bouix, 1983). Products based on nematodes, in their vigorously infective juvenile stage, like *Steinernema carpocapsae* (Nematoda: Steinermatidae) also provide a feasible control of *C. chalcites* in a wide range of crops (Brødsgaard & Albajes, 1999). Several strains of *Bacillus thuringiensis* (*Bt*) are commonly used for the control of *C. chalcites* in different regions; like in Sicily, Italy, to efficiently protect tomato crops grown under net protection on in open-air greenhouses

(Vacante et al., 1996). In Israel, *B. thuringiensis* var. Kurstaki is routinely used to protect horticultural and ornamental crops (Broza & Sneh, 1994). In the Canary Islands, at the moment, 4 biological control agent over 10 authorized phytosanitary products are permitted in banana plantations. According to Agricultural Services of Canary islands, the list was recently updated by the date of 19 of July, 2019 and these biological agents are: *Bacillus thuringiensis* Kurstaki 9.74% (SC) *Bacillus subtilis* 15.67% (WP) *Beauvesia bassiana*, *Paecilomyces lilacinus* (Government of Canary Islands, 2019). Recently, in the Canary Islands, researches focused on baculoviruses, brought an alternative to *B. thuringiensis* with high mortality rates of the local strains of the *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchSNPV), which may help during a period of high infestation of *C. chalcites* in banana crops in the Canary Islands. This virus has since gained particular interest as a potential biopesticide to control this pest (Bernal et al., 2013; Del Pino et al., 2011b; Hernández, 2007). Insect-infecting baculoviruses are promising control agents for a number of lepidopteran pests due to their excellent insecticidal properties, host specificity and outstanding safety records (Caballero et al., 2009; Moscardi, 1999). A number of baculoviruses have been developed as the basis of effective biological insecticides against different agricultural and forest pests and applied to large field areas (Caballero et al., 2009; Cherry & Williams, 2001; Moscardi, 1999).

3. BANANA PLANT

Bananas and plantains belong to the genus Musa, with most of the dessert bananas being triploids of the wild ancestor *Musa acuminata* Colla, Cavendish subgroup (Table 1.2).

Table 1.2. Taxonomical classification of the host plant.

Kingdom	**Plantae – plants**
Division	**Tracheophyta** – vascular plants
Subdivision	**Spermatophytina** – seed plants, phanerogams
Class	**Magnoliopsida**
Order	**Zingiberales**
Family	**Musaceae** – banana
Genus	**Musa (Linneo)**
Species	***Musa acuminata* (Colla)** – edible banana

Thousands of years of domestication have produced the delicious edible fruit that we can eat today. In fact, the plant's origin dates back thousands of years: as historic records witness, the plants of this genus were first cultivated for commercial purposes in Southeast Asia and Western Pacific. Here, as in many other tropical and subtropical areas, the cultivated banana is an important plant still today; it is a sort of all-purpose material, widely used in building shelters, waving carpets, making baskets, tanning material to color leather, and as wrapping material. It is also an important staple crop, since it provides energy, and nutrients. From Southeast Asia and the Western Pacific, the fruits of banana widespread in the Mediterranean countries around 650 b.C.; they arrived in the Canary Islands only during the 15th Century, and later to America in 1516 (Simmonds, 1966). The cultivars, that are currently planted in the Canary Islands, were first introduced on the island at the beginning of 19th Century, but their commercial cultivation began only at the end of the Century (Galán Sauco, 1992). During the first decades of the 20th Century the fruit was exported to the United Kingdom and France, however, nowadays, this variety is almost all marketed in Spain and Portugal.

3.1. Ecology

The plant can grow between 30º N and 30º S latitude, however the best conditions for its optimal growth are found in the geographic areas located between 15º N – 15º S latitude. Canary Islands are located at 28º N latitude, corresponding to the subtropical region. Here the highest altitude possible to plant banana is 500 masl (BOE, 2011). To ensure the best conditions for growth the temperature needs to be constantly at 20-25ºC, although it can be occasionally as high as 35ºC and as low as 15ºC (Robinson & Galán Saúco, 2012). Water disponibility in the soil and environmental humidity are key factors for growth of banana plants. As Canary Islands is considered a region suffering from water deficiency (the range of rainfall here is on average 500 mm/year based on the agroclimatic data of Canary Islands), the use of irrigation is essential for banana crops of this area. Optimal irrigation in this region is around 20 l/plant/day (100 mm of water/month), enough to obtain a cost-effective production, however, these values have to be adjusted, as the plant requirements greatly vary by location (Nort, South) and type of cultivation (open air, greenhouse) (Hernández, 2007).

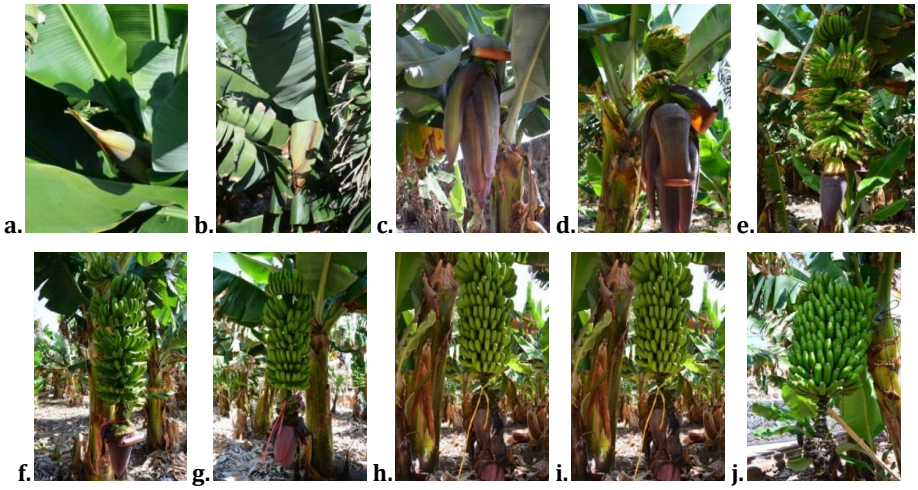

Figure 1.5. Banana Development Stages (a-j).

3.2. Economy and production

Banana production is one of the most relevant agricultural activities from the economic and social point of view. Even it has been cultivated commercially since the XIX century, the cultivated area has increased in the last years, and currently this crop represents almost 22% of total agricultural area. In 2018, banana crops in Canary Islands extended up to 8,679 ha (Asprocan, 2019) (Fig. 1.5.).

According to the available data reported in 2017 (ISTAC, 2017a), the production of "plátano" on Canary Islands amounts to 421,297 tons. Considering this number, banana plants represent the most important crop in terms of production amount on Canary Islands, more than tomatoes (74,131 ton) and potatoes (72,205 ton). The economic value of banana production in 2011 reached 201 million euros, about 39% of the total agricultural production of the islands (ISTAC, 2017a).

Due to this relevance of banana production within the local economy, many research efforts are exerted to give answers to demands from Canarian banana growers for the problems threatening the crop profitability. Among these threats, insect pests, as *Chrysodeixis chalcites* are one of the most often mentioned.

4. BIOINSECTICIDES

Bioinsecticides are substances that are derived from microorganisms, plants or minerals (Hall et al., 1995; Koul et al., 2004; Cantó et al., 2017; Pavela & Benelli, 2016). Also, they can be synthetic substances similar or identical to others found in nature. These insecticides are characterized by having a very low toxicity for humans and other vertebrates, decomposing within a few hours after being applied or being specific for the pests we wish to control. For these reasons they are considered environmentally benign. Its effect on wildlife and the environment is less harmful than that of conventional insecticides (Koul et al., 2004; Pavela & Benelli, 2016). For the control of pests in organic farming is authorized the use of some insecticide products and repellents, including plant extracts, soaps, clays and rock powders. Extracts from the Neem tree (*Azadirachta indica* A. Juss.) They are among the most widely used products of plant origin use in pest control. The main active principle found in this plant is azadirachtin, which acts as growth regulator of insects and other arthropods, by interfering with ecdysone activity and altering the process of the molt Azadirachtin is also known to have an antiapetitive effect e on some insects. Other group of botanical products, applicable to pest control in organic farming, are the oils essential. These oils, consisting mostly of a mixture of terpenoids, have biological activity against a broad spectrum of plant pests, on which they can act as fumigants, contact insecticides, repellents or affect feeding, development speed (Hall et al., 1995; Pavela & Benelli, 2016; Navarro et al., 2017).

4.1. Desirable characteristics

The first most important feature of a bioinsecticide as characteristic apart from being environmentally friendly and less toxic than synthetic chemicals currently available in the market, should be its less harmfull economic, social and scientific value on natural environment (Hall et al., 1995; Koul et al., 2004; Regnault et al., 2012) that might present a viable option. Secondly it might be good if it can be easily produced in large quantities. Thirdly efficacy of the product should be at least as good as the synthetic insecticides. On that concern so far countries are trying to figure out the growing markets demanding questions on that matter. The European Parliament and of the Council European establishes a framework for action to achieve use sustainable of phytosanitary products. Regulation No. 1107/2009 refers to the marketing of products for crop protection in the

European Union. A pesticide or crop protection product is used mainly in the agricultural sector to prevent, destroy or control a harmful organism or plague. It must contain at least an active substance that is a chemical or a botanical extract with effect against a plague (Koul et al., 2004; Cantó et al., 2017; González et al., 2017).

4.2. Advantages and limitations

One trend driving the adoption of biopesticides is their suitability in integrated pest management (IPM) programs, combined with other biological, cultural and pesticide approaches (Koul et al., 2004). A major advantage of biopesticides is their lack of toxicity to pollinators and compatibility with other natural enemies, such as hymenopteran parasitoids. Bioinsecticides can be used in rotation with synthetic pesticides to pause pest resistance by breaking pressure from a single mode of action, or in combination with synthetic pesticides providing additive if not synergistic effects (Hall et al., 1995; González et al., 2017). In addition to the nanotechnology associated with bioinsecticides, other technologies have been widely used in sustainable agriculture with the aim of reducing the population of insects that attack crops, such as the use of bioinsecticides (Cantó et al., 2017; Pavela & Benelli, 2016). Bioinsecticides are excellent alternatives to bypass the environmental pollution derived from chemical insecticides, besides providing the most selective control of insects (Hall et al., 1995; Navarro et al., 2017).

On the othe hand, the essencial oils have the disadvantage of being phytotoxic (toxic to plants) in times of high temperatures or drought. Tend to burn the foliage and tender parts of the plants. Phytotoxicity problems and limitations of their use are similar to the use of microbial agents in biological control (Navarro et al., 2017).

4.3. Massive production

Massive production of bioinsecticides is mainly based on extraction of active ingredient from the plant. In Spain, available ingridient of various commercial products are such as; clove, citronella, orange oil and peppermint essential oils are already commercially available in the market (Zehnder et al., 2007; Government of Canary Islands, 2019). Azadirachtin, pyrethrins, carvone, citral, eugenol and thymol are pure compounds and have been authorized in the agricultural market in the last decade. Garlic, trigonela, tea

tree or pepper extracts are also known to be authorized and products are available in the market (Hall et al., 1995; Cantó et al., 2017; González et al., 2017).

4.4. Formulation

For a succesfull bioinsecticide product, it is necessary to develop appropriate formulations which enhance the persistence of such active ingredient (plant extract etc.), by minimizing the volatility of the bioactive components through controlled release methods (Cantó et al., 2017; Navarro et al., 2017). Various techniques are used to overcome these issues, including encapsulation, nanoemulsion, nanoparticle synthesis, and emulsifiable concentrate formulations to enhance the solubility, persistence, and efficacy (González et al., 2017). These technics are providing a very positive approach for the future by accelarating the efficacy of the experiments on developing formulations with botanical extracts (Koul et al., 2004; Navarro et al., 2017).

4.5. Field efficacy

Bioinsecticides, differ from synthetic pesticides in fundamental ways and often require specific handling and application, which are not well understood by growers, sales representatives and consumers. The mode of action of these bioinsecticides is not the same for all the species (Hall et al., 1995; Zehnder et al., 2007; González et al., 2017). The effect of repellency or toxicity produced in a given species may vary by accentuating or decreasing in a different species. It is necessary to test different bioinsecticides, or pure compounds, for the control of different target species on the field (Navarro et al., 2017). On-farm demonstrations are the most effective method to demonstrate efficacy of bioinsecticides, and it is imperative that more field data are collected because proof of effect is the key to adoption. Stand-alone comparisons in laboratory trials with other products do not always reflect the added benefits gained from using bioinsecticides, Persistence of activity for 14 days is a realistic target for most applications on foliage. Persistence of activity for more than 21 days on foliage (Hall et al., 1995; Zehnder et al., 2007; Glare et al., 2012).

5. BACULOVIRUSES BASED BIOINSECTICIDES

Baculoviruses are infectious agents that cause fatal disease in arthropods. From an anthropocentric point of view, they are beneficial due to their potential for the control of insect pests. The spectacular symptoms induced by baculovirus infection were initially described two millennia ago in China in silkworm culture (Benz, 1986). Only much later, from 1950 to 1975, baculoviruses were observed to be effective biological control agents of insect pests (Ignoffo, 1981; Steinhaus, 1956). The first baculovirus registered as a pesticide in the United States was a commercial failure for a variety of reasons (Ignoffo, 1981). Nevertheless, the ever more obvious drawbacks of chemical pest control and the rapid build up of resistance to chemical pesticides has led to increased efforts to develop baculovirus insecticides and a concurrent increase in our understanding of the biology and ecology of these viruses.

5.1. Desirable characteristics

The ability of foliar-applied baculoviruses to protect crops from insect damage depends on the effective dose acquisition and the speed of action of the acquired dose (Black et al., 1997). Effective dose acquisition is critical to the success of any insecticide. In contrast with chemicals, which act by contact, baculoviruses need to be ingested in a sufficient dose to produce a systemic infection. The initiation of an infection is thus mainly reliant on the pathogenicity of the virus isolate or genotype forming the active compound. Consequently, the study of the natural diversity and the genotypic characterization of these viruses to select the genotype or mixture with improved phenotypic characteristics is a must.

Additionally, the success of baculovirus-based bioinsecticides relies on the intrinsic characteristics of baculoviruses as biocontrol agents. Among the desirable characteristics, bioinsecticides must have the following: (i) high virulence, (ii) high transmission capacity, (iii) high field persistence, (iv) feasibility for mass-production at practical and economic terms, (v) narrow host range, (vi) harmlessness to human, animals and environment, (vii) long shelf life, (viii) ability to be applied by conventional methods, and finally, (ix) susceptibility to be genetically modified (Ibarra & Del Rincón Castro, 2001).

5.2. Advantages and limitations

Baculoviruses have several inherent advantages that make them exceptional biological control agents, considering their safety, specificity and efficacy (Moscardi, 1999; Caballero et al., 2009; Rodgers, 1993). They are naturally occurring pathogens highly specific to insects and other closely related arthropods. Hence, they are safe in terms of pathogenicity against vertebrates, plants and other organisms (Gröner, 1986; Souza et al., 2007; Szewczyk et al., 2009). This fact coupled with their narrow host range, sometimes limited to one or two species, make them harmless for non-target organisms, principally beneficial insects that naturally suppress insect pest populations and thus contribute to keep the biodiversity in agroecosystems (Ahmad et al., 2011; Ashour et al., 2007; Gröner, 1986). However, while single chemicals can cover a large portion of the crop pest complex or even all of it, baculovirus-based biopesticides usually target one pest species in a crop (Eberle et al., 2012). Baculoviruses are also highly persistent under natural conditions (specially in soil and litter), where they constitute an inoculum source for subsequent pest generations (Carinhas et al., 2010) and favour the establishment of the viruses as a mortality factor to regulate population densities, and extend the effect of an application. The capacity of these viruses to cause natural epizootics relies sometimes on their persistence but depends principally on the density of the pest population. Unfortunately, despite their persistence in the environment they are susceptible to UV degradation, resulting in a rapid degradation rate. For this reason, an appropriate formulation including UV protectans is especially recommended (Copping & Menn, 2000).

5.3. Massive production

The selection of a virus for pest management programs depends not only on its bioefficacy but also on the ease to mass produce it (Claus & Sciocco de Cap, 2001; Sherman, 1985) given the great amounts of bioinsecticides needed for field applications. Thus, the inocula used for virus production should be selected carefully to maximize both insecticidal activity (pathogenicity and virulence) and OB yield (Shapiro, 1986). The mass production system is one of the greatest limitations for the use of baculoviruses as biocontrol agents. As obligate pathogens, baculoviruses need active host cells for their replication and, for that reason, viral production must be performed either in host larvae (*in vivo*) or in cell culture (*in vitro*). Associated costs of either of the

two systems make it difficult for viral products to compete in the marketplace (Grzywacz et al., 1998). *In vivo* production is, to date, the only feasible method for large scale propagation of baculoviruses (Gupta et al., 2007; Hunter-Fujita, 1998). Several authors have reviewed this system in the last 40 years (Hunter-Fujita, 1998; Jaques, 1977; Moscardi et al., 1997, Shapiro, 1986; Sherman, 1985; Shieh, 1989; van Beek & Davis, 2007). *In vivo* production involves the inoculation of massive numbers of larvae, the rearing of larvae while the virus replicates, and the recovery of OBs from infected larvae. To make it economically efficient, an ideal production system is one that yields the greatest amounts of active virus *per* larva with the lowest virus dose and in the shortest possible time while keeping contamination at minimally acceptable levels (Ravensberg, 2011). In consequence, the techniques and methodology should be specifically developed and adapted for each host-virus system. *In vivo* production has several advantages over *in vitro* production. However, it involves a number of limitations and the efficiency depends on both biotic and abiotic factors that affect virus replication and viability in the insect host.

5.4. Formulation

An efficient virus along with a viable production system cannot reach commercial success without a formulation that protects the virus from environmental degradation. The main objective of a formulation is thus to preserve the virus biological activity so as to deliver the product to the target system in time for the virus to be consumed by the host pest. It is also important to use conventional delivery techniques familiar to the end user. Appropriate formulations thus require previous studies to determine field persistence and storage stability.

Ultraviolet light is one of the most detrimental environmental factors affecting OB persistence (Támez-Guerra et al., 2005). During the last decades, several natural and artificial compounds have been evaluated as UV protectants, among them optical brighteners are the best studied. These stilbene derivates, which absorb energy in the UV portion of the spectrum and re-emit it in the blue portion of the visible spectrum, are commonly used in industrial processes to enhance the color appearance of fabric or paper, causing a whitening effect. Shapiro (2000) described their usefulness as UV protectants of LdMNPV OBs for the first time. Meantime, Shapiro & Robertson (1992)

demonstrated they enhanced viral activity in some virus-host systems by disrupting the peritrophic membrane (Okuno et al., 2003; Wang & Granados, 2000) or inhibiting midgut cellular sloughing (Washburn et al., 1998). Virus induced apoptosis in insect midgut cells may also be blocked in the presence of optical brighteners (Dougherty et al., 2006). Moreover, optical brighteners can alter the rate of feeding of larvae (Shapiro & Farrar, 2003) and also increase the susceptibility of later instars to NPV infection (Shapiro, 2000; Shapiro & Robertson, 1992) to such low levels that viral concentrations used for earlier instars can also control later ones. This is an interesting aspect of brighteners for field applications, as mixtures of larval stages are likely to be present simultaneously in the field due to overlapping of pest generations. However, field applications of optical brightener formulations have shown some negative effects: they may reduce the growth of monocot crops (Goulson et al., 2003) or induce modification in the behaviour of bee pollinators (Goulson et al., 2000).

5.5. Field efficacy

Once the efficacy of a NPV has proven useful under laboratory conditions, it is necessary to validate it under controlled field conditions. It is recommended to perform these assays first under greenhouse conditions that help identifying any potential variables (eg. percent leaf coverage, persistence, application rates, etc.) in the open field (Ibarra & Del Rincón Castro, 2001).

The success of this kind of products depends on a deep knowledge of the crop and the target insect pest, as applications must be designed to provide optimum deposits at the pest feeding site. Special interest has to be deposited in controlling young instars to prevent them to get more voracious and damaging in later instars (Briese, 1986). The biology of the target host insect, particularly its feeding preferences and habits, may significantly influence the rate of acquisition of a lethal infection upon consumption of contaminated foliage.

Formulation and cultural crop techniques should also be adapted to favour virus efficacy in each situation. On this respect, selection of the isolate with increased insecticidal characteristics is an important step in the implementation of control programs involving baculoviruses. Timing applications properly and larval density are also key features for a successful use.

6. SCOPE OF INVESTIGATION

(i) Improving the population monitoring through knowing distribution of the pest in the plant.

In order to improve the population monitoring of *C. chalcites* on banana plantations of Tenerife, we focused on three development stages of the plant. Ovipositional preferences of *C. chalcites* on banana plants were discussed on this matter. The aim of the study was to understand the vertical distribution of eggs + first instar larvae (EL) of *C. chalcites* on banana plants in mesh-built greenhouse conditions. Similar studies on banana plantations were carried out on the Arctiidae *Antichloris viridis* (Liscano & Domínguez Gil, 2004), while on the Lepidoptera *C. chalcites* or *C. includens* data on insect distribution were obtained in tomatoes and soybeans (Izquierdo et al., 1996; Mascarenhas & Pitre, 1997; Valverde, 2007).

The knowledge on distribution of eggs is especially important in the case of efficacy of biological control agents, e.g. *Trichogramma* species that only parasitizes eggs and is the most effective controller of *C. chalcites* and the only one that is being used commercially at present in the Canary Islands (Del Pino et al., 2013). The presence of five *Trichogramma* sp. have been recently reported from Canary Islands (Polaszek et al., 2012).

(ii) Development of an effective ChchNPV-TF1 formulation, improving its persistence on the leaf surface.

Baculovirus-based bioinsecticides are generally used through spray applications (Moscardi, 1999), although in certain situations other methods that take advantage of existing virus reservoirs in the environment are also possible (Fuxa, 2004). In spray applications, which basically make use of the same technology developed for conventional chemical pesticides, it is necessary to adjust a series of variables that are decisive for the effectiveness of the treatment to be desired. The concentration of virus applied has an important effect on the degree of pest control, therefore, the lowest concentration that allows adequate control must be adjusted (Cherry & Williams, 2001).

The number of treatments needed depends mainly on the persistence of the virus on the phylloplane that can be negatively influenced by UV radiation, which has been a constraint for its commercial development (Jaques, 1977; Ignoffo, 1992; Williams & Cisneros, 2001). Because the virus is generally ingested from the leaf, which is precisely the plant part most exposed to the UV radiation, rain and exudates from the plant, the quantification of the persistence of the virus in different types of cover depending on the crop is of special relevance to design more effective biological control programs (Dias Vasconcelos, 2001).

(iii) Determining the efficacy of bio-rational or natural products currently used on banana plantations on the control of *C. chalcites.*

The need on the knowledge of efficacy of available products in the market on the control of *C. chalcites* in banana plantations has brought us to study these products under laboratory and greenhouse conditions in order to compare benefits of each product to the farmer and adapt these products to GIP programs Currently, GIP programs depend to some extent on the use of biorational products with a relatively acceptable eco-toxicological profile and other products of natural origin (spinosad, abamectin, azadirachtin, *Bacillus thuringiensis,* etc.) (Williams & Cisneros, 2001; Gent et al., 2009; Government of Canary Islands, 2019).
In this concern it is possible to find a combination of several modes of actions or use biorational insecticides just to reduce, replace and/or avoid the applications of synthetic insecticides. Once the effectiveness of the bioinsecticides was evaluated, it might be interesting to make a compatibility test combining these products with virus ChchSNPV for the control of *C. chalcites* on banana crops of the Canary Islands.

Chapter 2

Chrysodeixis chalcites (Esper) (Lepidoptera: Noctuidae) Oviposition Preferences on Different Growing Stages of Banana (*Musa acuminate* Colla, Musaceae) Plants.

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Abstract

Banana (*Musa acuminate* Colla, cultivar Grand Nain) is the main crop in Canary Islands, covering an area of about 9,000 hectares with a production in 2017 of 437.782 tonnes of banana worth 104.4 million Euros. Today, one of the most used pest control method is the Integrated Pest Management (IPM), which takes into account the different management options for pest control, giving priority to the least harmful solutions. This research aims to improve the use of IPM to control populations of *Chrysodeixis chalcites* through understanding growing stage and specific preferences on banana plants as preliminary experiments. Our main focus was to investigate the oviposition preference of *C. chalcites* (Esper) adults by counting all present eggs and larvae on the plant seven days after adult release into cages. The combination of two plants through three growing stages (young plant, mature plants without bunch and mature plants with bunch) were tested over 48 banana plants into six different field cages. Significant difference in ovipositional behaviour was found between the three growing stages of banana plants. In order to detect *C. chalcites* populations on banana plantations, it can be suggested to look firstly to the 5th leaf on young plants, secondly to the 8th leaf on pants with bunch, and finally plants without bunch. In all cases, more eggs and first instar larvae were found on young plants and on the underside of the leaves.

Key Words: Spatial distribution, biological control, Integrated Pest Management.

1. Introduction

The island of Tenerife is the largest producer of bananas in the Canary Islands (43% of the total production), followed by La Palma (33%) and Gran Canaria (22%), while the remaining 2% of production occurs on the islands of La Gomera, El Hierro and Lanzarote. Most of the production is destined to markets in mainland Spain (91%), and only a small fraction is exported to Western Europe (0.1%), whereas the remaining (8.9%) is consumed locally (Mapama, 2016).

Numerous phytosanitary problems are present on banana crops around the world, with the most important and widespread pest being the banana weevil *Cosmopolites sordidus* (Germar) (Carval et al., 2016). Other pests on the other hand, have a local distribution; for example, the species *Antichloris viridis* Druce, 1884, *Caligo memnon* C. Felder and R. Felder, 1867 are present in Venezuela, Costa Rica and other countries of Latin America; *Spodoptera litura* Fabricius, 1775 and *Chrysodeixis acuta* Walker, 1858 are present in India (Tayade et al., 2014). In the Canary Islands, *Chrysodeixis chalcites* is one of the most important pest on banana crops (Fuentes et al., 2018). *C. chalcites* is within the polyphagous insect group, so that affects several fruit, horticultural, ornamental and forest crops such as cotton, alfalfa, cabbage, sunflower, geraniums, beans, corn, turnips, potatoes, cucumbers, peppers, bananas, soybeans, tobacco and tomato (Cabello et al., 1996). The damage of *C. chalcites* on banana appears not only on the leaves of the plant, but also on the bracts and fruits. Second and third stage larvae start to eat the leaf edges (Rashid et al., 1971), and in the following stages they become even more voracious: they eat the entire leaves but the central veins. During the night, the adults lay eggs on the underside of the leaf. The life cycle duration varies according to temperature, food composition and climate conditions. The full cycle is usually completed within 45 days at 20° C, while at temperature lower than 25° C the larvae cycle can be completed in 44 to 50 days (Del Pino 2011a). The activity and the development can be influenced to some extent by the photoperiod (Cayrol, 1972). *C. chalcites* deposit eggs either isolated or in small groups (Harakly & Farag, 1975), so that they are scattered in the crop (Linden, 1996). In some crops the larvae may eat the fruits, as it was observed in bananas by Vilardebo & Guèrout (1964) who witnessed a larger larvae placing themselves in the cigar leaf (the unopened leaf), which is upper part of the plant, where their formation is then completed. Feeding damage of *C. chalcites* to leaves and fruit of banana plants in Canary Islands was studied in a survey in 2018 (Fuentes et al., 2018). A foliar damage of

1.5–7.3% (depending on island) and a fruit damage of 1.0–5.7% was detected in commercial banana plantations.

Female phytophagous insect whose offspring develop at the oviposition site are under strong selection to optimize oviposition site, because plant resources are highly heterogeneous for offspring growth and survival (Denno, 2012). In 1988, Thompson pointed out that the relationship between oviposition preference and off-spring performance is central to our understanding of plant-insect interactions but that our knowledge of the relationship is preliminary and incomplete. Knowledge of the relationship between the insects and the plants is essential for understanding the distribution and population dynamics of phytophagous insects and information on this relationship can also provide insight into the evolution of host shifts (Craig et al., 1989).

The aim of the study was to understand the vertical distribution of eggs + first instar larvae (EL) of *C. chalcites* on banana plants in mesh-built greenhouse conditions. Similar studies on banana plantations were carried out on the Arctiidae *Antichloris viridis* (Liscano & Domínguez Gil, 2004), while on the Lepidoptera *C. chalcites* or *C. includens* data on insect distribution were obtained in tomatoes and soybeans (Izquierdo et al., 1996; Mascarenhas & Pitre, 1997; Valverde, 2007).

The knowledge on distribution of eggs is especially important in the case of efficacy of biological control agents, e.g. *Trichogramma* species that only parasitizes eggs and is the most effective controller of *C. chalcites* and the only one that is being used commercially at present in the Canary Islands (Del Pino et al., 2013). The presence of five *Trichogramma* sp. have been recently reported from Canary Islands (Polaszek et al., 2012).

Taking proactive decisions on IPM is getting more important as the application of pesticides are harmful to nature. According to Agricultural Services of Canary islands, the list of allowed active materials aganist lepidoptera species was recently updated by the date of 3 of September 2019, these active materials are: piretroide: lambda cihalotrin 2.5% (WG); Oxadiacina: indoxacarb 30% (WG) and Spinosina: Spinosad 48 % (SC) (Government of Canary Islands, 2019). Synthetic insecticide treatments require multiple applications which increase production costs, hamper the commercialization of products that can occasionally contain pesticide residues, and have led to the

development of resistance in certain populations of this pest (Horowitz et al., 1998). The amount of pesticides that are used in the banana fields can be reduced by knowing more about oviposition preferences of insect populations by the use of pesticides in specific stages and/or parts of the plants. This aspect may be useful for farmers to design more effective pest control strategies and to reduce the cost of phytosanitary application. Therefore, the knowledge about spatial distribution of insect populations is essential for IPM strategies.

2. Material and Methods

2.1. Greenhouse

The study was conducted during the spring-summer season from May to August 2017 under mesh-built greenhouse conditions at an experimental banana plantation belonging to the Agricultural Research Station (ICIA) at Valle de Guerra, Tenerife, at 28°31'38.4" N – 16°23'09.4" W). The greenhouse area was 7,200 m^2, located at an altitude of 100 masl. All practices in greenhouse maintenance and plant growth process were made according to the Integrated Pest Management (IPM) application methods and cultural growing technics. The average temperature was 21.5 ºC±2.08 with a maximum of 35.4 ºC and minimum of 15 ºC. Average humidity was 69 %±9.54 with a maximum of 90 % and minimum of 35.4 % during the experiment.

2. 2. Banana Growing Stages and Cage types

In order to demonstrate the specific preferences of *C. chalcites* on banana plants, the three main growing stages found in a banana orchard were selected: (1) Young plant (YP); which has a maximum of 6 to 9 leaves and are usually concerned as planting stage, (2) plant “no bunch” (PN); refers to plants on a vegetative growing stage with 10 to 20 leaves without bunch appearance, (3) plant "with bunch" (PB); where the plants were selected from elder plants with young banana bunches. The combination sets are described in Table 2.1.

Table 2.1. Description of Plant stages and parts of the plants that were used in the experiment.

	Code	**Description**
Plant Stage	1. YP	Young Plant 
	2. PN	Plant "no bunch" 
	3. PB	Plant "with bunch" 
Cage Type	A (PN+PB)	Two plants, one without bunch (PN) and the other one with bunch (PB).
	B (PN+PN)	Two plants, both without bunch (PN-PN).
	C (PB+PB)	Two plants, both with bunch (PB-PB)
	D (PB+YP)	Two plants, one plant with bunch (PB) and the other one young plant (YP)
	E (YP+PN)	Two plants, one young plant (YP) and the other one plant without bunch (PN).
	F (YP+YP)	Two plants, both young plant (YP-YP)
Leaf Position	1	Distal
	2	Median
	3	Proximal
Surface	A	Beam
	B	Underside

2.3. Experimental design

The parcel was planted with Grand Nain banana cultivar at the beginning of the planting season. Natural infestation of insects was avoided by isolating plants into four-meter-high mesh-built greenhouse cages, containing two banana plants in each one. Plants were selected from different growing stages of banana (Fig. 2.1.) and placed alone or combining different stages by putting them into the field-cages per pairs (Fig. 2.2.) Experimental plots were designed for adult release of *C. chalcites*. The adults used to infest artificially banana plants were obtained from a laboratory reared *C. chalcites* colony, obtained from greenhouse plants of banana farms in southern Tenerife and reared in the laboratory. The colony was maintained in the Instituto Canario de Investigaciones Agrarias (ICIA), Tenerife, at 25±1ºC, 60±80% relative humidity and a photoperiod of 16:8 h (light:dark) on a semi-synthetic diet based on cornflower, wheat germ and yeast Cabello (et al., 1984) Adults were fed with 10% v/v honey solution. The adults were allowed 5 days to mate before they were released. A total number of 20 females and 20 male *C. chalcites* adults were released into each cage.

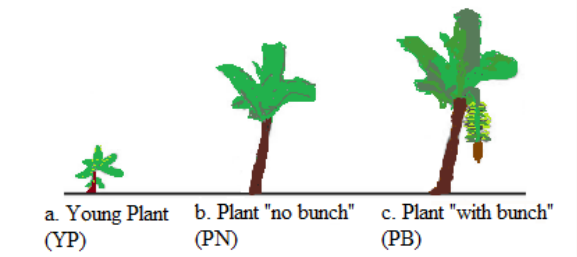

Fig. 2.1. Banana growing stages that were used in the experiment.

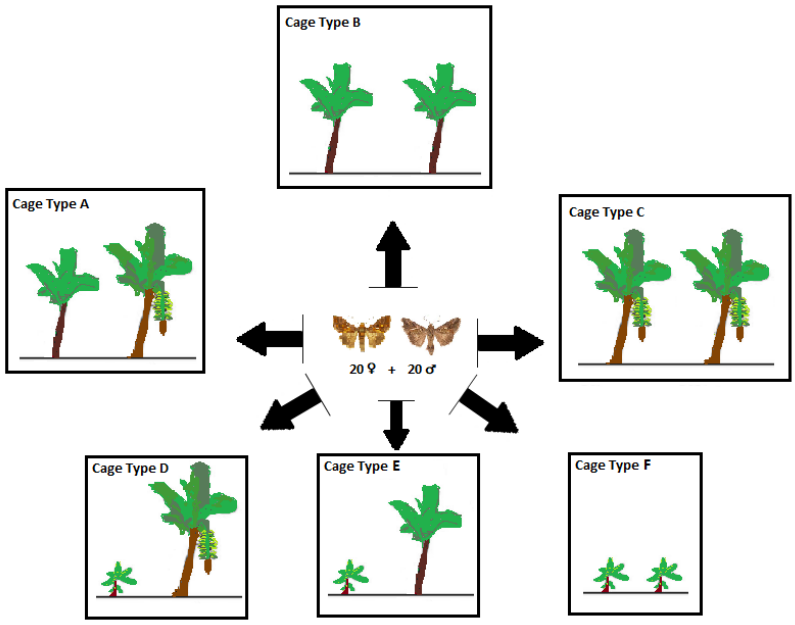

Fig. 2.2. Six field cage combinations that were used in the experiment.

Field cages were maintained seven days for ovipositional process. After 7 days, when the cages were opened, each plant was carefully cut from bottom to apex. It was found a mixture of eggs and hatching larvae together on the leaves. So that the term EL was used in all analysis which refers to the total number of eggs + first instar larvae (EL) found on each plant. All leaves were numbered being the first the youngest leaf and the last the oldest. Leaves were then cut in three parts; distal, median and proximal part (Fig. 2.3.). Each cage represents a combination of different stages of two banana plants (letters A, B, C, D, E, F in Fig. 2.2.), each combination was randomly distributed in the plantation and repeated four times during the experiment.

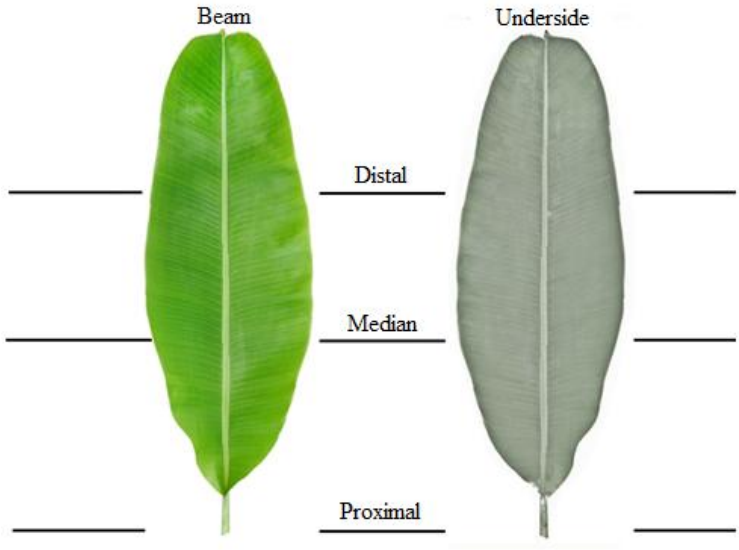

Fig. 2.3. Illustration of banana leaves and proportions used in the experiment.

First instar larvae and eggs (EL) of *C. chalcites* in both faces of each leaf were counted within the same plant. The number of EL were examined in order to determine the spatial distribution of EL comparing cage, plant stage, leaf position and the preferred leaf.

If exist, banana bunches were also cut and ovipositional data was obtained from all parts of the banana plants till the end of summer.

2.4. Statistical analysis

Ovipositional encounters were normalized by arctang transformation [arctang ($\sqrt{}$(x+0.5/100)] prior to analysis in order to stabilize variances. Data were subjected to an analysis of variance (ANOVA) when there were more than 2 groups (cage and plant comparisons) followed by Tukey's HSD test to separate means. The rest of the data were subjected to t-tests in order to compare the corresponding two groups. A 2-way ANOVA was performed in order to test interaction between cage and type of plant, followed by other ANOVA to compare those interactions.

In order to check whether the mean value (mean leaf position) represented the maximum amount of EL, the normality of the vertical distribution of EL was tested, using the Shapiro-Wilks statistic. The statistic W in this test examined for departure from normality. It ranges from 0 to 1, with one being a perfectly normal distribution. The mean value represents the maximum amount of EL One-way ANOVA was used to compare the mean number of EL laid on leaves. All analyses were performed using the SPSS Statistics v 20.0 (Chicago, IL, USA).

3. Results

3.1. Spatial distribution of eggs + L1 larvae (EL) of *C. chalcites*

3.1.1. Cage comparison

The amount of eggs + L1 larvae (EL) counted in the different cages was significantly different ($F=39.76$; $P<0.001$). The cages with "two young plants" (YP-YP) showed a higher amount of EL (162.96±19.2) than the rest of the cages, followed by the cages with young plants and plants "no bunch" (PN-YP) (118.67±6.6) and with "young plants" and plants "with bunch" (PB-YP) (106.94±20.8), and also cages containing plants "no bunch" and plants "with bunch" (PN-PB) (76.13±9.2). The cages with two "with bunch" (PB-PB) plants showed higher EL (24.4±2.6) than those cages with two plants "no bunch" (PN-PN) (11.69±2.6), but less than the other four types of cages (Fig. 2. 4.).

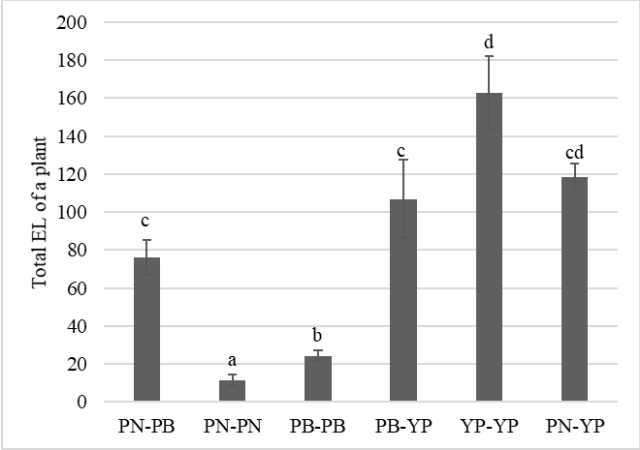

Fig. 2.4. Total EL counted in all leaves of a plant. Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (P<0.05).

3.1.2. Plant comparison

When pooling all the cages and attending only to the type of plant, different EL number were counted (F=51.059; P<0.001). A higher amount of EL were counted on "YP" (165.25±15.7) followed by adult plants with a bunch (PB) (49.52±5.5). Plants "PN" showed the lowest amount of EL (35.61±4.5) (Fig. 2. 5.)

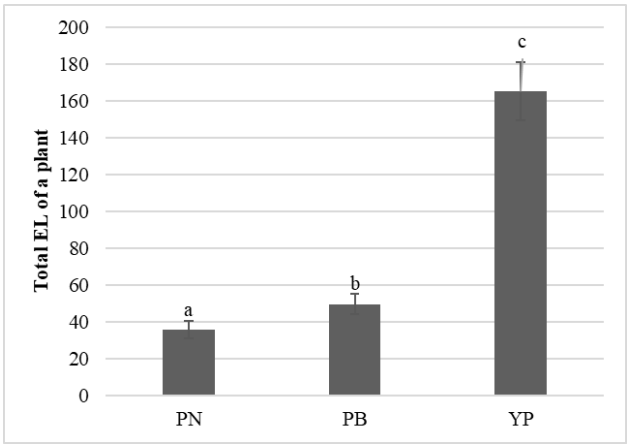

Fig. 2.5. Average of the total EL counted in all leaves of a plant. Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (P<0.05).

3.1.3. Leaf position

When the amount of EL was compared between the different parts of the leaves, no difference of EL was found between distal, middle and proximal parts (data not shown).

3.1.4. Beam/underside of the leaf

Higher amount of EL was counted on the underside of the leaves (119.02±3.9) compared to the beam (47.9±9.9) (t=-5.951; d.f. = 286; P<0.001) (Fig. 2. 6).

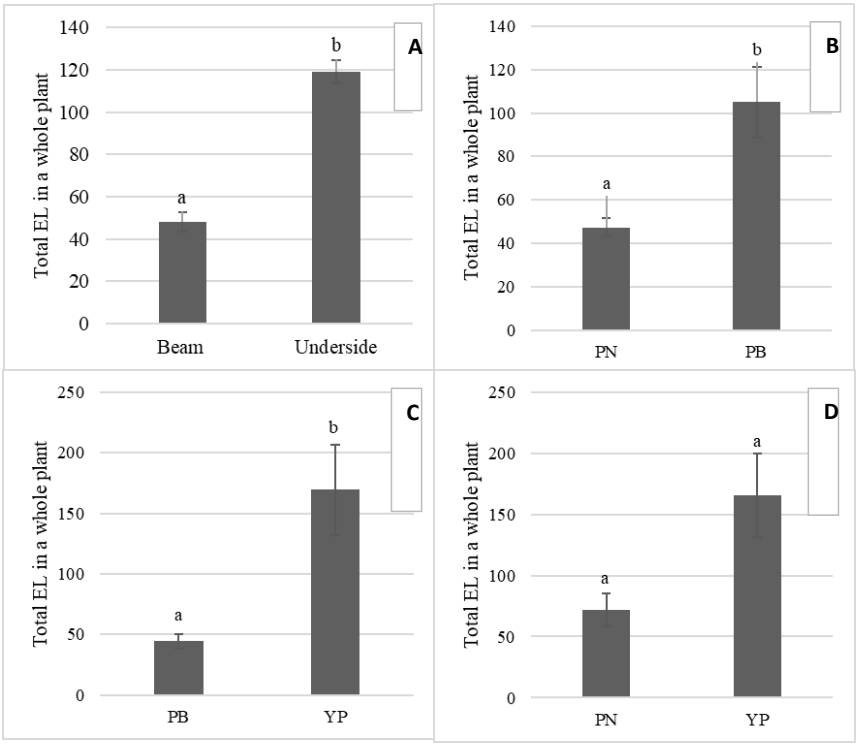

Fig. 2.6. A. Average of the total EL counted on the beam and underside of a single leaf. Different letters above bars indicate significantly difference among groups according to a T-test (P<0.05). **B**. Average of the total EL counted on plants "no bunch" and plants "with bunch" in the same cage. Different letters above bars indicate significantly difference among groups according to a T-test (P<0.05). **C**. Average of the total EL counted on plants with bunch and young plant in the same cage. Different letters above bars indicate significantly difference among groups according to a T-test (P<0.05). **D**. Average of the total EL counted on plants no bunch and young plants in the same cage. Different letters above bars indicate significantly difference among groups according to a T-test (P<0.05).

3.1.5. Comparison of plants within the same cage.

Higher EL were counted in the same cage, on plants "PB" (104.96±4.2) than on plants "PN" (47.29±4.2) (t=-3.227; d.f. =39,157; P=0.003) (Fig. 2. 6.). In those cages with "YP" and "PB", the "YP" showed higher EL (169.54±37.3) than the plants "PB" (44.33±5.9) (t=-2.792; d.f. =37.31; P=0.008) (Fig. 2. 6.). No difference was found in EL between "YP" (165.54±34.5) and plants "PN" (71.79±13.3) (Fig. 2. 6.).

3.1.6. Comparison of the same type of plant on different cages.

The amount of EL was lower on cages with two plants "PN" (11.69±2.6), than when the plant "PN" was with a "YP" (165.54±34.2) or a plant "with bunch" (76.13±9.2) (F=63.257; P<0.001). (Fig. 2. 7.).

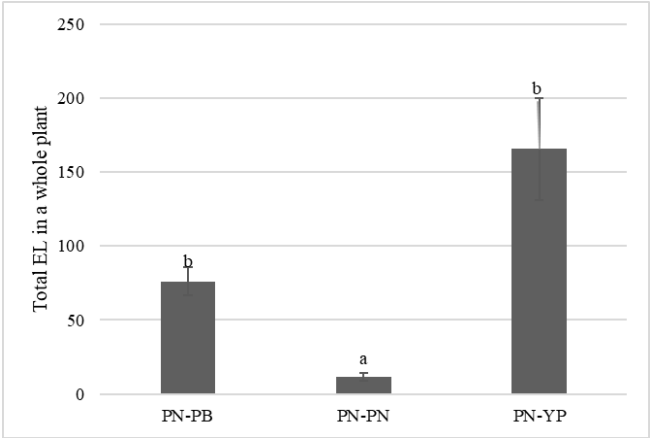

Fig. 2.7. Average of the total EL counted on plants "no bunch" in three different cages. Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (P<0.05).

Plants "PB" showed a higher amount of EL when they were in the same cage of a plant "PN" (104.96±5.9), compared to when they were with "YP" (44.33±2.6) and also more than when they were together with another plant "PB" (24.4±16.1) (F=26.65; P<0.001) (Fig. 2. 8.).

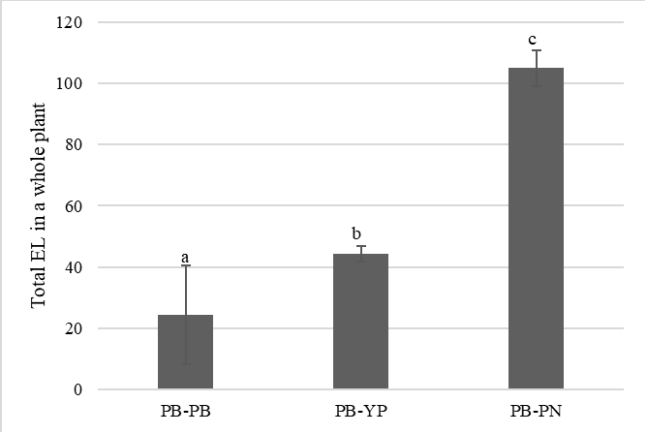

Fig. 2.8. Average of the total EL counted on plants "PB"in three different cages. Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (P<0.05).

No difference was found in the number of EL between "YP-YP" in the same cage compared to a "YP-PN".

3.2. Vertical distribution of eggs + larvae (EL) of *C. chalcites*.

3.2.1. EL mean position

The vertical distribution of EL are shown in Table 2.2. Five out of the plants from the 16 measured without bunch showed a normal distribution (null hypothesis not rejected according to Shapiro-Wilks test) of EL among the leaves, with a mean position of 7.98, indicating that this is the leaf with the highest EL in those plants. It was also checked whether or not the mean position among those 5 plants gather around the "mean" value, and indeed they followed a normal distribution (W=0,938; df=5; P=0,654). When all the plants without bunch (16) were studied, the target leaf would be 6.27, nevertheless as 11 out of those followed no normal distribution this number is not representative of a leaf with maximum count of EL.

Table 2.2. Vertical distribution of EL in every leaf of 48 banana plants, according to the type of plant and showing Shapiro-Wilks test.

Plant Number	Cage	Plant	nº leaves	Mean position	W	P-value
1	"PB"-"PN"	"PN"	12	5.57	0.842	0.029
2	"PB"-"PN"	"PB"	18	7.61	0.862	0.013
3	"PB"-"PN"	"PN"	17	4.86	0.455	<0,001
4	"PB"-"PN"	"PB"	24	12.88	0.877	0.007
5	"PB"-"PN"	"PN"	13	6.70	0.782	0.004
6	"PB"-"PN"	"PB"	14	6.18	0.887	0.073
7	"PB"-"PN"	"PN"	16	8.09	0.954	0.559
8	"PB"-"PN"	"PB"	16	10.91	0.946	0.431
9	"PN"-"PN"	"PN"	18	4.60	0.466	<0,001
10	"PN"-"PN"	"PN"	16	5.32	0.644	<0,001
11	"PN"-"PN"	"PN"	17	6.09	0.728	<0,001
12	"PN"-"PN"	"PN"	13	4.00	0.524	<0,001
13	"PN"-"PN"	"PN"	17	6.22	0.748	<0,001
14	"PN"-"PN"	"PN"	14	7.14	0.861	0.032
15	"PN"-"PN"	"PN"	20	5.24	0.534	<0,001
16	"PN"-"PN"	"PN"	15	4.65	0.827	0.008
17	"PB"-"PB"	"PB"	14	6.92	0.966	0.813
18	"PB"-"PB"	"PB"	14	7.84	0.939	0.411
19	"PB"-"PB"	"PB"	13	6.95	0.934	0.389
20	"PB"-"PB"	"PB"	14	8.08	0.941	0.432
21	"PB"-"PB"	"PB"	14	8.03	0.87	0.042
22	"PB"-"PB"	"PB"	14	8.78	0.893	0.09
23	"PB"-"PB"	"PB"	14	10.42	0.878	0.055
24	"PB"-"PB"	"PB"	14	9.29	0.854	0.025
25	"PB"-"PN"	"PB"	15	7.75	0.894	0.077
26	"PB"-"YP"	"YP"	8	5.77	0.708	0.003
27	"PB"-"YP"	"PB"	15	9.70	0.93	0.274
28	"PB"-"YP"	"YP"	7	4.19	0.925	0.51
29	"PB"-"YP"	"PB"	15	8.74	0.83	0.009
30	"PB"-"YP"	"YP"	8	4.33	0.957	0.784
31	"PB"-"YP"	"PB"	15	8.01	0.916	0.168
32	"PB"-"YP"	"YP"	8	4.89	0.904	0.314
33	"YP"-"YP"	"YP"	8	5.50	0.916	0.399
34	"YP"-"YP"	"YP"	8	5.06	0.871	0.155
35	"YP"-"YP"	"YP"	8	6.09	0.8	0.028
36	"YP"-"YP"	"YP"	8	5.40	0.954	0.756
37	"YP"-"YP"	"YP"	7	4.25	0.946	0.692
38	"YP"-"YP"	"YP"	8	5.49	0.921	0.438

39	"YP"-"YP"	"YP"	7	4.80	0.887	0.26
40	"YP"-"YP"	"YP"	7	4.23	0.899	0.327
41	"YP"-"PN"	"PN"	10	5.97	0.97	0.892
42	"YP"-"PN"	"YP"	8	4.92	0.975	0.933
43	"YP"-"PN"	"PN"	12	5.37	0.984	0.982
44	"YP"-"PN"	"YP"	9	4.48	0.962	0.837
45	"YP"-"PN"	"PN"	16	10.83	0.952	0.588
46	"YP"-"PN"	"YP"	8	6.05	0.975	0.933
47	"YP"-"PN"	"PN"	14	9.62	0.958	0.692
48	"YP"-"PN"	"YP"	8	4.13	0.936	0.569

For the plants with "PN", 11 out of 16 showed a normal distribution, and the average of those 11 showed that the value with the highest EL was 8.32. This mean value was representative of the maximum amount of EL for the plants with "PN" as they followed a normal distribution (W=0,944; df=11; P=0,572). When all the plants were studied the target leave was calculated as 8.63, which is very similar with the ones that followed a normal distribution.

Following the same procedure, 14 out of 16 leaves of the "YP" followed a normal distribution, and the average of mean position was the 4.84. This mean value was representative of the maximum amount of EL for the "YP" as they followed a normal distribution (W=0,819; df=14; P=0,209). When all the 16 plants were used for the target leave calculation the number was 4.97, which is very similar to the ones that followed a normal distribution. On the 48 plants of the trial, 25149 EL were counted, with a mean number of 500.77 ± 242.16 (mean ± SE).

3.2.2. Linear regression

A linear regression was used to model the amount of EL counted in the Distal/Middle/Proximal part of the leaves both in the beam and the underside. Results showed that the most accurate estimation of the models were those using the underside of the leaves, and among them, measuring the middle part of all the leaves did make a reliable estimation of the amount of EL in the whole plant. According to our results, it can be predicted as y=4.35x+68.12, where y= total EL in the whole plant and X= adding

of EL counted in the underside of the median part of all leaves. The Nash-Sutcliffe efficiency for the linear regression was 0,878, and the RMSE=167.61; r^2=0.877.

Other linear, exponential, potential equations were used in order to model the amount of EL in the whole plant counting only part of the leaves, such as using the underside of the proximal part of the "target leaf" (8^{th} in adult plants and 5^{th} in young plants), or even all the EL of those leaves, but we did not manage to find a reliable regression.

4. Discussion

Our data showed that *C. chalcites* EL were more abundant on the underside than on the beam. Previous studies show that in soybean, *C. chalcites* eggs were found also preferably on leaves than in other parts of the plant, but mainly in the underside of the leaf (Mascarenhas & Pitre, 1997; Valverde, 2007). In sweet pepper, *S. exigua* oveipositional distrubution was tested and within each leaf no differences were found in the number of eggs laid in the proximal, medial or distal portion (Cabello et al., 1992). In contrast, in soybean tomato and sweet pepper, oviposition was mainly in the upper and middle part of the plants (Izquierdo et al., 1996; Mascarenhas and Pitre, 1997; Valverde, 2007), or in the lower part of the plant (Cabello et al., 1992), with no studies taking into account the proximal, medial or distal part of the leaves, probably for being herbaceous plants with small leaves, compared to the big leaves of the banana plants. Abera et al. (1999) studied the timing and distribution of attack by the Banana weevil *Cosmopolites sordidus* in banana plantations in Africa and mentioned that the age of the plant was an important factor on dispersion of the insect. It was found that in older (i.e., flowered) banana plants, received more eggs than other plant stages. Additionally, egg density per unit surface area of the plant was greater on older plants suggesting that oviposition was not based on random encounter with hosts. Justin et al. (2006) studied the spatial distribution of banana skipper (*Erionota thrax* L.) on banana plantations in Malaysia and stated that the infestation levels and parasitism of *E. thrax* life stages were recorded from plants "PB", "PN", "YP", broad leaf followers and narrow leaf followers, as well as on well managed and poorly managed plants. Significant numbers of *E. thrax* immatures were recorded from broad leaf followers and "YP"; no eggs were found on plants "PB" and "PN". This states parallel results with our study since it was found significantly more number of EL on "YP" of banana. Raupp & Denno (1983) mentioned that the spatial

distribution of insects on plants is a reflection of the feeding and/or oviposition decisions (Okolle et al., 2006). Similarly, the random distribution of the EL in this study is therefore a reflection of the random dispersion of eggs. Udayagiri & Mason (1995) mentioned that chemical composition of plant tissue of certain regions of the plant might be involved in influencing females to select these regions as oviposition sites, in addition to factors such as temperature, light, humidity, leaf toughness, and predator/parasite avoidance. This might be one of the reasons of variance between different growing stages of plants.

Cage comparison **(i)** In the case of existence of "YP", *C. chalcites* adults prefers primarily those plants to lay eggs than in any other plant growing stage. When a "PB" is combined with a "PN", the EL number was found higher in "PB" plants. When the combination of the plant was; one "YP" and one "PB" or one "YP" and one "PN", it was measured that adults again are preferring for oviposition the "YP" more than any other growing stage.
Plant comparison(ii) Higher EL was found on "YP" then on "PB" and then on "PN". The soft epidermis tissue of young plant leaf surface, might be one of the factors of this preference. Moreover, late growing stages of banana plants have big canopy size and this might result as an unsafe oviposition site for adults of *C. chalcites*. This natural selection might be due to the existance of an enormous variety of microtextures on the plant surface and unicellular and multicellular outgrowths from the epidermis. These structures are, because of their small scale, usually indiscernible to the unaided human eye, but they are often of paramount importance to small herbivores and their natural enemies (Schoonhoven et al., 2005).

In 1998, in a study conducted to understand development time of hatching of forest tent caterpillar larvae, it was found the late hatching of larvae as result of changes in aspen foliar quality by time (Parry et al., 1998). The reduction in water content may exacerbate the decline in nitrogen available to caterpillars (Scriber & Slansky 1981). In addition to the seasonal decline in the nutritive value of leaves, secondary chemicals, in particular phenolic glycosides, may have played a role in the delayed development of later hatching larvae. In conjunction with lowered nitrogen levels, constitutive levels of phenolic glycosides have been shown to have significant negative effects on larval performance of

several aspen folivores including forest tent caterpillar (Bryant et al., 1987; Lindroth & Bloomer 1991; Lindroth & Hwang 1996). These effects are likely to have greater impact on young caterpillars because of the reduced sensitivity of older instars to dietary quality changes (Lindroth & Bloomer 1991).

Beam/underside of the leaf (iii) The data showed that the number of EL found underside of the leaves were higher than the ones counted on the beam of the leaves. This results have been demonstrated before by other researchers (Duffield et al., 2001). *Helicoverpa armigera* and *Helicoverpa punctigera* within plant distrubution on soybean was studied and more EL was found underside of the leaves. In the same way, the preference for leaves was characterized by a strong preference for their underside. Similar preferences for the underside of leaves in the top portion of the canopy have been recorded for both species on cotton (Mabbett & Nachapong 1984; Hassan et al., 1990), and for species of *Heliothis* on soybean in the United States (Hillhouse & Pitre 1976; Terry et al., 1987). Underside of the leaves is likely to facilitate a shelter for the EL.

Comparison of plants within the same cage *(iv)* The results of double combinations such as “PB” and “PN” showed higher EL on plants with bunch. On the other hand, when "PB" was combined with a "YP", EL number was higher in "YP" plants. Significantly more EL were found on "YP" stage and the lowest EL on plants "PN". *C. chalcites* adults prefer mostly early development stages, and when there are no young plants, they prefer late stages of banana plants. As generally banana plantations in green houses are found mostly mixed stages, this can be a useful information to find *C. chalcites* on banana plantations.

Comparison of the same type of plant on different cages (v) When we take plants "PN" and compared them with three stages (PN, PB, YP), the EL number was lower in combination of "PN-PN", than "PN-PB", and than "PN-YP" which means when there are plants "PN", the preference of *C. chalcites* is more likely to the "YP" and secondly to the "PB". However, when we choose as a comparison point the plants "PB" and compare it with (PB, PN, YP), we found more EL on the combination of "PB-PN" than on "PB-YP" than "PB-PB". In this case, when late stages of banana "PB" are together with plants

"PN", the adult's preferences has been switched from "YP" to elder stages of plants (PB-PN). As a result, different combination of cages showed that, in all cases, the tendency was firstly to young plants, if there is no young plant, then the elder plants had more EL. However old plants without a bunch had the lowest number of EL in all cases.

Leaf position (vi) No significant difference was found between distal, median and proximal parts of the same leaf. The distribution of the oviposition has not shown any special pattern on any part of the leaves.

It was not possible to estimate the amount of EL in the whole plant by measuring only one third of the underside of a leaf, instead a reliable approximation of the whole population on a plant can be predicted by counting the total number or EL in the middle part of the underside of all leaves with the expression y=4.35x+68.12, where y= total EL in the whole plant and X= adding of EL counted in the underside of the leaves.

Resuming all the data, when the different combinations of EL was measured between the three stages of banana plants, *C. chalcites* lays more eggs on "YP" than plants "PN", this is likely due to the fact that leaves are more tender in young plants.

It was determined that the adequate leaf to measure the maximum amount of EL on adult banana plants (PB) is on the "8th" (the 8th newest leaf) and on young bananas (YP) the 5th leaf. In order to detect *C. chalcites* on banana plantations, when necessary, it is suggested to look first to "YP" on the 5th leaf, then on "PB" on the 8th leaf and then "PN". In all cases, the underside of the leaves should be checked first.

Spatial distribution of insects on plants is an important aspect for a better understanding of population dynamics of *C. chalcites* on banana fields in the Canary Islands. Our findings could be useful on taking decisions on Integrated Pest Management (IPM) strategies. Further experiments are required to determine the mechanism, if any, of this behavior. Nonetheless, the results from this investigation suggest that in addition to distribution of EL, larval dispersal between different growing stages of banana plantations is also an essential aspect to understand the population dynamics of *C. chalcites* on banana plantations in Canary Islands.

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Universidad de La Laguna

Chapter 3

Effects of several UV protectant substances on the persistence of the insecticidal activity of the Alphabaculovirus of *Chrysodeixis chalcites* (ChchNPV-TF1) under laboratory and open field conditions on young banana (*Musa acuminata*, Musaceae, Colla) plants

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Abstract

The Alphabaculovirus of *Chrysodeixis chalcites* (ChchNPV-TF1) was probed as useful insecticide against *C. chalcites* (Esper) (Lepidoptera: Noctuidae) in banana crops in the Canary Islands. The present study aimed to study the effects of several substances on the persistence of ChchNPV-TF1 in field conditions. Natural photo protective substances such as: moringa, cacao, green tea, benzopurpurin, charcoal, benzimidazole, caolinite, bentonite, and congo red were firstly evaluated under laboratory conditions. The photo protective substances were divided into three groups: low protection (0-8%; caolin), intermediate protection (48-62%; green tea, moringa, bentonite and cacao) and high protection (87-100%; charcoal). Two of the best resulting substances from intermediate and high protection, 1% cacao and 1% charcoal, respectively, were selected for the open field experiment in a young banana plantation. The persistence of OBs on leaf surfaces with sunlight exposure was compared by determining initial mortality of 2nd instar *C.*

chalcites larvae on different time points post application. The percentage of mortality was significantly higher in 1% charcoal+ChchNPV-TF1 (54.89±9.34%) than on 1% cacao+ChchNPV-TF1 treatment and control plants (ChchNPV-TF1 alone) (9.74±4.0%) ($F_{2, 33}$ = 44.613, P = 0.001) after one hour of exposure to UV. The mortality rates decreased over time in all treatments, being always higher in the UV protected substance-treated parcels. 1% charcoal showed the highest protection both in the laboratory and field experiments. No specific interference of UV protected substances was observed on the maximum photochemical efficiency of banana plants in field conditions.

Key Words: baculovirus activity, *Chysodeixis chalcites*, field-biocontrol application, NPV, persistence, protectants, ultraviolet radiation.

1. Introduction

The Canary Islands is the biggest producer of banana plants (*Musa acuminata*, Colla, Musaceae) in Spain, covering an area of about 9,000 hectares (Asprocan, 2017). In 2017, the Canary Islands produced 437,782 tonnes of banana worth 104,416 thousands euros (Asprocan, 2017). In Tenerife, banana-growing industry is a significant contributor to the agricultural economy with 43% of the total production, followed by La Palma (33%) and Gran Canaria (22%). Most of the production is destined to markets in mainland Spain (91%), and only a small fraction is exported to Western Europe (0.1%), whereas the remaining (8.9%) is consumed locally (Fuentes et al., 2017).

Chrysodeixis chalcites (Esper) (Lepidoptera Noctuidae) is one of the most important pest on banana crops. Larvae are highly polyphagous, feeding on several fruit, horticultural, ornamental and forest crops such as banana (*Musa* sp. L.), cotton (*Gossypium* L.), alfalfa (*Medicago sativa* L.), cabbage (*Brassica oleracea* var. *capitata* L.), sunflower (*Helianthus annuus* L.), geraniums (*Pelargonium* sp Charles L'Héritier), bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), turnip (*Brassica rapa* L. subsp. *rapa*), potatoes (*Solanum tuberosum* L.), cucumber (*Cucumis sativus* L.), pepper (*Piper nigrum* L.), soybean (*Glycine max* L.), tobacco (*Nicotiana tabacum* L.) and tomato (*Solanum lycopersicum* L.) (Cabello et al., 1996). Chemical insecticides are normally used to control this pest (Fuentes et al., 2017a). However, chemical applications have enormous negative consequences for both farmers and nature. In addition, beneficial insects are eliminated from agricultural ecosystems and pesticide residues restrict the commercialisation of banana crops (Del Pino et al., 2011b). Currently, a low number of active substances are authorized for *C. chalcites* control in banana crops. Insecticides that does not leave xenobiotic residues in fruit are today essencial and more importantly compatible with IPM systems that aim to conserve natural enemy populations. These facts motivated the researchers for alternative control methods, such as biological insecticides (O'Callaghan & Brownbridge, 2009; Killick, 1990; King & Coleman, 1989). Such products as ChchNPV-TF1 may become an alternative for *C. chalcites* control in future (Weinberg & Bealer, 2004; Cinelli et al., 2019; Komalamisra et al., 2005).

The *C. chalcites* single nucleopolyhedrovirus (ChchNPV-TF1), isolated and characterized from the banana farms in southern Tenerife (Bernal et al., 2013), was probed as an effective insecticide of *C. chalcites* by Fuentes et al. (2017b). Currently, the efficacy of

the virus still requires further improvements for its successful use in Integrated Pest Management (IPM) programs. One of the factors that clearly influence their efficacy is the solar ultraviolet (UV)-radiation, as it affects the persistence of occlusion bodies (OBs) deposited on plant surfaces (Ignoffo & Garcia, 1992). The efficacy of these viruses drastically decreases within the first 24–48 h post-spraying due to solar radiation (Bullock et al.,1970; Young et al., 1986). A number of natural substances provided UV protection to the virus product; such as Dyes, Fluorescent brightener or lignin derivates. Antioxidant or oxidative enzyme such as Dilodin, Inol, Vitamins, Folic acid, Riboflavin, and Pyridoxine are all promising compounds for the protection of entomopathogenic viruses from UV-rays (El Helaly et al., 2013). However, those substances might affect the photosynthetic activity. Chlorophyll fluorescence (Photoquimical Efficacy) technique has been amply used in the diagnosis on plant health during decades (Baker N. R. 2008; Baker et al., 2004; Maxwell & Johnson, 2000). Many fluorescence parameters or indexes have been applied to study the effect of different environmental stresses (light, temperature, heavy metals etc.). Among them, the Fv/Fm parameter is the most known, and is closely linked to the photosynthetic activity reflecting the maximum photochemical efficiency of photosystem II (Björkman & Demmig, 1987).

In the present study several substances, which were previously found to have photo protective capacity such as green tea, moringa, cacao, caolin, iron oxide, charcoal, bentonite, benzopurpurin and benzimidazole (Asano, 2005; El-Helaly et al., 2013; Choi et al., 2010; El-Salamouny et al., 2009; Arivudainambi et al., 2000; Shaphiro et al., 1983) were tested with ChchNPV-TF1. For doing that, first the optimal dose of ChchNPV-TF1 against *C. chalcites* was determined. After, that the natural photo protective substances were selected under laboratory conditions and were proved in field. The persistence of ChchNPV-TF1 OBs on banana plants was measured as viral induced mortality on *C. chalcites* larvae. Finally, the influence of those substances on maximum photochemical efficiency of banana plants in field conditions was also determined.

2. Material and method

2.1. Insects

The larvae used to infest artificially banana young plants were obtained from a laboratory reared *C. chalcites* colony, obtained from greenhouse plants of banana farms in southern Tenerife and reared in the laboratory. The colony was maintained in the Instituto Canario de Investigaciones Agrarias (ICIA), Tenerife, at 25±1ºC, 60±80% relative humidity and a photoperiod of 16:8 h (light:dark) on a semi-synthetic diet based on cornflour, wheat germ and yeast. Adults were fed with 10% v/v honey solution (Cabello et al., 1996).

2.2. Virus Strain

The ChchNPV-TF1 was mass-produced by infecting 6th instar *C. chalcites* with 1×10^{8} OBs/ml by the droplet feeding method (Bernal et al., 2013). Inoculated larvae were placed in 24 well tissue culture plates with semi-synthetic diet and incubated at 25ºC. Larval mortality was revised daily. Dead larvae with polyhedrosis symptoms were collected and stored at -20ºC. OBs cadavers were homogenised, filtered through muslin and centrifuged at 3,800 *x g* for 5 minutes. The resulting OBs pellet was resuspended in sterile water, and OB concentration was determined by counting triplicate samples using an improved Neubauer hemocytometer (Superior Marienfield, Laude-Koeningshofen, Germany) under phase contrast microscopy at x400. Purified OBs were stored at 4ºC until use. The identity of these OBs was confirmed by restriction endonuclease analysis using *Bgl*II (Bernal et al., 2013, Bernal A., 2014).

2.3. Determining the optimal ChchNPV-TF1 concentration

In order to determine the optimal OB concentration to apply against 2nd instar *C. chalcites*, five different viral concentrations were tested. Density of viable OBs on banana leaf surface was estimated by a calibration curve of the bioassay. To this end, 5-fold dilution series were used as concentrations: 160, 800, 4000, 2x10^{4}, and 1x10^{5} OBs/ml, or water, as control, were applied to the banana leaves collected from untreated part of the parcel using a compressed-air hand sprayer (DEA 2000, Italia). These concentration ranges were previously determined to kill between 95 and 5% of the experimental insects for each instar (Bernal, 2014). All treatments included 0.1% (v/v) Agral (Syngenta Agro S.A., Madrid, Spain) as wetter-sticker. 30 min post-application, when banana leafs plants were completely dry, five cm diameter of banana leaf discs were

carefully cut and placed into petri dishes. Each petri dish was infested with 30 2nd instars *C. chalcites*. As control, 30 larvae from the laboratory colony were fed with banana discs. Larvae were individualized in 25 ml plastic cups with artificial diet 24h post-infection and under controlled conditions at 25±1 ºC, 70±5% humidity, and a 16:8 h (light:dark) photoperiod. Larvae were inspected daily until death or pupation. Whole process was repeated on five occasions.

2.4. Determination of photo protective activity of several substances under laboratory conditions

In Table 3.1., the photo protective activity of several substances is shown. All these substances were assayed under laboratory conditions by spraying along with the virus in banana leaves. Virus suspension was applied at 10^5 OBs/ml, which was obtained from the optimum concentration assay and expected to produce up to 80-100% mortality. The photo protective substances were firstly applied at 10%, however, a thick layer of substances remained on banana leaves that might inhibit the photosynthesis metabolism as act as a physical barrier. Therefore, all substances were prepared at 1%.

A positive control, which contained only the virus without photo protective substance, and a negative control, only water solution, were included in the analysis. These suspensions were sprayed onto banana discs. After 30 minutes, letting them to dry, leafs were placed in a cross-linker at 200 J/cm^2 (Stratalinker Stratagene 1800 UV Crosslinker). Additionally, banana leaves were sprayed with the virus only and were included in the analysis, which might be served to know if crosslinker irradiation has worked well. Finally, non-irradiated discs sprayed only with water were also included as negative control. A foliar surfactant 0.1% (v/v) Agral (Syngenta Agro S.A., Madrid, Spain) as wetter-sticker was included to each suspension. Banana disks of each treatment were placed in Petri dishes and were infested with 30 larvae. 24 hours after, larvae were individualized at 24-well Petri dishes containing artificial diet. After a week, the virus induced mortality was evaluated. A total of 3 replicates were performed.

Table 3.1. Natural substances that were assayed as UV protectors under laboratory conditions.

Substance	Original Activity Remaining (OAR* %) (compared with control)	Reference
Moringa	1%; 93%	El-Helaly et al. (2013)
Cacao	10%; 50% 1%; 96% 1% 85-100%	El-Helaly et al. (2013) El-Helaly et al. (2013) El- Salamouny et al. (2009)
Green tea	1% 85-100% 1%; 66% 1% 100%	El- Salamouny et al. (2009) El-Helaly et al. (2013); El Salamouny et al. (2009)
Benzopurpurin 4B	1%; 80% 1% 75%	Ignoffo et al.,(1997)
Charcoal	1% 75% 5% 50%	Ignoffo et al. (1977); Shapiro et al. (1983); Shapiro et al. (2000); Jacques (1971)
Benzimidazole	High protection	Shapiro et al. (1983)
Caolinita	1%; 80%	Filho et al. (2001)
Bentonite	1% 96%	Arivudainambi et al. (2000); Choi et al. (2010)
Iron oxide	1-4mg/ml (UV 1/6-1/18)	Asano (2005)
Congo red	10%; 72%	Baskaran et al. (1998)

2.5. Determination of the UV protection efficacy of 1% cacao and 1% charcoal under field conditions

The field experiment was realized in young banana plants (Gran Enana variety) at open air conditions in the property of ICIA, during summer time in two consecutive years, from 19/06/17 to 19/07/17 and from 27/09/18 to 11/10/18, exposed to strong solar radiation. First, the air temperature (ºC), photosynthetically active radiation (PAR) (Minikin QTi, EMS, Brno, CZ) and air humidity (%) (Minikin RTHi, EMS, Brno, CZ) data

were measured both years during the course of the experiments. Internal data loggers continuously recorded 30 min-averages of all measurements taken every 5 minutes.

In a plantation of approximately 700 m^2 16 blocks were marked, each block consisted of three banana plants surrounding a central plant of an average number of seven leaf each plant. The trials were applied in a randomized block design with four treatments, which included a negative control composed by water only, a positive control that consisted in ChchNPV-TF1 at 10^9 OBs/l (10^{12} OBs/ha), a suspension that included virus and 1% charcoal, and finally virus with 1% cacao. Foliar surfactant was included to each suspension. These suspensions were sprayed directly to the banana plants and exposed to natural solar radiation. Viral application (3 litres per plant) was made with a manual backpack sprayer with 10-liter capacity of spraying. Suspensions were applied between 8.00 and 11.00 am.

The effect of cacao and charcoal on the persistence of ChchNPV-TF1 on banana plants was measured by the mortality induced by banana leaves at 1hour, 24h, 72h, 120h and 168h post-treatment. For doing that, five cm diameter of banana leaf discs from the three central plants of each treatment were carefully cut and placed into petri dishes. The leaf discs were brought to the laboratory and stored at 4 ºC until the bioassay was made. After 24 hours post application, each leaf disc was sealed with the 30-40 hungry 2^{nd} instar *C. chalcites* during 24h. Thereafter, larvae were individualized in 24-well Petri dishes containing artificial diet and virus induced mortality was measured after one week. A total of 4 replicates were performed until the end of summer season. Larval mortality caused by protected virus treatment was compared with that produced by non-protected virus treatment at each time. Sealed larvae were maintained in a laboratory rearing chamber at 25±1ºC, and 16:8 hours (L:D) photoperiod, until death or pupation. Larvae were monitored daily and those dead with the typical signs of polyhedrosis disease were individually frozen at 60±80 % R.H for subsequent analysis.

2.6. Influence of cacao and charcoal on the photosynthetic activity of banana plants

The chlorophyll fluorescence was measured at midday in the middle-section of sun-exposed leaves after 1, 3, 5, 7 and 14 days post application in 2018. The measurements were made with a portable fluorimeter (Handy PEA, Plant Efficieny Analyzer, Hansatech,

UK) on dark adapted leaves (30 min) to determine basal fluorescence (Fo). After saturating red light pulse (650 nm, 3000 µmol photons m^{-2} s^{-1}] flashed by an array of ultra bright red light emitting diodes, the maximum fluorescence (Fm) was determined. From these parameters, the maximum photochemical efficiency (Fv/Fm) was calculated as the ratio (Fm-Fo)/Fm according to (Genty et al., 1989).

2.7. Statistical analysis

In order to determine the optimal concentration, Probit linear regression (SPSS. Ver 23) was used to estimate the lethal concentration 50 (LC_{50}). Percentage mortality for laboratory and photochemical and Pl abs data was calculated for each treatment and subject to analysis of variance (ANOVA), as data were normally distributed. The significance between treatment for each concentration was determined by between-subject comparisons among the estimated means with Tukey HSD test ($P \leq 0.05$). A GLM repeated measure ANOVA was conducted to evaluate the effect on the larval mortality over time on the field trials. All the analysis were done using SPSS (IBM SPSS Statistics v. 23).

3. Results

3.1. Determination of the optimal dose of ChchNPV-TF1 OBs against *C. chalcites*

The *Bgl*II profile of the virus produced in laboratory-infected larvae was identical to that described previously (Bernal et al., 2013; Bernal, 2014)(Fig. 3.1A), which confirmed the identity and the absence of cross-contamination during laboratory production of OBs. Furthermore, LC_{50} of laboratory produced ChchNPV-TF1 OBs for 2nd instars of *C. chalcites* was 8.47x 10^3 OBs/ml (Fig. 3.1B). This value is relatively similar to that of Bernal et al. (2013). Data analysis revealed that there was a significant difference in larval mortality ($F_{5,24} = 46.586$, $P < 0.0001$).

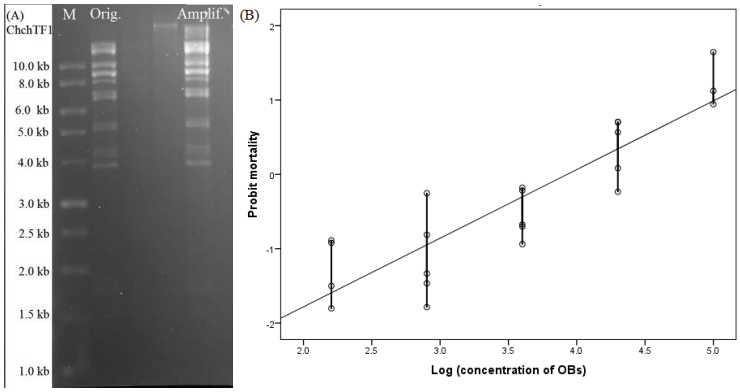

Fig. 3.1. (A) Restriction endonuclease analysis (*Bgl*II) following digestion of genomic DNA of the original inoculum (Orig.) and the laboratory-amplified (Amplif.) OBs of ChchNPV-TF1. The DNA 1 kbMarker Ladder (Nippon, Tokyo, Japan) was used as a molecular size marker (M). (B) Probit regression line obtained after laboratory production was determined by bioassay in 2nd-instar *Chrysodeixis chalcites* larvae ($y = 0.92x - 3.63$, $R^2 = 0.78$).

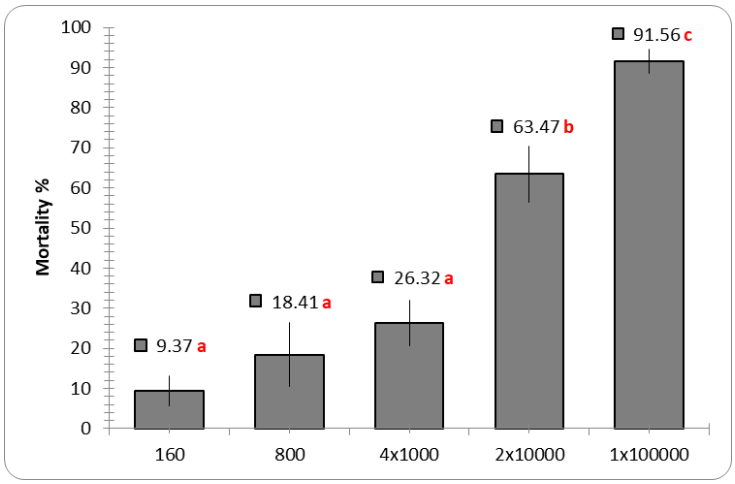

Fig. 3.2. Mortality distribution over time of *C. chalcites* 2nd instars killed by 160, 800, 4000, $2x10^4$, and $1x10^5$ OBs/ml

3.2. Laboratory Trials

There was a significant difference between the nine different UV protected substances evaluated under laboratory conditions ($F_{11,24}$ = 11.56. P < .0001) (Fig. 3. 3.). It was shown that three main groups were obtained by the means of virus induced mortality on 2nd instar *C. chalcites* larvae. It was determined that, the group that presents a high photo protection, obtaining mortalities similar to the non-radiated virus, which suggests its protection against UV such as charcoal and iron oxide which produced mortality up to 87 – 100 %. Secondly those that presented an intermediate photo protection were cacao, green tea, moringa and bentonite. The mortality of *C. chalcites* from this second group ranged from 48 – 62 %. Finally, there were the group of UV protected substances which did not present photo protective activity to the virus since it only caused about 0 – 8% mortality of *C. chalcites* post-UV exposure. This group consisted of caolin, benzopurpurine and benzimidazole. In field assay however, two products, charcoal and cacao, were selected based on the protective activity under laboratory conditions and the market price.

3.3. Field Trials

Cumulative radiation measurements (W/m²) during the experiments in 2017-2018 were registered. The mean radiation was significantly higher (t = 3.535, df = 60, P = .001) in 2017 (235.5 W/m²) than in 2018 (181.6 W/m²). The cumulative radiation was higher in 2017 than in 2018 throughout the entire study period. During the experiment in 2017, the average temperature was 23.81 ºC, minimum temperature was 17.64 ºC and the maximum temperature was 28.21 ºC. The average temperature in 2018 was 18.41 ºC with a minimum of 17.55 ºC and a maximum of 26.08 ºC. The registered average humidity was 52.79 % in 2017 and 77.67 % in 2018 during the experiments.

The percentage of mortality of 2nd instar *C. chalcites* obtained in 2017 is presented in Fig. 3. 4. and that of 2018 Fig. 3. 5. In 2017 at first day, mortality rates were significantly higher in 1% charcoal+ChchNPV-TF1 (50.16±10.79%) than in 1% cacao+ChchNPV-TF1 treatments (43.43±11.95%) ($F_{2,28}$= 36.021, P < .0001) although the mortality in 1% cacao+ChchNPV-TF1 was not significantly greater than the mortality in control plants (36.56±7.62%). At 24 hours post application, 1% charcoal+ChchNPV-TF1 (40.88±9.44%) produced higher mortality rates than on 1% cacao+ChchNPV-TF1

treatment (25.57±6.89%) ($F_{2,33}$ = 29.959, P < .0001). There was still significant difference ($F_{2,33}$ = 4.361, P =0.047) between the treatments and control plants at three days' post application; *C. chalcites* mortality rates continued such as: 1% charcoal+ChchNPV-TF1 (25.37±5.44%), 1% cacao+ChchNPV-TF1 (15.21±2.32%) and control plants. Seven days post application, the mortality rates did not differ significantly ($F_{2.33}$ = 0.862, P = 0.454) between treatments and control plants (ChchNPV-TF1 alone).

In 2018 persistence of ChchNPV-TF1 varied significantly between days-post application ($F_{4,50}$= 21.450, P < .0001) and also between four treatments ($F_{3,14}$= 17.869, P < .0001). The mortality rates after one hour of exposure to UV was significantly ($F_{3,33}$ = 44.613, P < .0001) higher in 1% charcoal+ChchNPV-TF1 (54.89±9.34%) than on 1% cacao+ChchNPV-TF1 treatment and control plants (ChchNPV-TF1 alone) (9.74±4.0%). After 24 h, the mortality from the plants treated with 1% cacao+ ChchNPV-TF1 was significantly ($F_{2,33}$ = 29.959, P < .0001) higher (61.80±7.57%) than the plants treated with 1% charcoal+ ChchNPV-TF1 (48.95±12.19%) and control plants (10.32±3.11%). At 3 days-post application, similar tendency was observed. The mortality of *C. chalcites* was significantly higher in 1% cacao+ChchNPV-TF1 treatment (59.80±5.91%) than on 1% charcoal+ChchNPV-TF1 (31.83±9.27%) and control plants (9.66±3.03%) ($F_{2,33}$ = 43.178, P < .0001). Five days post application, the percentage mortality was significantly different between treatments 1% charcoal+ChchNPV-TF1 samples showed the higher percentage mortality (30.47±7.37%), followed by 1% cacao+ChchNPV-TF1 (21.93±6.32%) treatment and control plants (ChchNPV-TF1 alone) (9.13±2.5%) ($F_{2,33}$ = 10.321, P < .0001). At the last day of sampling, percentage mortality was significantly different ($F_{2,33}$ = 12.089, P < .0001); mortality rate remained around 30.72%±9.86 on 1% charcoal+ChchNPV-TF1 and 10.90±4.34% on 1% cacao+ChchNPV-TF1 on *C. chalcites* 2nd instar larvae. Control plants had 6.32%±2.99 mortality at 7 days.

Further data exploration revealed that in 2017 cumulative virus-induced mortality of *C. chalcites* was significantly higher in 1% charcoal+ChchNPV-TF1 treatment than in 1% cacao+ChchNPV-TF1 at all sampling times ($F_{2,33}$ = 3.044, P < .0001) (Fig. 3. 6.). At all-time points, control treatment mortality values were below UV-protectant treatments. mortality values. In 2018, cumulative virus-induced mortality was significantly higher on the parcel of 1% cacao+ChchNPV-TF1 treatment, followed by 1% charcaol+ChchNPV-

TF1 treatment, and then control treatment for the first 2 time points ($F_{2,33}$ = 55.399, P < .0001) (Fig. 3. 7.). At all-time points, control treatment mortality values were below UV-protectant treatments.

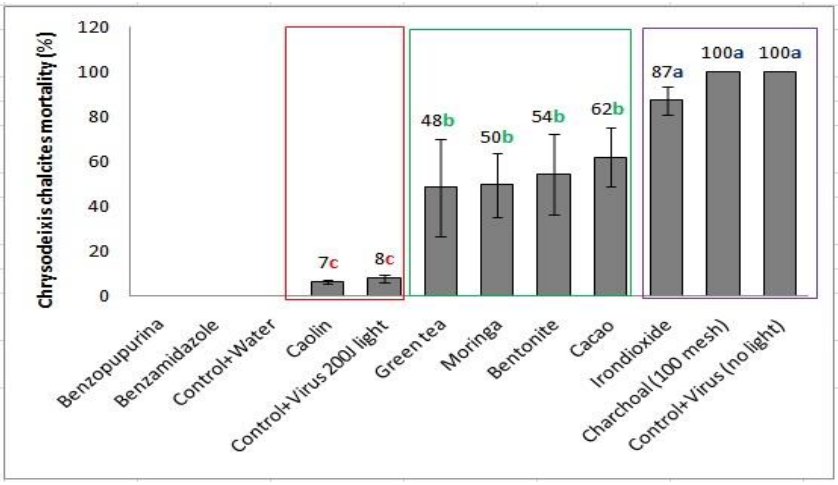

Fig. 3.3. Percentage of virus induced mortality of *C. chalcites* with selected UV protected substances after exposed to 200J/cm² radiation at cross-linker.

3.4. Influence of ChchNPV-TF1 protectant on photosynthetic activity

No significant difference was found between the treated and control plants after 1, 3, 5, 7 and 14 days post application in 2018 (Table 3. 2.; Table 3. 3.). The mean Fv/Fm values per treatment ranged from 0.74 for water and ChchNPV-TF1 treatments and 0.75 for 1% charcaol+ChchNPV-TF1 and 1% cacao+ChchNPV-TF1, respectively.

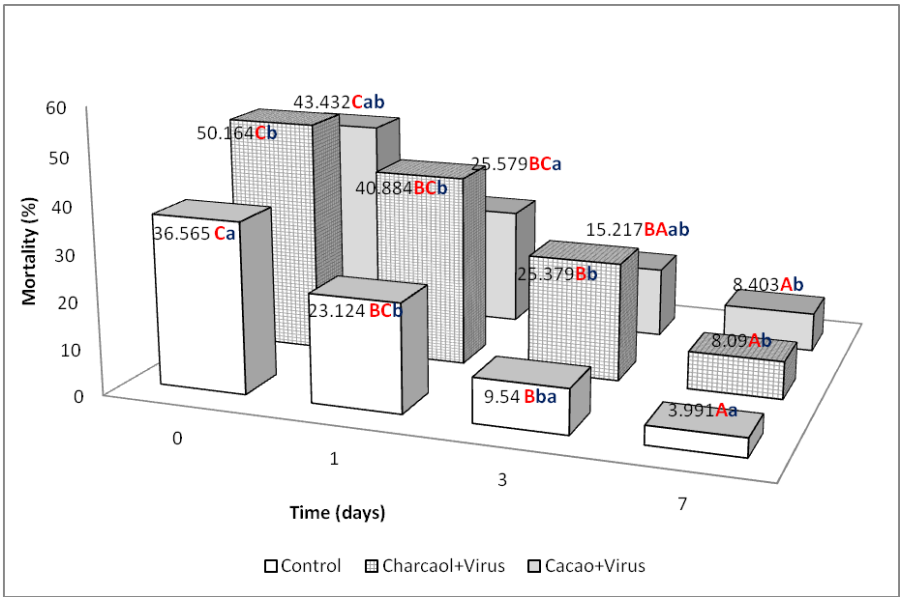

Fig. 3.4. Percentage of mortality of 2nd instar larvae of *C. chalcites* (2017).

Comparison of percentage mortality of 2nd instar larvae of *C. chalcites* (2017) (i) Charcoal 1%, (ii) Cacao 1%, (iii) Control (+) (water+virus) and (iv) Control (-); (water only) with time post application. *Capital letters represents differences between the treatments, lowercase letters represents differences between the days post application).

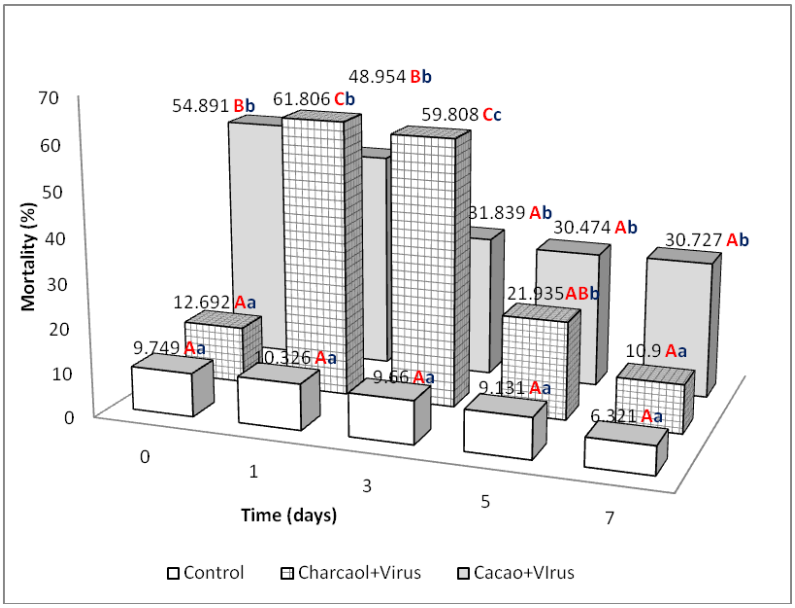

Fig. 3.5. Percentage of mortality of 2nd instar larvae of *C. chalcites* (2018).

Comparison of percentage mortality of 2nd instar larvae of *C. chalcites* (2017) (i) Charcoal 1%, (ii) Cacao 1%, (iii) Control (+) (water+virus) and (iv) Control (-); (water only) with time post application. *Big letters represents differences between the treatments, small letters between the days post application).

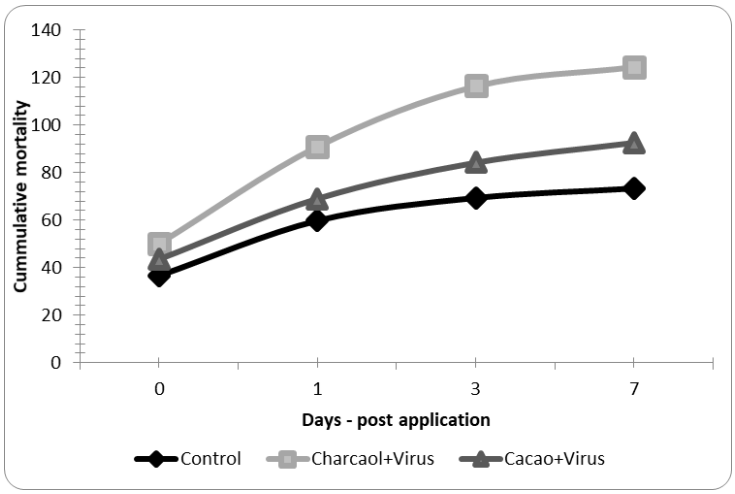

Fig. 3. 6. Cummulative larval mortality (2017).

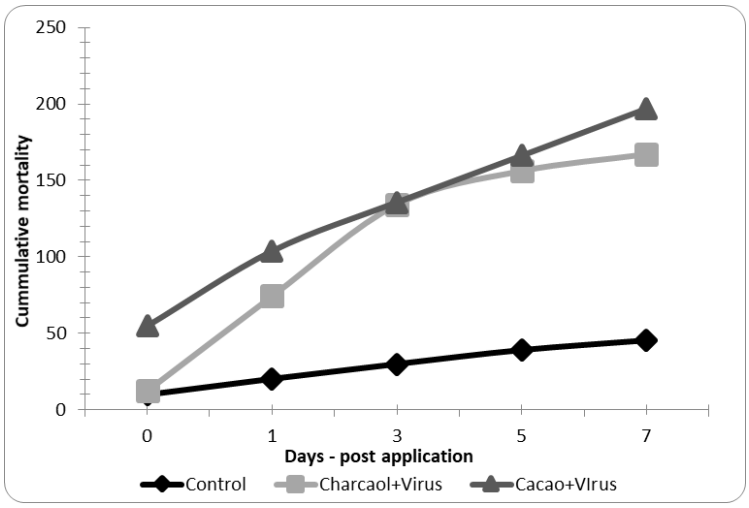

Fig. 3.7. Cummulative larval mortality (2018)

Table 3. 2. Photoquimical Efficacy

	Photoquimical Efficacy					
Treatments	0 day	1 day	3 days	5 days	7 days	14 days
Charcoal	0.76 ± 0.02	0.73 ± 0.03	0.72 ± 0.02	0.75 ± 0.01a	0.79 ± 0.02	0.74 ± 0.01
Cacao	0.71 ± 0.02	0.76 ± 0.01	0.74 ± 0.02	0.75 ± 0.01a	0.82 ± 0.01	0.74 ± 0.02
Control(-)	0.74 ± 0.01	0.78 ± 0.01	0.70 ± 0.03	0.72 ± 0.01b	0.76 ± 0.02	0.73 ± 0.02
Control(+)	0.72 ± 0.06	0.76 ± 0.01	0.71 ± 0.01	0.74 ± 0.01ab	0.78 ± 0.01	0.74 ± 0.02
Df	3,6	3,35	3,35	3,43	3,44	3,44
F	0.254	1.326	0.510	3.236	1.860	0.064
P	0.856	0.282	0.678	0.031	0.150	0.979

Means in the same column with the same letter(s) are not significantly different (Tukey HSD, $p < .05$).

Table 3.3. Performance Index (PI) values

	Pl abs – values					
Treatments	0 day	1 day	3 days	5 days	7 days	14 days
Charcoal	1.16 ± 0.01	1.19 ± 0.21	1.58 ± 0.48	1.61 ± 0.17	3.10 ± 0.49	1.50 ± 0.26
Cacao	1.04 ± 0.33	1.62 ± 0.30	1.95 ± 0.41	1.69 ± 0.21	3.52 ± 0.35	2.22 ± 0.78
Control(-)	1.24 ± 0.05	1.9 ± 0.13	1.45 ± 0.50	1.16 ± 0.11	2.23 ± 0.46	1.36 ± 0.23
Control(+)	1.80 ± 0.40	2.03 ± 0.48	1.08 ± 0.12	1.58 ± 0.29	2.62 ± 0.49	1.75 ± 0.50
Df	3,6	3,35	3,35	3,43	3,44	3,44
F	0.284	1.517	0.669	1.376	1.578	0.583
P	0.835	0.227	0.577	0.263	0.208	0.629

Means in the same column with the same letter(s) are not significantly different (Tukey HSD, $p < .05$).

4. Discussion

Solar radiation is one of the most important factors affecting the persistence of OBs [Ignoffo et al., 1977; Krieg et al., 1980; Martignoni & Iwai, 1985). Natural sunlight, especially the ultraviolet (UV) portion of the spectrum (UV-B, UV-A) is responsible for inactivation of the microbial insecticides (Shaphiro, 2000). Ultraviolet light inactivates baculoviruses rapidly (Asano, 2005). In 2017, natural occurrence and efficacy of the ChchNPV-TF1 was determined under greenhouse and open field conditions in Canary Islands (Fuentes et al., 2017). Infestation of ChchNPV-TF1 under greenhouse conditions observed four-fold more densities than on the open-air conditions. The present study aimed to evaluate the persistence of the ChchNPV-TF1 on banana plants with selected natural substances under laboratory and field conditions. Moringa, cacao, green tea, benzopurpurin, charcoal, benzimidazole, caolinita, bentonite, iron oxide and congo red were subjected to UV radiation with ChchNPV-TF1 under laboratory conditions. Two of the substances (1% cacao and 1% charcoal) was subjected for the open field experiment in a young banana plantation. There are several reasons that we have chosen charcoal and cacao for the field study. First of all, these products are readily available, and secondly, they are relatively cheaper compared to other protectants. Although iron oxide showed a high protection under laboratory conditions together with charcoal, we selected cacao. The price of iron oxide is around 100 €/kg, while cacao is 12 €/kg and charcoal´s price is 5.95 €/kg. When compared, the accessibility of the product and the

price of cacao and charcoal were more reasonable than that on iron oxide. 1% charcoal added parcels showed the highest protection both under laboratory and field conditions. The persistence of ChchNPV-TF1 with %1 cacao was significantly different than on ChchNPV-TF1 alone. The mortality rates reduced in all treatments but it was higher in the substance added parcels. These results are in agreement with those reported by several authors (Ignoffo et al., 1997; Shaphiro et al., 1983; Shaphiro, 2000), where they discussed photo protective effect of charcoal with *Heliotis* nucleopolyhedrosis virus (*Hz*SNPV). Cacao were studied as UV protector before by several authors both in the laboratory and open field conditions for *Spodoptera littoralis* nucleopolyhedrovirus (*Spli*MNPV) (Arivudainambi et al., 2000) and for the beet armyworm nucleopolyhedrovirus (*Se*MNPV) (El-Salamouny et al., 2009) and demonstrated that cacao at 1.0% concentration provided excel-lent UV protection (95% OAR) during a 15-min UV exposure.

The essential substance of cacao is theobromine, formerly known as xantheose, a bitter alkaloid of the cacao plant (Weiberg et al., 2004), which remains and colours the leaves when applied. On the other hand, charcoal is a lightweight black carbon residue produced by removing water and other volatile constituents from animal and plant materials. Such kind of protectants could have an effect the photochemical efficacy. No differences were observed on photochemical activity between the treatments and control plants during all experiments. This implies that the protectants used in this study did not affect the photosynthetic rate of the plants. This result shows that cacao and charcoal are safe to be used as UV-absorber to ChchNPV-TF1 since they provide protection to the virus thus increasing its efficacy overtime and do not interfere with photosynthesis.

Cultivation of banana plants use to be in high radiation rate regions (Cinelli et al., 2019). In both years of the assays there was a high degradation on the number of viable OBs on direct UV light. Introducing these natural substances into the development of baculovirus formulations seem to have a key role since they have a significant effect on the number of viable OBs against direct sun light. These are some of the factors that are limiting baculoviruses application efficacy on the field conditions

In Tenerife, the most used products to control *C. chalcites* are indoxacarb and *Bacillus thuringiensis* (Fuentes et al., 2017a) which provide a rapid peak in mortality that

declined over a 7-day sampling period. The sensitive period of *C. chalcites* is the first three days after application. For a high lethal doses in the first days of the experiment, charcoal or cacao addition protects the ChchNPV-TF1 more, especially at the beginning of the experiment (1-3 days). And that period is critical for virus to acquire a lethal infection (Simón et al., 2015). In the field work done with on banana plants in 2017, application of 1×10^8 OBs/ml resulted in 54±10% (20.85 ± 10.42) lethal infection in larvae sampled at 1 day post-application, on the other hand application of 1×10^9 OBs/ml resulted in 86±7% lethal infection in larvae collected at 1 day post-application to 92±5% lethal infection in larvae sampled at 3 days post-application, which shows a crucial peak at that doses . Higher OB application rates are likely to be necessary to protect fully grown banana plants efficiently, given the high volume applications needed effectively to cover the foliage of large-sized plants [Bernal et al., 2013; Simón et al., 2015). These results indicate a greater persistence of ChchNPV-TF1 OBs on the banana plant with respect to virus alone application. Therefore, addition of 1% charcoal or 1% cacao to ChchNPV-TF1 10^9 OBs/l solution may extend the period of pest control, protecting OBs from UV degradation by producing larval mortality for longer periods. The duration of activity of ChchNPV-TF1 virus protected with these natural substances may increase the performance of virus application even in high UV radiation zones.

The results obtained in this study is providing promising information that can be a useful tool for the future of biological control of *C. chalcites* with ChchNPV-TF1 formulation. However, it is also necessary further studies to obtain results under more different conditions to develop a better formulation for ChchNPV-TF1.

Acknowledgment

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Chapter 4

Efficacy of biorational insecticides for control of *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) on banana plant under laboratory and screenhouse conditions

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Abstract

The golden twin-spot moth, *Chrysodeixis chalcites,* is one of the most important pests in banana production in the Canary Islands, causing damage to the epidermis of banana fruit, then reducing their market suitability. In this regard, the aim of the present study was to assess the efficacy of different biorational insecticides against *C. chalcites* to improve the management of this pest. Products based on bioenzyme complex (Intruder®), Rutaceae and Piperaceae (Avenger®), Rutaceae and Lauraceae (BioKnock®), Cinnamon, Citronella and Menta (Cinamite®), Alliaceae and Solanacea (Garlitrol-Forte®), Citrus plant extracts (Prevam®) and neem oil (Indasol®) were evaluated. Laboratory assays: (i) choice, (ii) no choice and (iii) contact toxicity were performed on *C. chalcites* 2nd instar larvae feeding on treated discs of banana leaves. In choice and no choice assays the consumed leaf area was recorded. The higher repellent effect (choice) observed with Prevam® (85.19±1.7 %), followed by Garlitrol® (68.44±5.7 %), and Intruder® (67.54±4.3 %). In no choice assays, the best results were again with Prevam® (0.92±0.4 %), followed by Indasol® (0.98±0.33 %) and Intruder® (2.7±0.33 %). Direct effect, evaluated through larval mortality, showed differences at 1, 3 and 7 days post application. At 1 day post application, the highest mortality was detected in Intruder® treated leaves (20.22±2.98 %), followed by Indasol® (18.44±3.8

%) and Bioknock® (10.44±4.7 %), whereas 7 days post application, the best results were obtained on Intruder® treated leaves (77.77±5.7%), followed by Indasol® (76±9.27%) and Prevam® (58±11.13%). A screenhouse trial was conducted to compare the efficacy of the products on damage level by 2nd instar *C. chalcites* on banana bunches. One week after the treatments, *C. chalcites* mortality, fruit damage level, and classification of fruit by quality categories were registered, using these last data for an estimation of economic losses for each treatment. Prevam® treated bunches showed 100.0 ±0% efficacy on *C. chalcites* 2nd stage instars, followed, with significant differences, by Indasol® (42.53 ± 21.47), Intruder® (36.25 ± 23.75) and Ripelser® (36.74 ± 12.74). The lowest damage level in fruit was observed with Intruder® (10.54 %), Prevam® (17.63 %) and Indasol® (24.11 %). Consequently, fruit in these treatments had the highest amounts of fruit classified as Category 1 (best quality), the lowest amounts of fruit under Category 3 (unmarketable), and the best estimated incomes.

Key words: banana, golden twin-spot moth, Integrated Pest Management, plant extracts.

1. Introduction

Banana (Musacea, *Musa acuminate* Colla) is the main crop of the Canary Islands, representing about 39% of the total agricultural production of the islands (ISTAC, 2011). The golden twin-spot moth, *C. chalcites* is responsible for annual losses of approximately 2.68 million euros despite the adoption of control measures in this crop (Fuentes et al., 2017). This insect is native from the Mediterranean and tropical regions (Rashid et al., 1971; Murillo et al., 2013) and it is a serious pest of screenhouse crops in Europe, affecting more than 30 different plant species (Cabello et al., 1996; van Oers et al., 2004). In recent years, it has been causing economic damage in banana plants in many banana-growing areas of Canary Islands. Larvae feeding on banana bunches produce significant damage to fruit skin, which is usually located in the upper hand of the bunch, the one closest to the leaves (Del Pino et al., 2007). These hands are those of the highest quality within the bunch and *C. chalcites* damage reduce their commercial value. Moreover, this insect has become resistant to several pesticides (Alonso, 2009; Del Pino et al., 2011). Currently, Integrated Pest Management (IPM) is a mandatory issue in all EU (Directive 2009/128/EC, OJEU 24-XI-2009, L309: 71-86), including Spain (RD 1311/2012, BOE-A-2012-11605), promoting more environmentally friendly methods in pest control. Bio insecticides are characterized by having a very low toxicity for humans and other vertebrates, decomposing within a few hours after being applied or being specific for the pests we wish to control. For these reasons they are considered environmentally benign. Intruder® (Bioenzyme Complex: 10%, and vegetable extracts: 5%), Avenger® (Rutaceae and Piperaceae: 12.0%), BioKnock® (Rutaceae and Lauraceae: 22%), Cinamite® (Citronella, Cinnamon 30% and Mint 13%), Ripelser® (Spicy chile and capsicin extract), and Garlitrol-Forte® (Alliaceae and Solanacea extract) are acting based on disintegrating molasses, blacks, fumagines, and ovicidal action, Prevam® is based on citrus plants (orange oil) and causes dehydration of insect cuticles, and Indasol® (neem oil) acts as a repellent by reducing insect feeding. The aim of this work was to evaluate their repellent effect, antifeeding effect and direct toxicity in the laboratory, and efficacy against *C. chalcites* larvae under screenhouse conditions.

2. Material and Method

2.1. Products applied

Intruder®, BioKnock®, Cinamite®, Avenger®, Ripelser®, Garlitrol-Forte®, Prevam® and Indasol® were the products evaluated both under laboratory and screenhouse conditions. In all trials, the application rates (dose) were the one recommended in the product label for banana cultivation (see Table 4.1.), and treatments were carried out at 20-24 ºC water temperature, and 7.0 water pH.

Table 4. 1.
Description of products used.

PRODUCT	COMPOSITION (%w/w)	DESCRIPTION	CHARACTERISTICS AND UTILITY	DOSE
Intruder®	Bioenzyme Complex: 10%, Plant extract: 5% pH: 5,8 (LS)	Enzymatic soap	Hydrolytic and degrading effect of chitins, sugars and others secreted by insects and mites.	1,2 ml/l
Avenger® MW	MW Plant extracts and oils (Rutaceae and Piperaceae): 12.0% pH: 7.8 (MW)	Enhacer of plant protection product.	Insecticidal effect on white flies, mealybugs, moths, psyllids, aphids and other insects.	1,4 ml/l
BioKnock ®	Plant extracts and oils (Rutaceae and Lauraceae): 22% pH: 8.0 (MW)	Insecticide (repellent)	Insecticidal effect against pests such as green mosquito and some lepidoptera.	2,4 ml/l
Cinamite®	Plant extracts (Citronella, cinnamon 30% and Mint 13%) pH: 7.2 (MW)	Insecticide (repellent)	*T. urticae, P. citri*, *Vasates* and *E. vitis.* Insecticidal effect on thrips (*T. tabaci, Frankliniella* sp. and others) in citrus, fruit and vegetables.	2,4 ml/l
Ripelser®	Spicy chile extract Capsicin	Insecticide (repellent)	Insecticidal effect against pests such as green mosquito and some lepidoptera.	2 ml/l
Garlitrol Forte®	Alliaceae and Solanacea	Insecticide (repellent)	Insecticidal effect against pests such as green mosquito and some lepidoptera.	5ml/l
Prevam®	Orange oil 6%	Insecticide	Causes dehydration of insect cuticles.	3 ml/l
Indasol®	*Azadiractina indica* (neem) oil	Insecticide (repellent)	Reduces insect feeding.	3 ml/l

2.2. Laboratory experiment

The efficacy of Intruder®, BioKnock®, Cinamite®, Avenger®, Ripelser®, Garlitrol-Forte®, Prevam® and Indasol® on the 2nd instar larvae of *C. chalcites* was evaluated under laboratory conditions. The tested products, their descriptions and application dose are shown in Table 4. 1. Three types of bioassays were carried out:

2.2.1. Choice assay: repellent effect

In Petri dishes of 140 mm diameter, 2 discs of 2 cm^2 banana leaves sprayed with test product and 2 discs sprayed only with water were placed. Five repetitions (dishes) per treatment were prepared. Leaves were let to dry for 30 min. Two 2nd instar larvae of *C. chalcites* were added in each per Petri dish and were let to feed on leaves for 24 h, in order to choose between treated or untreated discs. After 24 h, leaves were scanned with a standard image scanner. Scanned leaf samples were subjected to Image Analysis Software ImageJ (Schneider et al., 2012) in order to calculate the area of the leafs eaten by 2nd instar larvae.

2.2.2. No choice assay: damage rate

In this case, 4 discs of 2 cm^2 banana leaves sprayed with the product (or only with water, in the case of control treatment) were placed in each Petri dish. Five repetitions (dishes) per treatment (also five dishes for the control) were prepared. Leaves were let to dry for 30 min.

Two 2nd instar larvae of *C. chalcites* were added in each per petri dish and were let to feed on leaves for 24 h, forcing the larvae to eat on the treated leaves. After 24 h, leaves were scanned with a standard image scanner. Scanned leaf samples were subjected to Image Analysis Software ImageJ (Schneider et al., 2012) in order to calculate the area of the leaves eaten by 2nd instar larvae.

2.2.3. Contact toxicity of products on *C. chalcites* larvae

To evaluate the direct effect of each product on *C. chalcites* 2nd instar larvae, one disc of banana leaf with 5 cm diameter was placed into a Petri dish and infested with 10 2nd instar larvae of *C. chalcites*. Each tested product, including control, was then sprayed on

the leaf using a 1-liter manual hydraulic sprayer (five repetitions-boxes per product). After 1 h, the number of dead larvae in each disc was registered, and alive larvae were carefully transferred into individual disposable cups with standard diet and kept at 25 ± 1ºC, 60% RH and 16 hs light: 8 hs dark photoperiod. Larvae were observed at 1 day, 3 days, and 7 days post-application, and time to death (if that was the case) was recorded.

2.3. Screenhouse experiment

2.3.1. Experimental plot and treatments

The trials were conducted in a screenhouse of the Canary Islands Institute of Agricultural Research (ICIA) located at Pajalillos (Valle de Guerra, La Laguna, Tenerife; 28°31'38.4" N – 16°23'09.4" W), during seasons 2016-2017 and 2017-2018. The trials were designed in a randomized pattern.

Each treatment consisted in four banana bunches close to harvest stage, selected from var. Gran Enana, hung on a mobile metal wire and placed 2 m one from each other. Forty 2nd instar larvae of *C. chalcites* per banana bunch were used for artificial infestation. After 24 hs, treatments were sprayed on banana bunches using a 2 liters capacity compressed-air hand sprayer (DEA 2000, Italy). The products applied were: Intruder®, BioKnock®, Cinamite®, Avenger®, Ripelser®, Garlitrol-Forte®, Prevam® and Indasol®, all of them including 0.1% (v/v) Agral wetter-sticker. All trials were applied between 8.00 and 11.00 am. A data logger Omega OM-92 was set for registering temperature and humidity inside the screenhouse during the experiment.

2.3.2. Evaluation of the treatments on larvae survival and fruit damage

Survival. Estimation of larval mortality was made by larval counting 1 day before, and 1, 3 and 7 days after treatments, in each bunch.

Efficacy. Efficacy of the products was calculated by using the Henderson-Tilton's formula:

Efficacy % = [1 – (n Co before treatment * n T after treatment/ n Co after treatment * n T before treatment)]*100

n = insect population (number of larvae)

T = treated

Co = control

Damage. One week after application of the products, banana bunches were hook off and hands and fingers were cut and separated. The number of hands, fingers (total and damaged by *C. chalcites* larvae feeding) and larvae by bunch were counted. Hands from each bunch were classified by their *C. chalcites* damage through three EU categories of quality standards, and weighed: Category 1 corresponds to banana hands with no damage, or damage smaller than 1cm^2, which fulfils requirements of European norms; Category 2 has damaged areas larger than 1cm^2 on one finger or smaller than 1cm^2, distributed on different fingers; and Category 3 has damaged areas more than 1cm^2 and in more than one finger. Categories 1 and 2 are marketable fruit, whereas Category 3 is considered as not marketable.

2.3.3. Estimation of economic losses caused by *C. chalcites*

In order to make an estimation of the economic impact of the damage caused by *C. chalcites* on banana fruit, the incomes to be received by the farmer were calculated for each product, taking into account the prices of banana fruit in high prices season and low prices season, and the weight of fruit in the different categories.

When banana fruit prices are high, Category 1 is paid, in average, 0.97 €/kg and Category 2, 0.81€/kg. When prices are low, Category 1 is paid around 0.73 €/kg and Category 2, 0.52€/kg. Category 3 was considered as not marketable, with 0€/kg value.

The cost of the applied products was calculated taking into account a volume of prepared product of 1300 l per ha (application to banana bunch and bracts of the plants) using the recommended dose (as indicated in the label), and with a retail price of the product provided by the local distribution company (Annex 1). It was assumed that 50% of the plants had banana bunches, as emergence of fruit is unevenly synchronized, which corresponds to 900 bunches per ha, as the usual plantation density for banana plants in the Canaries is 1,800 plants per ha.

The avoided economic losses estimation was calculated by gross income in the treatment (the total economic value (€) of fruit in each treatment, as classified in category 1, category 2 or category 3, and taking into account the price of the category in one season) minus cost of the treatment (based on the dose of application and the retail price of the applied product), minus gross income value (€) of the control bunches (calculated as in for the treated bunches). The final value represents the avoided

economic losses for each product. The same procedure was applied for low and high banana prices.

2.4. Data analysis

The eaten leaf area (damage index) from choice and no-choice experiments, cumulative mean mortality percentages in the contact toxicity experiments, damaged banana fingers and categorization of damaged banana fingers were subjected to one-way ANOVA.

Damage index values in choice tests under laboratory conditions were presented (Fig. 4. 1.) as repellence percentages. In non-choice test, the mean percentage of consumed area of the treated leaves (anti-feeding effect) is presented in Fig. 4. 2. In both assays, it was compared the eaten area of leaves treated with product against control leaves.

The analysis of the data of larval mortality for the estimation of contact toxicity of the products was made by Henderson-Tilton formula, and the efficacy values obtained were used in a repeated measured analysis to evaluate the mortality percentage of *C. chalcites* over time (day 1, day 3 and day 7). Keplan-Meier curve was used to determine the fractions of larvae dying in treated units to control units over time. Cox's regression analysis was used to estimate the Hazard ratios (HR). All analysis were done with SPSS (ver. 23). In the events of significant mean differences ($P < .05$), means were separated using Tukey HSD test.

3. Results

3.1. Choice assay (% repellence)

The results of the choice assay (% repellence test) are shown in Fig. 4. 1. The avoided consumption of the treated leafs showed that Prevam® (85.19±1.7 %) significantly differed *(P< 0.05)* from the other products. The second group was constituted by Garlitrol® (68.44±5.7 %), Intruder® (67.54±4.3 %), Indasol® (60.44±5.7 %) and Cinamite® (57.46±3.3 %). Avenger® (50.79±2.9 %), Bioknock® (38.64±3.6 %) and Ripelser® (10.77±7.9 %) showed the lowest repellent effect on the choice assay.

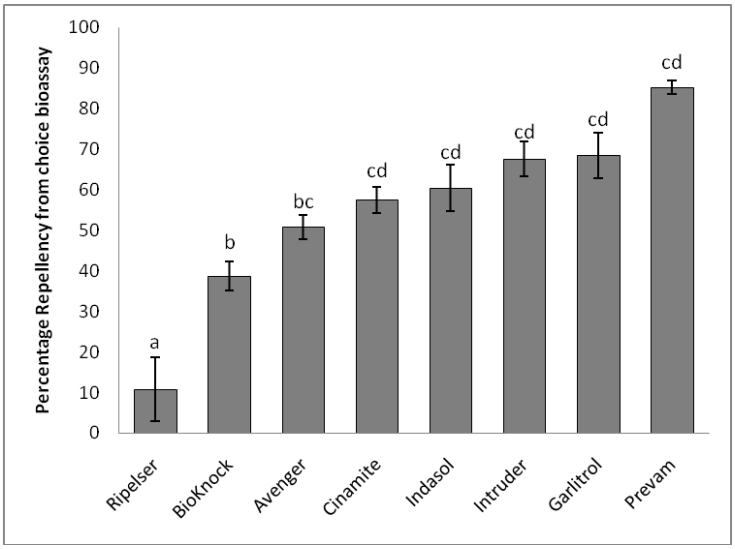


Fig. 4. 1. Repellence test of selected products on the 2nd instar larvae of *C.chalcites* under laboratory conditions (choice assay). Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (*P<0.05*).

3.2. Non-choice assay

The mean percentage of consumed area of the treated leafs in the non-choice assay is shown in Fig. 4. 2. The effect of each product on treated leaves was compared with control leaves. Significant difference *(P< 0.05)* was detected between treatments. Prevam® (0.92±0.4 %) and Indasol® (0.98±0.33 %) had the lowest consumed area 24 h post application. Intruder® (2.7±0.33 %) was the intermediate group. The leaves treated with Avenger® (16±2.55), Bioknock® (16.86±2.43 %), Ripelser® (17.59±2.9 %), Garlitrol® (19±4.5 %) and Cinamite® (27.06±1.67 %) showed a high consumption by the 2nd instars of *C. chalcites* compared with control leaves.

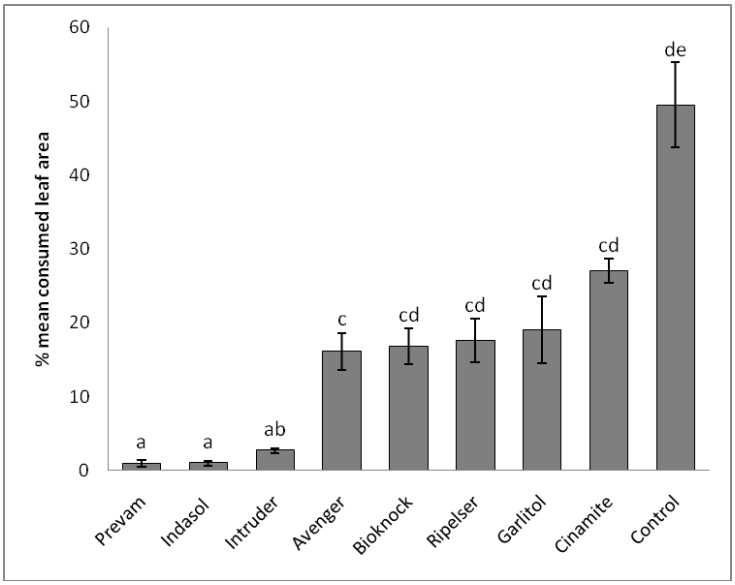


Fig. 4. 2. Mean area (%) of leaves consumed by 2nd instar larvae of *C. chalcites*). Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (*P<0.05*).

3.3. Contact toxicity of products on *C. chalcites* larvae

The contact toxicity of treated leaves is shown in Fig. 4. 3. The cumulative mortality values were evaluated 1 day, 3 days and 7 days post application. At 1 day post application, the highest mortality was detected in Intruder® treated leaves (20.22±2.98 %), followed by Indasol® (18.44±3.8 %) and Bioknock® (10.44±4.7 %). Three days post application, Intruder® had the highest contact toxicity effect (49.11±4.04 %), followed by Indasol® (47.33±6.8%) and Prevam® (26±9.27%). Seven days post application, the highest mortality was detected again on Intruder® treated leaves (77.77±5.7%), followed by Indasol® (76±9.27%) and Prevam® (58±11.13%) treated leaves.

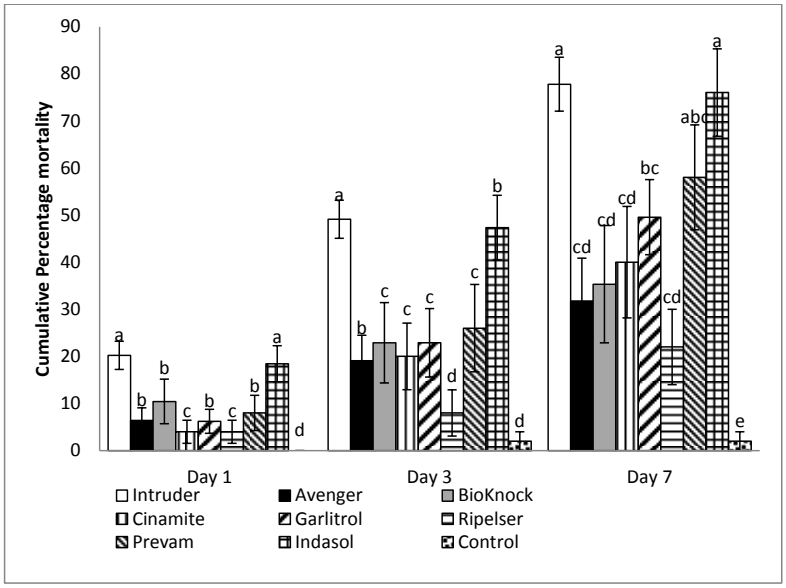
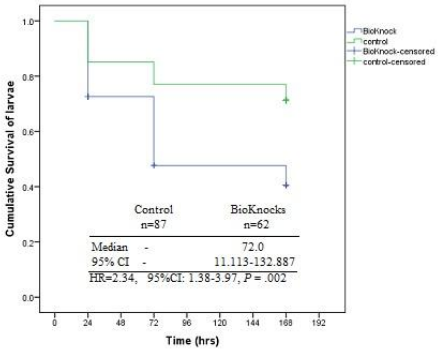


Fig. 4. 3. The contact toxicity of products on 2nd instar larvae of *C. chalcites*). Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (P<0.05).

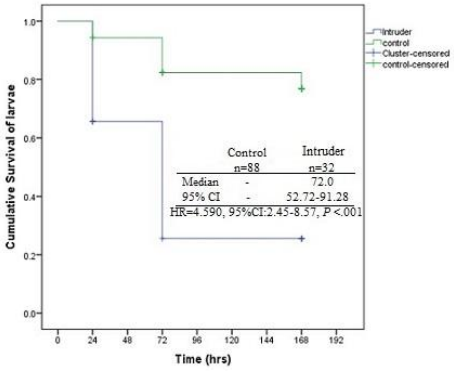
3.4. Screenhouse experiment

The average temperature and humidity of the plot in the period of the assay were 22ºC and 72%, respectively, which are favourable conditions for *C. chalcites* development. The number of alive *C. chalcites* larvae obtained 1 day before the application, and 1 day, 3 days and 7 days after the application of each product is presented in Fig. 4. 4.

a.



b.



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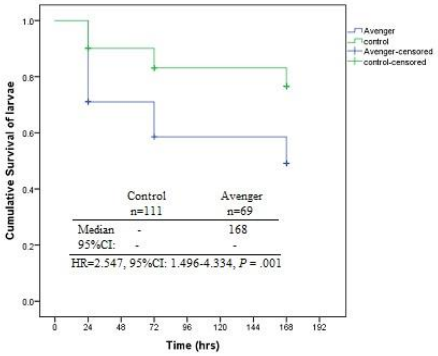

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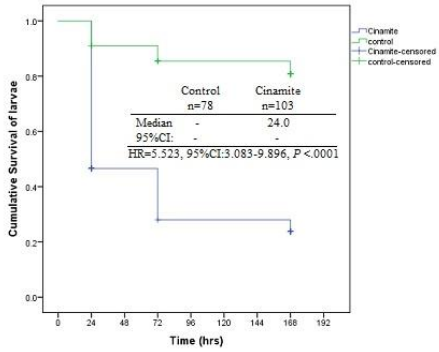

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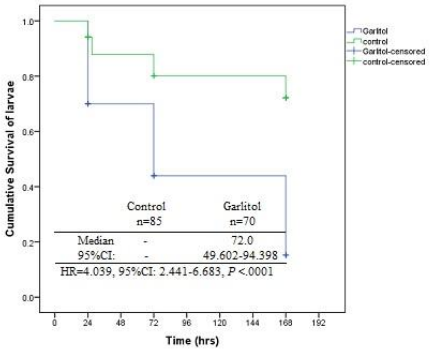
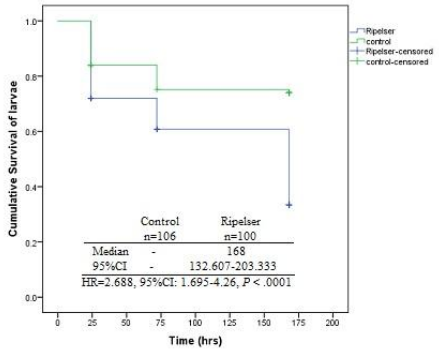


f.



g. h.

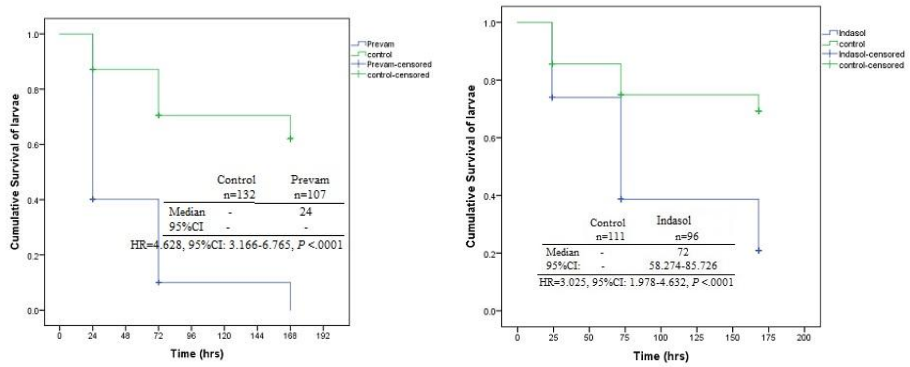


Fig. 4. 4. Survival analysis of *C. chalcites* larvae 1 day before, 1, 3, 7 days after the application of Bioknock® (a), Intruder® (b), Avenger® (c) Cinamite® (d), Garlitrol Forte® (e), Ripelser® (f), Prevam® (g) and Indasol® (Neem oil) (h).

The initial number of larvae decreased both in treated and control plots, but in the treated plots this decrease after application of the products was significantly higher than in the control. In fact, *C. chalcites* scores were significantly reduced *(P< 0.05)* on all treated plots 3 days after spraying. In consequence, the total number of alive larvae after the product application was always lower on the treated plants.

The efficacy of each treatment was calculated using Henderson-Tilton's formula on the results of 1 day, 3 days and 7 days after the application of products. According to Henderson-Tilton formula, at 1 day post application, it is possible to make three groups (most effective, intermediately effective and less effective products). The most effective product was Prevam® (58.92 ± 12.18), followed by Cinamite® (58.63 ± 4.27) and Intruder® (51.18 ± 15.14). In the intermediate group at 1 day were Bioknock® (25.73 ± 8.94), Avenger® (30.80 ± 15.56), Garlitol® (24.39 ± 8.77), Ripelser® (26.50 ± 6.59) and Indasol® (26.55 ± 9.20).

In day 3, the most efficient product was again Prevam® (66.58 ± 10.67), followed by Indasol® (46.21 ± 9.62). The intermediate group of the 3rd day consisted of Bioknock® (27.90 ± 7.78), Intruder® (21.08 ± 15.35), Cinamite® 833.68 ± 10.86) and Garlitol® (34.68 ± 8.61). The less effective products at the 3rd day were Avenger® (11.76 ± 7.8) and Ripelser® (6.10 ± 4.93). Seven days after the application, Prevam® treated bunches showed (100.0 ±0) efficacy on *C. chalcites* 2nd stage instars. Indasol® (42.53 ± 21.47),

Intruder® (36.25 ± 23.75) and Ripelser® (36.74 ± 12.74) were in the intermediate group. Moreover, Bioknock® (11.16 ± 6.47), Avenger® (9.88 ± 5.71) and Cinamite® (15.59 ± 9.03) remained the less effective group at the 7th day post application.

Table 4. 2. Henderson-Tilton efficacy values on banana bunches 1, 3, and 7 days post application[a].

Treatment	Day 1	Day 3	Day 7
Prevam®	58.92 ± 12.18a**B**	66.58 ± 10.67a**B**	100.0 ± 0.0a**A**
Cinamite®	58.63 ± 4.27a**A**	33.68 ± 10.86bcd**A**	15.59 ± 9.03d**B**
Intruder®	51.18 ± 15.14ab**A**	21.08 ± 15.35bcde**A**	36.25 ± 23.75cd**A**
Avenger®	30.80 ± 15.56abc**A**	11.76 ± 7.88de**A**	9.88 ± 5.71d**A**
Indasol®	26.55 ± 9.20bc**A**	46.21 ± 9.62abc**A**	42.53 ± 21.47ab**A**
Ripelser®	26.50 ± 6.59bc**A**	6.10 ± 4.93e**B**	36.74 ± 12.74cd**A**
Bioknock®	25.73 ± 8.94bc**A**	27.90 ± 7.78bcde**A**	11.16 ± 6.47d**A**
Garlitol Forte®	24.39 ± 8.77bc**B**	34.68 ± 8.61bcd**A**	65.78 ± 12.0abc**A**

[a]Lowercase letters shows differences within each day, capital letters resemble the difference between the post application days.

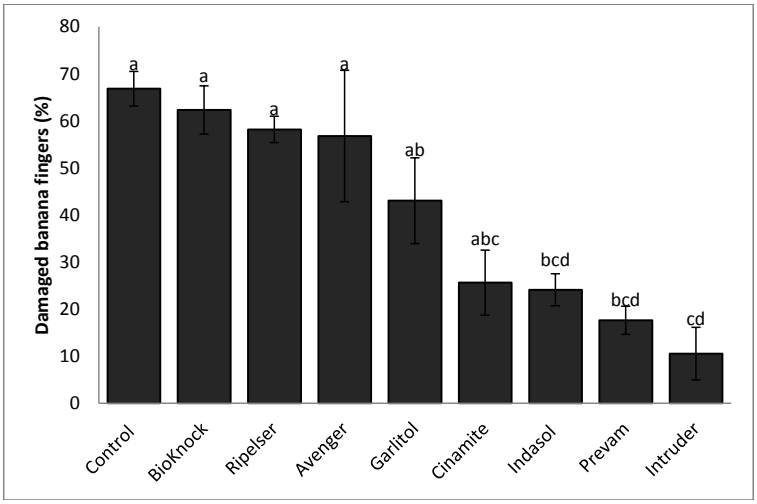


Fig. 4. 5. Percentage of damaged banana fingers per treatment. Different letters above bars indicate significantly difference among groups according to ANOVA followed by Tukey's HSD test (P<0.05).

In brief, all applied products decreased initial populations of *C. chalcites* (number of larvae). However, not all of them had a significant effect on reducing the damage. The percentage of damaged fingers is presented in Fig. 4. 5. According to this results, treatments were assigned in one of three groups: low, intermediate and high damage. Application of Intruder® (10.54 %) and Prevam® (17.63 %) appear to be in the group with lowest damage levels (high protection), while in the intermediate damage group were Indasol® (24.11 %) and Cinamite® (25.61 %). Garlitrol® (43 %) was in the intermediate damage level group as well, but less protective than Cinamite® and Indasol®, whereas in the high damage group (more than 50% damaged fingers, low protection) were Avenger® (56.17 %), Ripelser® (58.17 %) and Bioknock® (62.3 %). It is noteworthy that any phytotoxic effect of the applied products was observed.

Fruit classification by quality categories is presented in Fig. 4. 6. It was found that bunches in treatment Control were highly damaged, being mostly grouped into Category 3 (56.9%), and in Category 2 (33.3%). The highest percentage of fruit in Category 1 was found in treatments with Indasol® (76.81%), followed by Intruder® (73.68%) and Prevam® (71.42%). Fruit in Category 1 was 36.04% with Bioknock®, 25.12% with Cinamite®, 23.67% with Garlitrol®, 18.18% with Avenger®, and 11.11% with Ripelser®.

Fruit in Category 2 was 77.78% for Ripelser®, 64.15% for Cinamite®, 47.15% for Avenger®, 31.40% for Garlitrol®, 26.32% for Intruder®, 24.75% for Prevam®, 16.15% for Indasol®) and 9.17% for Bioknock®. Bioknock® and Garlitrol® appear to be the most damaged treatments with fruit in Category 3 by 54.79% and 44.93%, respectively. In the rest of the treatments, fruit classified into Category 3 was: Avenger® (34.67%), Ripelser® (11.11%), Cinamite® (10.73%), Indasol® (6.97%) and Prevam® (1.18%). Treatment with Intruder® did not have fruit within Category 3.

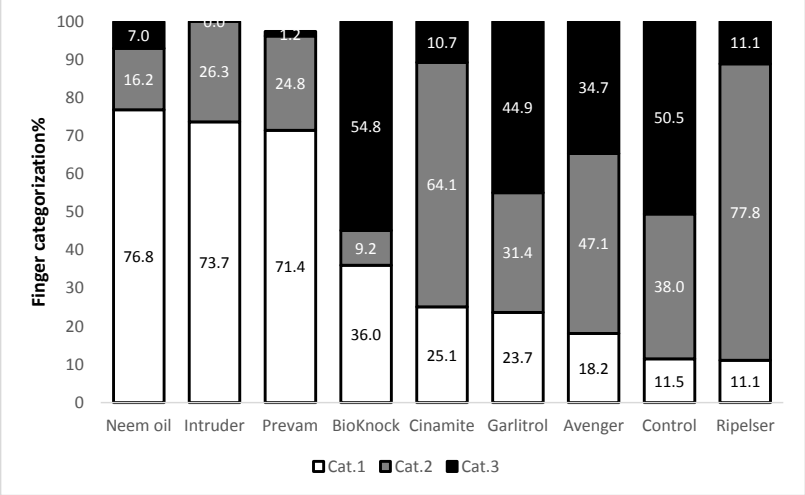


Fig. 4. 6. Damage level categorization in each treatment according to European Standards (EPPO, PP1). Cat. 1: Without damage and/or the damage is <1 cm^2 at 1 finger; fulfilling European norms; Cat. 2: Damage >1cm^2 on 1 finger, or <1 cm^2 on some fingers; Cat. 3: Damage >1cm^2 more than 2 fingers. Mean percentage were separated with Tukey's HSD. Means with the same letter(s) are not significantly different. Capital letter(s) compare means within the bars, small letter(s) compare means across the bars for the different categories.

3.5. Estimation of the economic losses

The results of the estimation of the economic losses respect to the untreated control, assuming 50% of plants with banana bunches, are shown in Table 4. 3. According to this calculation, avoided losses are calculated by the value of gross income of the treatment minus cost of the treatment and the gross income in the control. The highest avoided losses value were found with Intruder® (high price: 12689.6 €/ha, low price: 9055.1 €/ha). Prevam® provided the second highest economic income (high price: 11714.7 €/ha, low price: 9080.7 €/ha), followed by Indasol®, which provided 11287.7 €/ha on high price season and 8953.8 €/ha on low price season.

Table 4. 3. Economic analysis assuming 50% plants with bunch in 1 ha (€)

	Gross income		Cost of the treatment	Avoided losses *	
	High price	Low Price		High price	Low price
Intruder®	23197.4	16117.3	22.8	12689.6	9055.1
Prevam®	22331.2	16251.7	131.5	11714.7	9080.7
Indasol®	21896.8	16117.3	124.1	11287.7	8953.8
Cinamite®	19081.7	12923.8	43.6	8553.1	5840.7
Ripelser®	18444.4	12138.9	67.5	7892.0	5031.9
Avenger®	13956.1	9447.1	14.1	3457.1	2393.6
Garlitrol®	12099.0	8402.2	88.4	1525.6	1274.3
BioKnock®	10596.4	7769.3	40.6	70.7	689.2
Control	10485.0	7039.4	0	0.0	0.0

* Avoided losses = gross income of the treatment - cost of the treatment - gross income in the contro

4. Discussion

These experiments allow a preliminary estimation of the potential of plant extracts based products, the so called biorational insecticides, to be used for the integrated management of *C. chalcites* in banana crops. Taking proactive decisions on IPM is getting more important as the application of synthetic pesticides is harmful to nature. Chemical-based control measures usually involve applications of chlorpyrifos, fenamiphos or indoxacarb, among others (Hernández et al., 2009). Synthetic insecticide treatments require multiple applications which increase production costs, hamper the commercialization of products that can occasionally contain pesticide residues, and have led to the development of resistance in certain populations of this pest (Horowitz et al., 1998). The amount of pesticides that are used in the banana fields can be reduced by increasing our knowledge on the use of biorational insecticides in specific stages and/or parts of the plants. This is especially important not only to protect the environment but also to maintain the efficacy of biological control agents, e.g. *Trichogramma* species that

only parasitizes eggs and is the most effective natural enemy of *C. chalcites* and the only one that is being used commercially at present in the Canary Islands (Del Pino et al., 2013). Also plant extract based products may be useful if alternated with *B. thuringiensis* application in organic banana production system with low populations of *C. chalcites*. Our results show that Intruder®, Prevam® and Indasol® are highly efficient insecticides against 2nd stage larvae of *C. chalcites* both in laboratory and screenhouse experiments, and could be used with low populations of *C. chalcites*. This is in agreement with previous works, when significant reductions in reproductive parameters were observed on adults of *Spodoptera littoralis* (Adel and Sehnal 2000), *S. exempta* Walker (Lepidoptera: Noctuidae) (Tanzubil and McCaffery, 1990), *Plutella xylostella* (L.) (Schmutterer, 1990), and *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) (Musabyimana et al. 2001) treated with azadirachtin. Prerviously, the efficacy of Prevam was also tested on larvae and eggs of *Tuta absoluta* (Meyrick) (Hafsi et al., 2012). Experiments on larvicide and ovicide activity of Prevam® in semi-natural conditions showed a high efficacy on all instars of *Tuta absoluta*. In our assays it was observed that the effectiveness of some of the products decreased over time (Cinamite®, Avenger®, Intruder®, Bioknock®), while in others it increased (Prevam®, Garlitrol Forte®), and in others it went down and then rose (Ripelser®, Indasol®), which seems to indicate different modes of action, which may be studied in the future. *C. chalcites* is cited in the literature as hosts of 35 species of different plants and belonging to 16 different families. In an internal report of FGULL-ASPROCAN (Pérez et al., 2009)

The interactions of *C. chalcites* with banana, tomato, pepper, cabbage, Moorish tobacco, Tomatillo del Diablo, Geranium, Millo and tobacco were evaluated. The most surprising results were that banana is a less prefered food for *C. chalcites,* when we compare it with other host plants. So the attacks observed in banana plantations can be “casual”, either because there are no other host plants nearby or because, due to the large foliar cover of this crop, inevitably adults of *C. chalcites* use the large leaves as a place of oviposition. Corn, cabbage and geranium plants have the possibility of being used as trap plants, since, being hosts for *C. chalcites,* their nutritional qualities are not very adequate. Screenhouse experiments showed that Prevam®, Intruder® and Indasol® reduced number of larvae and damage level on banana fruit, and had around 70% bunches classified into Category 1. In this assay, the application of these products doubled fruit

incomes respect to the control. BioKnock®, Avenger®, Cinamite®, Garlitrol®, and Ripelser® had an intermediate protection against *C. chalcites* larvae on banana bunches after 7 days of application, damage level was high and more than 50% bunches were considered as unmarketable fruit on this treatment. Avenger® is a product that was normally recommended as an enhancer or a secondary product but in this case we evaluate it as separately. It has been tested previously against lepidopteran species such as *Tuta absoluta* which creates a natural barrier on plant surface, and recommended by the producer against attacks of mite and erythide pests, and some species of thrips (White & Blue, 2016). On the other hand, Bioknock® is mentioned as a natural physical-barrier based on botanical extracts of *Fabaceae* and *Rutacea* which is recommended in protecting plants against attacks of psyllids, *Empoasca* sp., and species of aphids and whiteflies (White & Blue, 2016). The avoided economic losses results are parallel to those obtained from laboratory and screenhouse efficacy tests. As a result, a complete analysis of this work (laboratory assays, screenhouse experiment and estimation of avoided economic losses) showed that Prevam®, Intruder® and Indasol® were the three most successful products against 2nd stage larvae of *C. chalcites*. Moreover, any phytotoxic effect of these products was observed. From the results of this study, it can be concluded that the new generation of biorational insecticides would be effective against 2nd stage *C. chalcites* larvae. A second application may increase efficacy and, consequently, also reducing damage in the fruit. Further field experiments are needed for a better understanding of these products: studying damage level in larger scales (more units of study), also in different phenological stages of banana bunches, and in various seasons of the year.

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Universidad de La Laguna

Chapter 5
General Discussion

Chrysodeixis chalcites is a polyphagous insect, native to the Mediterranean basin, which currently became worldwide risk that it will be introduced and established in different environments where it has already been frequently reported in many countries (Murillo et al., 2013).

Importantly, EU has established a strategy on the sustainable use of phytosanitary products (Directive 2009/128 / EC) whose main objective is to adopt a series of measures that mitigate the risk posed by the use of pesticides and enhance the implementation of Integrated Pest Management (GIP); This community strategy has resulted in a National Action Plan (NAP) that is mandatory since January 1, 2014, which establishes, among its general principles, that biological methods or other sustainable non-chemical methods should be preferred to chemical methods, provided they allow satisfactory control of pests.

This study aimed to improve the knowledge on the control strategies of *C. chalcites* in Canary Islands. In this contribution we tried to answer 3 questions related to present issues on improving the control applications of *C. chalcites* based on alphabaculoviruses ChchNPV-TF1 application, where;

In the **Chapter 2,** we discussed "Improving the application through knowing distribution in the plant." In this chapter, we investigated where exactly *C. chalcites* adults lays eggs on banana plants on different growing stage in order to localize eggs on each banana plant. Our data showed that *C. chalcites* EL were more abundant on the underside than on the beam. Previous studies show that in soybean, *C. chalcites* eggs were found also preferably on leaves than in other parts of the plant, but mainly in the underside of the leaf. This information is practically bringing a proactive concept by detecting first the insect preferences on the host plant. In large banana plants the accumulation of the eggs+ L1 larvae (EL) was more central to the plant, in the 8th leaf, on the other hand in young banana plants it was the 5th leaf. It already simplfies the field work of *C. chalcites.* More eggs+L1 larvae were found on young plants. So that it

might be useful to check first small banana plants as the first preference of the adult is the small plant.

In the **Chapter 3**, we discussed "Development of an effective ChchNPV-TF1 formulation, improving its persistence on the leaf surface." In this chapter, we tried to increase and stabilize the effectiveness of virus on the field conditions by including natural additives, which facilitates protection to virus from UV radiation in open field conditions. In this way we tried to maximize the effectiveness of the virus, extending the period of virus infection up to 5-7 days post application. In Tenerife, the mostly used treatments to control *C. chalcites* are indoxacarb and *Bacillus thuringiensis* treatments which are providing a rapid peak in mortality that declined over the 7-day sampling period. The sensitive period is the first three days after application. For a high lethal doses in the first days of the experiment, charcoal or cacao addition protects the ChchNPV-TF1 more, especially at the beginning of the experiment (1-3 days). And that period is critical for virus to acquire a lethal infection. In the field work done with on banana plants in 2017, application of 1×10^8 OBs/ml resulted in 54±10% (20.85 ± 10.42) lethal infection in larvae sampled at one day post-application, on the other hand application of 1×10^9 OBs/ml resulted in 86±7% lethal infection in larvae collected at 1 day post-application to 92±5% lethal infection in larvae sampled at 3 days post-application, which shows a crucial peak at that doses. Higher OB application rates are likely to be necessary to protect fully grown banana plants efficiently, given the high volume applications needed effectively to cover the foliage of large-sized plants. These results indicate a greater persistence of ChchNPV-TF1 OBs on the banana plant with respect to virus alone application. Therefore, addition of 1% charcoal or 1% cacao to ChchNPV-TF1 10^9 OBs/l solution may extend the period of pest control, protecting OBs from UV degradation by producing larval mortality for longer periods. The duration of activity of ChchNPV-TF1 virus protected with these natural substances may increase the performance of virus application even in high UV radiation zones.

In the **Chapter 4**, we tried to find an answer on "The effectiveness of nine bioinsecticides that are available on the market against *C. chalcites,* firstly on laboratory conditions and than under the greenhouse conditions". The idea was to demonstrate the larvae/bioinsecticide interaction by following a series of experiments. First step was to

evaluate these products in the laboratory. We selected three different analyses for this step which are consist of: (i) Choise test, (ii) Non-choise test and (iii) Contact toxcity test which can be found in detail in Chapter 3. After that we continued under greenhouse conditions with banana bunchs, applying these natural products on 40 second instar larvae of *C. chalcites* on bunches. The whole process took 14 days' post application and then banana bunches were cut till fingers and a damage analysis was made. Categories were designed by the quality of the commercial fruit. So that we could compare each product in front of control plants. Results were simply showing that alternating the application of synthetic insecticides by using less harmfull products available on the market, may control *C. chalcites* on banana platations in the Canary Islands. In this way, a sustainable environment will help to protect also *Trichogramma* species that only parasitizes eggs and is the most effective controller of *C. chalcites* and the only one that is being used commercially at present in the Canary Islands (Del Pino et al., 2013). Unfortunatelly, from another point of view, bioinsecticides are still not as much commonly used in the agricultural society, as they used to with synthetic insecticides. On the other hand, the effectiveness of new generation bioinsecticides are so far poorly demonstrated and there is need of more investigation on this demand. In our study we could conclude that some of these bioinsecticides (Intruder®, Indasol® and Prevam®) may be as affective as sythetic insecticides especially in banana plantations.

Results of this study, conclude that the new generation biorational insecticides would be effective against 2nd stage *C. chalcites* larvae. Moreover, any phytotoxic effect of these products was observed. A second application may increase efficacy by reducing the larval activity and, consequently, also reducing damage in the fruit. Further field experiments are needed for a better understanding of these products. In future, it will be necessary to study damage level in larger scales (more units of study), also in different phenological periods of banana plants, and in various seasons of the year.

Combining all our data, it could be adressed firstly the 5th leaf on the young plant and the 8th leaf on the plant with bunch appearance and without bunch appearance to detect *C. chalcites* in banana plantations. Taking into account that adults have the tendency to lay eggs underside of the leaves more than beam part of the leave. This may facilitate

much time and enery saving and minimize the volume of applied product, by targeting the insect on the exact point of the plant.

This opens a specific possibility for alternating the control of *C. chalcites*, in which then we may introduce virus application with one of the selected additives in our case 1 % cacao or 1 % charcoal. In our study 1% charcoal resulted the most protective additive in both experiments. In this way these additives may extend the period of efficacy of the virus on the field applications up to 5 days' post application. This extended period may give the possibility of the adaptation to the virus with other less harmfull phytosanitary products. Afterwards, depending on the treshold and damage level, a second or third applications would control *C. chalcites* without using any synthetic insecticides.

It can be concluded that the formulation of the virus might get much better with more protective additives which needs to be studied in the future. The most limiting factor for baculoviruses efficacy in the open air conditios is the UV radiation. However, the UV radiation is less intense into banana greenhouses. So that it can be suggested to continue more focused on the greenhouse plantations rather than open field plantations. Natural additives together with the application of baculoviruses might be more effective when they applied under greenhouse conditions then on the open air plantations.

Summarizing, this work has provided knowledge on (i) *C. chalcites* oviposition distribution in the plant, which leads to a more easy and precise monitoring of the insect presence in the field, and enhances effectiveness of field applications of natural or synthetic insecticides, minimizing the amount of phytosanitaries applied, (ii) the effectiveness of natural substances as UV protectants for baculovirus formulations, to improve their efficacy, finding that cacao (1 %) and charcoal (1%) are two promising additives that may help, especially in open field conditions, (iii) the efficacy of bioinsecticides against *C. chalcites* larvae under greenhouse condions, finding that some of them are highly effective. The use of these biorational products, and the formulated baculovirus within an Integrated Pest Management programme may help not only to decrease the insect damage in banana fruit, but also the issues related to the intensive use of synthetic insecticides on the control of *C. chalcites* in the Canary Islands. In future

studies, it would be interesting to evaluate the compatibity between virus and bioinsecticides, both under greenhouse and in open field conditions.

Conclusiones

Los resultados que se han presentado a lo largo de esta tesis permiten extraer las siguientes conclusiones:

1. Cuando se contaron el número de huevos y larvas de primer estadio de *C. chalcites* en hojas de platanera con diferente estado fenológico, se encontraron diferencias significativas entre los tres estados evaluados, de forma que *C. chalcites* pone más huevos en plantas jovenes (YP) seguidas de las plantas maduras con racimos (PB) y por último plantas maduras sin racimo (PN).

2. Independientemente del estado fenologico de la planta de platanera, *C. chalcites* prefiere la oviposición en el envés de las hojas al haz, aunque no muestra preferencias por una zona concreta de la misma.

3. Para detectar *C. chalcites* en fincas de plátano, se sugiere buscar los huevos en la quinta hoja de las plantas jóvenes; cuando todas las plantas son maduras, tengan o no racimmo, se recomienda muestrear en la octava hoja. En todos los casos, primero se debe revisar el envés de las hojas.

4. Para el desarrollo de una formulación efectiva del ChchNPV-TF1, que mejore su persistencia en la superficie de la hoja de platanera en campo, se probaron como sustancias fotoprotectoras el carbón vegetal (charcoal) al 1% y el cacao al 1% previamente seleccionados de entre diez sustancias en un ensayo en laboratorio. La adición de un 1% de cualquiera de los dos fotoprotectores naturales a la solución de ChchNPV-TF1 10^9 OBs / l permitió extender el período de control de *C. chalcites* en campo, protegiendo a los OBs de la degradación UV sin afectar la fotosíntesis de la planta. El carbón vegetal resultó más eficaz en la protección del virus.

5. Por primera vez se han valorado nueve sustancias naturales en laboratorio y campo como posibles formulados bioracionales en el control de *C. chalcites* en

platanera. En estos ensayos Prevam®, Intruder® e Indasol® fueron los formulados más eficaces para el control de las larvas jóvenes de *C. chalcites*, redujeron el daño en la epidermis del plátano, y darían el mayor retorno económico.

Conclusions

The results that have been presented throughout this Thesis allowed us to draw the following conclusions:

1. When the number of eggs and larvae of first stage of *C. chalcites* in banana leaves with different phenological stages were counted, significant differences were found between the three evaluated stages, so that *C. chalcites* lay more eggs in young plants (YP) followed by mature plants with bunches (PB) and finally mature plants without bunches (PN).
2. Regardless of the phenological state of the banana plant, *C. chalcites* prefers oviposition on the underside of the leaves to the beam, although it does not show preferences for a specific area of them.
3. To detect *C. chalcites* in banana, it is suggested to look for eggs in the fifth leaf of young plants; When all plants are mature, whether or not they have clusters, it is recommended to sample on the eighth leaf. In all cases, underside of leaves should be monitored.
4. For the development of an effective formulation of ChchNPV-TF1, which improves its persistence on the surface of the banana leaf in the field, 1% charcoal and 1% cocoa were tested as photoprotective substances, previously selected between ten substances in a laboratory test. The addition of 1% of any of the two natural photoprotectors to the ChchNPV-TF1 10^9 OBs / l solution allowed extending the control period of *C. chalcites* in the field, protecting the OBs from UV degradation without affecting the photosynthesis of plant. Charcoal was the most effective in protecting the virus.
5. For the first time nine natural substances have been evaluated as possible biorational formulations in the control of *C. chalcites* in banana under laboratory and field conditions. In these tests Prevam®, Intruder® and Indasol® were the most effective products against *C. chalcites* young larvae, reducing damage in banana fruit skin and providing higher revenues.

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Cakmak T., Piedra-Buena Diaz, A., Hernández Suàrez E. (2017). Efficacy of biorational insecticides to prevent damage of *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) on banana fruit. *SEEA Congress; Congreso Nacional de Entomología Aplicada - XVII.* Poster Presentation.

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