Chrysodeixis chalcites nucleopolyhedrovirus: a useful component for IPM programs on the Canary Islands banana crops

ERNESTO G. FUENTES BARRERA
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Programa de Doctorado en Biotecnología
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TESIS DOCTORAL

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ERNESTO GABRIEL FUENTES BARRERA

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Chrysodeixis chalcites nucleopolyhedrovirus: a useful component for IPM programs in the Canary Islands banana crops

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INFORMAN:

que la presente memoria de Tesis Doctoral titulada "**Chrysodeixis chalcites nucleopolyhedrovirus: a useful component for IPM programs in the Canary Islands banana crops**" elaborada por D. **ERNESTO GABRIEL FUENTES BARRERA** 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 Pamplona a 1 de junio de 2017,

Fdo. Dr. Primitivo Caballero Murillo

Fdo. Dra. Oihane Simón de Goñi

Fdo. Dra. Estrella Hernández Suárez

A mis abuelos

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RESUMEN

La platanera (*Musa acuminata* Colla) es uno de los principales cultivos de las Islas Canarias, representando el 30% de la producción agrícola total del archipiealago. Dicho cultivo se realiza bajo invernaderos de malla principalmente en las vertientes cálidas del sur de las islas, y al aire libre en las frías vertientes del norte. Por ello, los cultivos bajo malla presentan mayores poblemas fitosanitarios. *Chrysodeixis chalcites* (Esper, 1789) (Lepidoptera: Noctuidae) ha sufrido cambios importantes en su hábitos alimenticios. Actualmente se alimenta principalmente de los frutos de platanera, produciendo daños en la epidermis que reducen claramente su valor comercial. El control de dicha plaga implica el uso repetido de un reducido número de materias activas, lo que favorece la aparición de resistencia, y por lo tanto disminuyendo su efectividad. Además, desde el año 2014 la gestión integrada de plagas (GIP) es de obligado cumplimiento en los sistemas de cultivo españoles. El objetivo de esta tesis ha sido abordar varios algunos aspectos importantes para la implementación de un programa de GIP efectivo en los cultivos de platanera de las Islas Canarias, España.

La toma de decisiones efectiva en GIP se basa en la comprensión de las relaciones que se producen entre el número de individuos plaga, la respuesta de las plantas a los daños producidos por dichos individuos y las pérdidas económicas resultantes. Por ello, en la presente tesis se estimó en primer lugar la incidencia y el daño alimenticio producido por *C. chalcites*, así como las pérdidas de producción debidas al daño directo a la fruta y al coste indirecto derivado de la compra y aplicación de insecticidas. La prevalencia de infestaciones varió entre el 42 y 100% y fue similar en los dos años de prospecciones. El daño foliar medio (1.5-7.3%) y el daño en fruta (1.0-5.7%) varió significativamente en las islas según el tipo de plantación (vertientes orientadas al norte o al sur) y estación. En general, los daños resultaron similares entre los dos tipos de plataciones, , excepto en Gran Canaria y Tenerife, donde se registaron más daños foliares y en fruto, respectivamente, en la vertiente sur. Los daños también fueron similares entre las estaciones, lo que indica que *C. chalcites* está presente en el cultivo durante todo el año. El peso de plátanos dañados respecto a la fruta cultivada varió significativamente entre las islas, desde el 0,2% en Tenerife hasta el 4,2% en El Hierro, siendo este daño especialmente

importante en primavera. Dicho periodo es el más susceptible para determinar la cantidad y calidad de la fruta cosechada, ya que coincide con el desarrollo del fruto tras la floración. En total se perdieron unas 3.155 toneladas de platano/año, lo que representa el 1,5% de la producción anual (2,68 millones de euros/año). Además, los costes de control con indoxacarb, el insecticida más utilizado (73%), supondrían unos 240€/ha por ciclo de cultivo. Dado que su uso continuado probablemente favorezca el desarrollo de resistencia, se necesitan nuevos insecticidas para rotar con los pocos productos autorizados. Entre ellos el nucleopoliedrovirus de C. chalcites, ChchNPV (Género *Alphabaculovirus, Baculoviridae*) resulta ser un insecticida seguro, eficiente y sostenible.

El siguiente objetivo fue evaluar el uso potencial de ChchNPV como un nuevo agente de control biológico en GIP. Para ello, primero se estimó la prevalencia y diversidad genética de las variantes de ChchNPV presentes en las poblaciones naturales de *C. chalcites* en Canarias y después se evaluó la eficacia insecticida del aislado más prevalente en comparación con dos insecticidas usados frecuentemente, indoxacarb y *Bacillus thuringiensis* (Bt), a pequeña escala en plantas jóvenes de platanera en condiciones de invernadero y aire libre. En general, la prevalencia de infección por ChchNPV fue del 2,3%, siendo de 0-13,8% en El Hierro, 0-5,6% en La Palma, 0-4,8% en Tenerife, 0-1% en La Gomera y 0% en Gran Canaria. En plantaciones bajo invernadero, la prevalencia de infección fue el doble (3%) que al aire libre (1,4%). ChchNPV-TF1 fue la variante más abundante (82%) y extendida, por lo que se seleccionó para los ensayos de campo. La aplicación 10^9 cuerpos de oclusión (OBs)/l de ChchNPV-TF1 redujo significativamente la densidad larvaria y el daño foliar en plantas jóvenes de platanera, al igual que los productos convencionales indoxacarb y Bt. Sin embargo, la mayor mortalidad producida por ChchNPV-TF1 en las larvas recolectadas a lo largo del tiempo sugiere que adquisición de una infección letal ocurre durante un periodo de tiempo más extendido, en comparación con el breve periodo que manifiestan las larvas tratadas con los insecticidas convencionales. Estos resultados indican una mayor persistencia de ChchNPV-TF1 en la planta de platanera.

El último objetivo de este trabajo abordó la evaluación de la eficacia de ChchNPV-TF1 para proteger los frutos de platanera de los daños producidos por *C. chalcites* en invernaderos de malla en plantaciones comerciales, durante un ciclo de cultivo completo en comparación con el tratameitno convencional. En los ensayos de 2014, el daño foliar fue similar entre las dos estrategias de control en las tres islas, mientras que el daño en fruto en las parcelas tratadas con ChchNPV-TF1 fue sorprendentemente mayor en Tenerife, pero similar en Gran Canaria y La Palma. En Tenerife y Gran Canaria la mortalidad larvaria fue mayor en la parcela tratada con ChchNPV-TF1, mientras en La Palma la baja incidencia no permitió determinar la mortalidad larvaria. En un segundo ensayo en Tenerife en 2015 los tratamientos con virus se aplicaron varios meses antes del dasarrollo del fruto, y esta vez ChchNPV-TF1 proporcionó un control efectivo, similar al proporcionado por los insecticidas convencionales.

En conclusión, este nuevo agente de control biológico resulta útil para ser incluido en la gestión integrada de *C. chalcites* en cultivos de platanera de Canarias. Los resultados de esta tesis forman parte de un trabajo más amplio que tiene como objetivo definir los umbrales de daño de *C. chalcites* en cultivos de platanera y el uso potencial de este nuevo insecticida biológico.

SUMMARY

Banana (*Musa acuminata* Colla) is one of the main crops on the Canary Islands, representing 30% of the total agricultural production of the archipelago. Bananas are cultivated in mesh-built greenhouses on the warmer southern slopes and in the open-field on the cooler northern areas of the islands. As such, crops grown under mesh tend to be more prone to phytosanitary problems. *Chrysodeixis chalcites* (Esper, 1789) (Lepidoptera: Noctuidae) has shown marked changes in its feeding habits. Currently larvae feed mainly on banana fruits, causing injuries to the epidermis that greatly reduce their market value. Control of this pest mainly involves the repeatedly use of a low number of active compounds, favouring the appearance of resistance, and hence reducing their effectiveness. In addition, since 2014 integrated pest management (IPM) is mandatory in crops grown in Spain. The aim of the present thesis has been to address several aspects important for the implementation of an effective IPM program in banana crops in Canary Islands, Spain.

Effective decision-making in IPM relies on understanding the relationships between pest numbers, plant responses to injury and the resultant economic losses. Therefore, in the present thesis firstly the incidence and feeding damage inflicted by *C. chalcites* was estimated as well as the production losses due to direct damage to fruit and indirect costs involved in the purchase and application of insecticides. The prevalence of infestations, which varied from 42 to 100%, was similar during the two years of prospections. Mean foliar damage (1.5-7.3%) and fruit damage (1.0-5.7%) varied significantly across the islands, plantation location (northern- or southern-facing slopes) and season. In general, damages were similar in both plantation locations except in Gran Canaria and Tenerife, where more foliar and fruit damage were registered in southern-facing slopes, respectively. Damages were also similar across the seasons, which means that *C. chalcites* is present throughout the year in this crop. The weight of damaged bananas varied significantly across the islands, from 0.2% of harvested fruit in Tenerife to 4.2% in El Hierro, being Spring damage especially important, which is the most susceptible period for determining the quantity and quality of the harvested fruit as it coincides with the fruit development after flowering. Overall 3,155 tonnes of bananas are likely to be discarded annually,

representing 1.5% of the annual production (2.68 million €/yr). In addition, control costs with indoxacarb, the most used insecticide (73%), average 240 €/ha per crop cycle. Given that continuous use of a single dominant insecticide is likely to lead to the development of resistance, novel insecticides are required for use in rotation with the handful of authorized compounds. Among these the nucleopolyhedrovirus of *C. chalcites*, ChchNPV, (Genus Alphabaculovirus, Baculoviridae) is a secure, efficient and sustainable insecticide.

The next objective was to evaluate the potential use of ChchNPV as a novel biological control agent in IPM. Therefore, firstly the prevalence and genetic diversity of ChchNPV variants present in *C. chalcites* populations on the Canary Islands were estimated, followed by an evaluation of the insecticidal efficacy of the most prevalent isolate in comparison with two frequently used insecticides, indoxacarb and *Bacillus thuringiensis* (Bt) on young banana plants grown in small-scale greenhouse and open-field conditions. Overall the prevalence of ChchNPV infection was 2.3%, but varied from 0-4.8% on Tenerife, 0-5.6% on La Palma, 0-13.8% on El Hierro, 0-1% on La Gomera and 0% on Gran Canaria. The prevalence of infection was twice higher under greenhouse structures (3%) than open-field plantations (1.4%). ChchNPV-TF1 was the most abundant (82%) and widespread variant, which prompted us to use it for field trials. Application of 10^9 viral occlusion bodies (OBs)/l of ChchNPV-TF1 significantly reduced *C. chalcites* prevalence and foliar damage in young banana plants as did the commonly used indoxacarb and Bt. However, the increased mortality produced by ChchNPV-TF1 over time suggested that the acquisition of a lethal dose occurred over an extender period compared to a brief peak in larvae on plants treated with conventional insecticides. These results indicate a greater persistence of ChchNPV-TF1 OBs on the banana plant.

The last objective relies on determining the efficacy of ChchNPV-TF1 in protecting banana fruits from *C. chalcites* damage in commercial banana mesh-built greenhouses during a complete crop cycle in comparison with conventional treatment. In 2014 trials, foliar damage was similar in both control strategies in the three islands, while fruit feeding damage in ChchNPV-TF1 treatment plot was surprisingly higher in Tenerife, but similar in Gran Canaria and La Palma. In Tenerife and Gran Canaria larval mortality was higher in ChchNPV-TF1 treatment plots than in conventional treatments, while in La Palma the low pest incidence did not enable us to measure larval mortality. In a second trial in Tenerife in 2015, treatments were applied several months before fruit development and this time ChchNPV-TF1 provided an effective control, similar to that provided by conventional insecticides.

In conclusion, this novel biological control agent proves useful for its inclusion in integrated management of *C. chalcites* in banana crops on the Canary Islands. The results of the present study are part of a wider approach to define *C. chalcites* damage thresholds in banana crops and the potential use of this novel biological insecticide.

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CHAPTER I

Introduction

Content

1. GENERAL INTRODUCTION

Banana (*Musa acuminata* Colla) is one of the most important crops on the Canary Islands, representing 30% of total agricultural production of the archipelago (Cáceres-Hernández et al., 2013; González-Concepción et al., 2008). Banana crops present different phytosanitary problems that include a variety of pests and diseases. One of the main pests is the tomato lopper, *Chrysodeixis chalcites* (Esper, 1789) (Lepidoptera: Noctuidae) (Del Pino et al., 2011; Domínguez et al., 2012). Currently, *C. chalcites* control in banana crops is mainly based on the use of chemical insecticides. However, the need to apply several treatments each year and the reduced number of active substances allowed by the European Union in this crop (Méndez-Hernández et al., 2017) will likely lead to the selection of biotypes of *C. chalcites* that are resistant. The selection of resistant populations of this insect will generate other important problems related to the sustainability of this crop and the international commercialization of bananas, as well as side effects for public health and the conservation of the environment. Moreover, most chemical insecticides are incompatible with the use of natural enemies (Cloyd, 2012; Stanley et al., 2009; Williams et al., 2003).

To avoid or alleviate these problems associated with chemical control, since 2014 an integrated pest management (IPM) approach is mandatory in all crop production systems in Spain (RD 1311/2012, which incorporates European Directive 2009/128/EEC). This directive promotes the implementation of IPM programs as the most favorable scenario for the sustainable use of chemical pesticides. IPM advocates making use of all available control methods combined in the most compatible and effective way possible, while establishing a clear priority of the most sustainable methods versus the least sustainable (Pedigo, 1996; Shepard et al., 2009). Microbial insecticides can contribute greatly to the implementation of IPM programs because of their high compatibility with other control agents (predators and parasitoids) and chemical insecticides. The nucleopolyhedrovirus of *Chrysodeixis chalcites* (ChchNPV) (*Alphabaculovirus*: *Baculoviridae*) has been found to be an effective biological control agent against this pest (Bernal et al., 2013; Simón et al., 2015). Several isolates have been characterized (Bernal et al., 2013; Murillo et al., 2000; van Oers et al., 2004), but the indigenous ChchNPV-TF1 isolate was the most pathogenic and fastest killing under laboratory conditions (Bernal et al., 2013), and presented a similar activity to conventional insecticides under greenhouse conditions with young banana plants (Simón et al., 2015). However, for the development and use of ChchNPV-TF1 as biological control agent in Canary Islands' banana crops, and its integration in IPM programs, it is necessary to evaluate several aspects related to the pest and to test the efficacy of the virus under the conditions of commercial production of this crop.

Therefore, the aim of this thesis was to evaluate aspects of the development of integrated *C. chalcites* management in banana crops in the Canary Islands involving the virus as an insecticide. For this purpose, firstly the incidence of *C. chalcites* on banana plants was estimated as well as the damage and losses caused as a consequence of larval feeding on leaves and fruit. In addition, indirect costs, derived from the purchase and application of insecticides, were also measured. Secondly, the efficacy of ChchNPV in controlling the damage produced by this pest was measured. For this, the natural incidence of ChchNPV in *C. chalcites* populations on Canary Islands banana crops was determined and the effectiveness of most abundant variant, ChchNPV-TF1, controlling *C. chalcites* damage was evaluated in young banana plants under greenhouse and open-field conditions in comparison with conventional insecticides. Finally, ChchNPV-TF1 efficacy was measured under the commercial crop production conditions in plantations on the archipelago. The results obtained in the present thesis will be of special interest as the basis for establishing *C. chalcites* damage thresholds, as well as for using the virus insecticide as a key component for implementing IPM programs in banana crops.

2. BANANA CROPS ON THE CANARY ISLANDS

Banana variety Cavendish is the main crop on the Canary Islands, which represents 30% of final agricultural production (Cáceres-Hernández et al., 2013; González-Concepción et al., 2008). In 2015, the cultivated area reached ~8,975 hectares (23% of the total cultivated area), mainly on the islands of Tenerife (4,095 ha), La Palma (3,033 ha) and Gran Canaria (1,766 ha). The average annual production of bananas is approximately 382,000 tonnes, which represents 0.3% of world production (MAPAMA, 2016; FAOSTAT, 2016). This production is distributed across the islands of Tenerife (45%), La Palma (33%) and Gran Canaria (20%), whereas the production is much smaller in the islands of La Gomera and El Hierro (2%) and in Lanzarote it is minimal (ASPROCAN, 2016). Most of the banana production goes to the national market in Spain (91%), just under one-tenth is destined for the domestic market of the Canary Islands and only a small part (0.1%) is sold in international markets (ASPROCAN, 2016). Banana production is of great social importance in the Canary Islands, as it generates over 7,000 jobs, which represents up to 10% of jobs on some islands. In addition, many other sectors provide services, or depend on banana production, such as irrigation services, transportation, cardboard packaging, pallets for transportation, importation and sales of machinery, fertilizers, pesticides, plastics and fuel, or even tourism sectors which adds 130 million euros/year to the island's economy (Cáceres-Hernández et al., 2013; González-Concepción et al., 2008).

Bananas are cultivated in the Canary Islands in two different environmental conditions, in open-field (65%) (Fig. 1A), more common on the cooler northern-facing slopes of the islands, or in mesh-built greenhouses (35%) (Fig. 1B), in the warmer southern-facing slopes of the islands (Galán-Saúco, 1992; Robinson and Galán-Saúco, 2010).

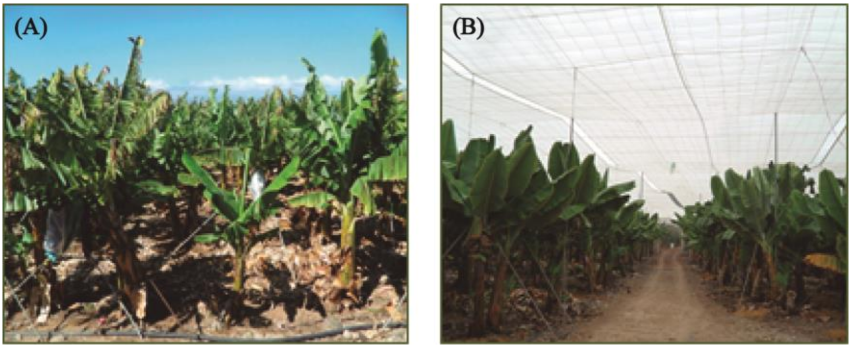

Figure 1. Banana crops on the Canary Islands; (A) open-field conditions and (B) mesh-built greenhouses.

The production cycle of bananas in greenhouses lasts about 15 months, it begins with the planting of young banana plants produced *in vitro* during the summer months of July-September (Fig. 2A-B), continues with the production of the banana inflorescence and fruiting between April and July (Fig. 2C) and ends with bunch harvesting between October to February (Fig. 2D) (Cabrera and Galán-Saúco, 2005; Galán-Saúco et al., 1984).

Banana crops on the Canary Islands are affected by numerous pests, among which the following are notable for their incidence and damage: red spider mite *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae), spiral whiteflies *Aleurodicus dispersus* Russell, 1965 and *Aleurodicus floccissimus* Martin et al., 1997 (Hemiptera: Aleyrodidae), mealybug *Dysmicoccus grassii* Leonardi, 1913 (Hemiptera: Pseudococcidae), banana weevil *Cosmopolites sordidus* Germar, 1984 (Coleoptera: Dryophthoridae), and tomato lopper *Chrysodeixis chalcites* Esper, 1789 (Lepidoptera: Noctuidae) (Perera and Molina, 2002).

Figure 2. Phenological stages of banana crop: (A) banana new planting; (B) young banana plants; (C) production of banana bunch; (D) adult plant with banana bunch.

Over the past few decades *C. chalcites* has become an important pest of banana crops, as this pest has changed its feeding habits. Formerly, *C. chalcites* produced feeding damage to leaves of young plants, but currently this pest feeds directly on fruit which completely eliminates the commercial value of damaged fruits (Del Pino et al., 2011; Dominguez et al., 2012). Nevertheless, the losses caused by the direct damage on the fruits have not been the object of previous studies, in order to estimate the value of these losses.

3. *Chrysodeixis chalcites*

3.1. Taxonomy and classification

Chrysodeixis chalcites is an insect belonging to the order Lepidoptera, Noctuidae family and Plusiinae subfamily. The generic and specific name of this lepidopteran has undergone numerous changes that have generated a long list of synonyms. The first description was made in 1789 by Esper who assigned the scientific name of *Phalaena chalcites*. Afterwards, this insect was renamed as *Phalaena chalsytis* Hübner, 1790, *Noctua bengalensis* Rossi, 1794, *Noctua quaestionis* Fabricius, 1794, *Plusia verticillata* Guenée, 1852, *Plusia integra* Walker, 1858, *Plusia adjuncta* Walker, 1865 and *Plusia buchholzi* Plötz, 1880.

C. chalcites is known by several common names in English, including "golden twin spot" (Zhang, 1994), "tomato looper" (Harakly and Farag, 1975) or "green garden looper" (Zimmerman, 1958). In Spain, *C. chalcites* is known as "oruga medidora" (De Liñan, 1998), and in the Canary Islands as "lagarta" (Lorenzo, 2005) or "bicho camello" (Perera and Molina, 2002).

3.2. Morphology

C. chalcites is a holometabolous insect and therefore its biological cycle comprises four distinct stages of development: adult, egg, larva and pupa (Fig. 3).

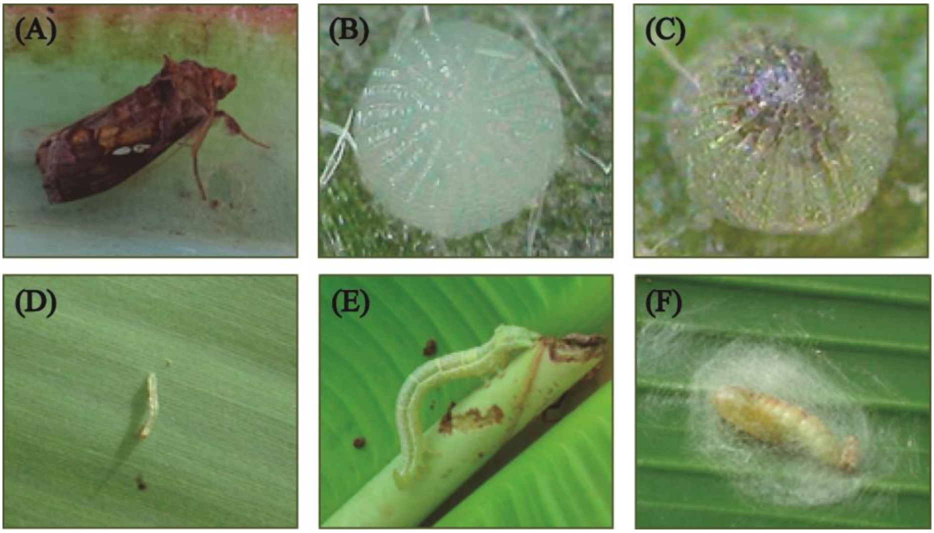

Figure 3. Different stages of development through which *C. chalcites* passes to complete its biological cycle: (A) adult; (B) young egg; (C) mature egg; (D) early instar larva; (E) sixth instar larva; and (F) pupa.

The adult is a moth with wingspan of approximately 4 cm and a brown coloration with purple spots (Fig. 3A). Head is ochre with filiform brown antennae. Ochre patches dorsally cover thorax and forewings have earthy-brown color with two silver drop-shaped spots, while hindwings are paler. Abdomen is pale-brown dorsally and lighter ventrally (Cayrol, 1972; Bretherton, 1983). Male and female adults are similar, differing in that the male has two yellowish hair plumes laterally at the end of the abdomen (Goodey, 1991).

The female adult lays eggs isolated or in small groups on the plant surface (Cayrol, 1972). *C. chalcites* eggs are translucent, whitish-greenish, dome-shaped and striated with 28 to 32 longitudinal stretch marks from the micropyle to the base (Fig. 3B), with a width of 0.8 mm and 0.6 mm high (Bretherton, 1983; Goodey, 1991), and shortly before hatching they darken (Fig. 3C) (Harakly and Farag, 1975).

C. chalcites larvae are eruciforms (Fig. 1D-E) and can go through six larval instars followed by a pre-pupal instar (Amate et al., 1998a; Goodey, 1991). The first three instars are less than 1 cm (Fig. 3D), while the last instar reaches a size of 3.8 cm. Mature larvae are, pale yellow-green with six white lateral lines (Fig. 3E). Below the head, the thorax is composed by three segments, each one with a pair of legs composed by five segments. Above the black spiracles, on each side of the body, there is a thin dark green or black line stretching from the head to the seventh abdominal segment; below there is a thicker white line that starts from the head, passing through each spiracle until the tip of the anal extension. The ventral region is speckled with white dots (Bretherton, 1983; Passoa, 1995). Larvae have only three pairs of prolegs, instead of the usual five, resulting in the looping gait, giving rise to some of the common names such as semi-looper or tomato looper.

The change from larva to pupa usually occurs on the leaf underside or along the central vein. The pupa has a fusiform aspect, and is wrapped in a white silk cocoon made by the larva when it reaches its maximum stage of development (Fig. 3F). The pupa has a length between 2 to 2.5 cm and a width between 0.4 and 0.45 cm; it is pale green color at the beginning, becoming a hazel or dark brown at the end of development (Bretherton, 1983; Goodey, 1991; Harakly and Farag, 1975).

3.3. Geographical distribution and host plants

C. chalcites is distributed mainly between 45º N and 35º S, in the area that ranges from southern Europe, including the Mediterranean and Middle East, to southern Africa (CABI, 2014) (Fig. 4).

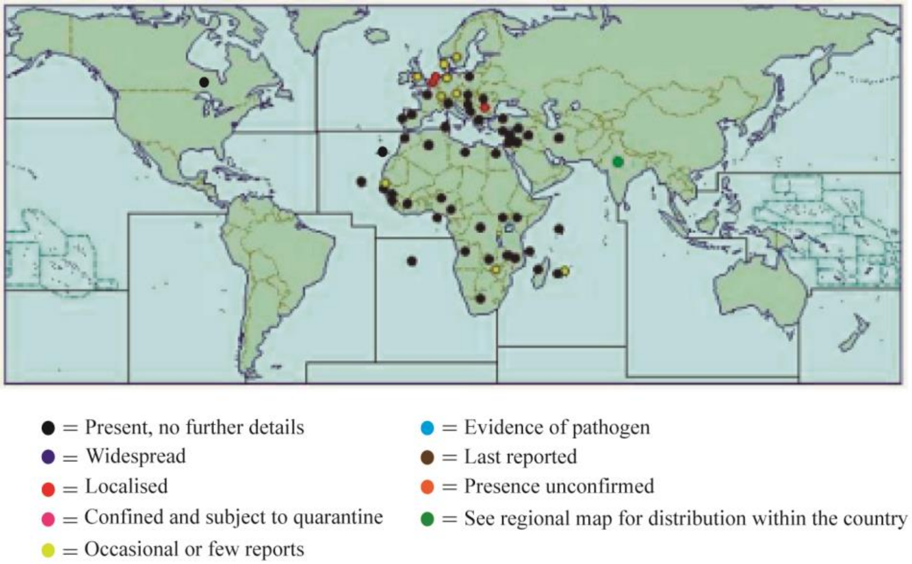

Figure 4. Geographical distribution of *C. chalcites* (CABI, 2014).

This species is a highly polyphagous insect that can be found feeding on numerous fruit, horticultural, ornamental and forest crops of different botanical families, such as: cotton, alfalfa, cabbage, sunflower, geraniums, beans, corn, turnips, potatoes, cucumbers, peppers, bananas, soybeans, tobacco and tomato (Cabello et al., 1996; Passoa, 1995). *C. chalcites* can be a pest in both open-field and protected crops, causing damage during the flowering, formation of the fruit, seeding and vegetative stages of the plant. Damage can be located on fruits, leaves or the whole plant and can also feed on numerous weeds or spontaneous plants (CABI, 2007).

In Africa, *C. chalcites* is usually present, being a quarantine pest in South Africa (EPPO, 2013), while in Egypt it is present on field fruit and vegetables (Anon, 1984). In Asia, this species is a major pest of tomato, lucerne, alfalfa and clover in Israel (Avidov and Harpaz 1969; Broza and Sneh, 1994). In North America, since 2008 specimens of *C. chalcites* have been found in southwestern Ontario, Canada, in tomato and green bean crops where it has the potential to become an important insect pest. In Ohio it was also identified on greenhouse crops of *Pelargonium* spp. (Passoa, 1995).

In Europe, *C. chalcites* has been reported as an important pest in Bulgaria and Turkey (Loginova, 1992; Uygun and Ozgur, 1980) affecting tomato, cucumber, peppers and ornamentals. In northern Italy, *C. chalcites* is one of the principal pests on soybean (Zandigiacomo, 1990), although it also attacks artichokes (Ippolito and Parenzan, 1985), and is one of the four main noctuid pests of glasshouse crops in Sicily (Inserra and Calabretta, 1985). *C. chalcites* is present all year long in the Netherlands greenhouses (de Vos and Rutten, 1995; Lempke, 1982), and established populations have also been found in greenhouses of Belgium (van de Veire, 1993). There are around 50 records of *C. chalcites* as a migrant insect in the UK between 1943 and 1990 (Bretherton, 1983), and a recent study has revealed its presence in the UK (Woods, 2011). However, there is no evidence that *C. chalcites* survives during the winter in Northern Europe outdoors (Lempke, 1982).

In the Iberian Peninsula, *C. chalcites* is a pest of horticultural, ornamental and industrial crops. In the south of Spain this species is important in the Guadalquivir Valley, the fertile lowland of Granada crops and in the greenhouses of Almeria, feeding on lucerne, maize and soybean (Amate et al., 1998b). On the Canary Islands, although this insect was described for the first time in 1904 on the island of Tenerife (CIE, 1977), nowadays it is present on all the islands (Bacallado-Aránega, 1972; García et al., 1992; Hernández-Santana, 2007). For many years, it was considered as a secondary pest of banana crops (Perera and Molina, 2002), but currently it is one of the most important pests mainly in greenhouses (Del Pino et al., 2011; Dominguez et al., 2012).

This widespread distribution may be a result of the horticultural and ornamental trade, as in Hungary, where it arrived from Germany on *Pelargonium* (Meszaros and Tusnadi, 1994), in the UK, where it was introduced from the Canary Islands on *Chrysanthemum morifolium* Ramat, 1792 (Seymour and Kilby, 1978) or in Italy it was introduced on exported bananas from the Canary Islands (Jannone, 1966).

3.4. Biology and ecology

C. chalcites adults emerge, and start immediately to fly and mate. Males mate as soon as they emerge from the pupa, while females mate within 1 to 4 days after emergence (Amate et al., 1998a). Adults are semi-nocturnal and generally avoid strong sunlight (De Liñán, 1998b). Adult moths lay eggs overnight on the underside of leaves (Cayrol, 1972), either in isolation or in small groups (Harakly and Farag, 1975), and are usually widely dispersed in the crop (van der Linden, 1996). Amate et al. (1998a) found an average longevity of *C. chalcites* females of 12.2 days with a mean fecundity of 1060.6 eggs/female, while Del Pino (2011) reported 20.5 days longevity and 1263.7 eggs/female at 25º C. Egg hatching occurs after 5-6 days at 25ºC (Gaumont and Moreau, 1961), while Del Pino (2011) observed emergence after 4.1 days. At the optimal temperature of 25°C *C. chalcites* goes through six instars of around 2.5 to 3.5 days each (Rashid et al., 1971; Harakly and Farag, 1975). At the end of the last instar, larvae stop feeding and create a silk white cocoon, where metamorphosis takes place (Passoa, 1995; Cabello et al., 1996).

Occasionally, cocoons can be found on the soil (Harakly and Farag, 1975). The pupal period at 25°C is between 8.8 days (Goodey, 1991) and 7.8 days (Del Pino, 2011). The full cycle is usually completed within 44 to 50 days at temperatures lower than 25°C (Gaumont and Moreau, 1961). However, the life cycle duration varies according to the temperature, food composition and climatic conditions. Activity and development can be also influenced by the photoperiod.

The cycle of *C. chalcites* is bivoltine. However, it is probably a species with optional diapause, and so, some years this insect may present supernumerary generations especially in protected crops (De Liñán, 1998).

C. chalcites has a strong flight capacity, being a migratory species from Northern Africa or Southern Europe, and mainly flies in summer and autumn (García et al., 1992), although it can also be transported by the southerly winds to central and northern Europe in late summer or autumn (Bretherton, 1983; Hachler et al., 1998).

In North Africa this species is present throughout the year (Cayrol, 1972), with up to eight or nine generations per year in Egypt (Rashid et al., 1971; Harakly and Farag, 1975). In Spain, it seems to have a sedentary behavior with catches of adults throughout the year in the south-east (Cabello and Belda, 1994; Cabello et al., 1996). Finally, in the Canary Islands it is also present throughout the year and can present up to 6 generations per year (Del Pino, 2011), although two main peak flights have been described, one in May-June and the other in September - October (Del Pino et al., 2012).

3.5. Damage

C. chalcites is considered an important pest in many countries (CABI, 2007), although there is little or no detailed information on its economic impact.

Early instar larvae are found on the underside of leaves feeding on parenchyma, and larvae, and the injuries they produce, are quite difficult to detect (Fig. 5A). Second and third instars begin to reach the leaf edges and perforate their surface, reducing the photosynthetic area (Goodey, 1991). The last two instars are the most voracious and eat the entire leaf but may avoid large veins (Fig. 5B). The main injury caused by the pest is defoliation, especially in young plantations. However, *C. chalcites* larvae also attack the fruits of some crops, such as in tomato or banana, reducing crop yields severely (Broza and Sneh, 1994; Del Pino et al., 2011; Vilardebo and Guérout, 1964). On legumes, they may excavate deep into pods, sometimes cutting them in two (Zandigiacomo, 1990).

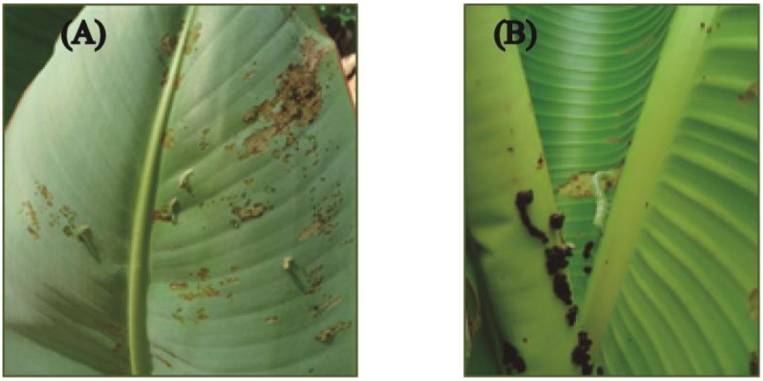

Figure 5. Feeding damage of *C. chalcites* in banana leaves: (A) early instar larvae feeding on leaf underside; (B) last instar larva feeding on leaf upperside.

C. chalcites was considered an occasional or secondary pest of banana crops on Canary Islands, as its damage was limited to the tenderest leaves of young seedlings (Perera and Molina, 2002). As the plant grows, the damage decreases and pest can be more easily kept under control. On open-field traditional plantations damage is 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 and Molina, 2007; Cabello, 2009; Del Pino et al., 2011).

In banana crops, *C. chalcites* larvae prefer to feed on unopened young leaves – called “cigar” leaves, cutting and perforating the leaves (Fig. 5B and 6A) (Perera and Molina, 2007). The presence of small larvae in a fully developed crop does not cause any appreciable damage, moreover, when *C. chalcites* attacks a plant, it destroys a relatively small area of the total foliar surface, rarely surpassing the critical threshold of 10% (Vilardebo and Guérout, 1964). As the crop develops, the levels of leaf damage decrease dramatically.

However, since 2000 this insect has become one of the most serious pests of banana crops in greenhouses of El Hierro, Southern Tenerife and La Palma and, most recently, also in southern Gran Canaria (Del Pino et al., 2011, Dominguez et al., 2012). Currently, *C. chalcites* feeding habits have changed, causing fruit damage (Fig. 6B and 6C). This resurgence as an emerging pest could be related to the reduction of authorized insecticides in banana crops, from 24 active substances in 2006 to 12 compounds in 2014 (López-Cepero, 2015). However, the reasons behind the changes in feeding behaviour directed towards fruit are still unclear. Usually, late instars feed on the epidermis (Fig. 6C), affecting the highest quality bunch handles, thereby reducing their commercial value and producing economic losses (Del Pino et al., 2011; Dominguez et al., 2012).

3.6. Integrated management of *C. chalcites*

In principle, *C. chalcites* control may seem to be relatively easy, since its whole life cycle occurs outside the plant. It is therefore exposed to the action of different control agents, both chemical and biological. However, control is more complicated inside greenhouses, as conditions favor pest development and the insect is exposed to few natural control agents (Cabrera et al., 2009).

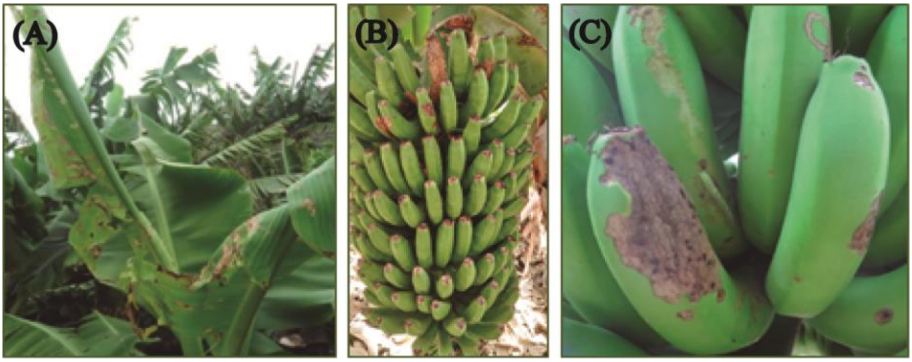

Figure 6. Damages of *C. chalcites* in banana crops: (A) leaf damage in young banana plant; (B) fruit damage in banana bunch; (C) last instar larva feeding on banana fruits.

Currently, synthetic chemical 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. Applications are made to cover the entire leaf underside and all plant parts where larvae may take refuge (Cabrera et al., 2009).

In general, *C. chalcites* control in banana crop becomes difficult due to the low number of chemical and biological insecticides authorized for this crop, the continuous use of the same compounds and the incorrect application of products that do not reach the pest, favoring the appearance of resistance in *C. chalcites* populations (Del Pino et al., 2011). In addition, chemical insecticide residues can hinder the commercialization of bananas from the Canary Islands. Moreover, the presence of toxic residues has to be continuously monitored in order to comply with the European Union established limits. Furthermore, the incompatibility of many broad-spectrum chemical insecticides and natural enemies is well recognized (Cloyd, 2012; Cloyd and Bethke, 2011; Regan et al., 2017). Therefore alternative control methods involving secure, efficient and sustainable strategies, are required urgently.

Integrated pest management (IPM) is a broad-based approach that integrates practices for economic control of pests, by suppressing pest populations below the economic injury level (EIL) (Pedigo, 1996; Pedigo et al., 1986; Shepard et al., 2009). The UN's Food and Agriculture Organization defines IPM as "the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms." Entomologists and ecologists have urged the adoption of IPM-based pest control since the 1970s. Since 2014, IPM is mandatory in any crop system in Spain (Royal Decree 1311/2012, which incorporates European Directive 2009/128/EEC). IPM allows for safer pest control, including several control methods that are more sustainable, such as managing insects, plant pathogens and weeds, and the use of natural and entomopathogenic microorganisms, with the aim of reducing chemical dependence.

However, to design an IPM approach several aspects have to be considered related mostly to the pest, with the aim of determining treatment thresholds for decision making. A number of IPM components are considered in the following sections.

3.6.1. Recognition of *C. chalcites* and its natural enemies

The first basic step in any IPM program is the correct identification and assessment of the importance of the pest species present in the crop, as well as their natural enemies.

In the Canary Islands, different species of lepidopteran pests have been found in banana crops: *Autographa gamma* Linnaeus, 1758, *C. chalcites*

Esper, 1789, *Cornutiplusia circumflexa* Linnaeus, 1767, *Paradrina clavipalpis* Scopoli, 1763, and *Spodoptera littoralis* Boisduval, 1833 (Camacho-Pérez, 2006). However, *C. chalcites* and *S. littoralis* are the two main pests, which present different types of crop damage. While *S. littoralis* mainly affects leaves (Fig. 7A), *C. chalcites* can produce severe damage to fruit (Fig. 7B) (Perera and Molina, 2002).

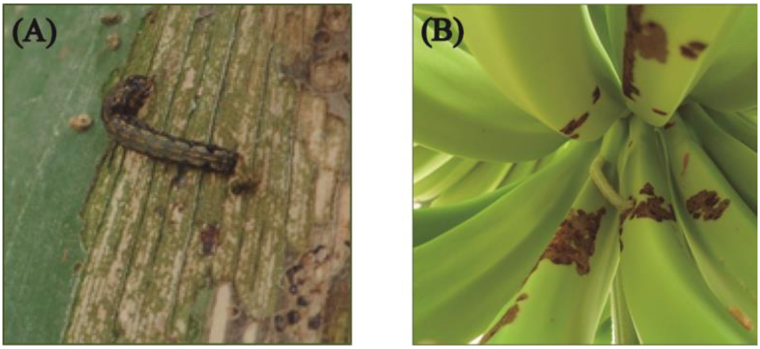

Figure 7. Crop damage by *S. littoralis* and *C. chalcites*, (A) *S. littoralis* larvae feeding on banana leaf, (B) *C. chalcites* larvae feeding on banana fruit.

Numerous *C. chalcites* natural enemies have been found during sampling in banana crops on the islands, especially parasitoid species of both larvae and eggs. Among them, hymenopteran species of the genera *Cotesia* (Fig. 8A) and *Hyposoter* (Fig. 8B), and the dipteran species *Aplomyia* and *Exorista* as larval parasitoids, and the hymenopteran *Trichogramma* as egg parasitoids (Del Pino, 2011). Species of predators have also been found, such as the hymenopteran *Delta dimidiatipenne* Saussure, 1852 (Camacho-Pérez, 2006), the generalist predator *Chrysoperla carnea* Stephens, 1836 (Del Pino, 2011) or the spider species *Neoscona crucifera* Lucas, 1839 and *Cyrtophora citricola* Forsskål, 1775 (Lorenzo, 2005; Camacho-Pérez, 2006).

Equally, entomopathogenic organisms have also been found including *Bacillus thuringiensis* (Berliner, 1915) and a baculovirus, the *C. chalcites* nucleopolyhedrovirus (ChchNPV) (Bernal et al., 2013; Hernández-Santana, 2007). *B. thuringiensis* var. *kurstaki* is a commonly used insecticide against this pest in Canary Islands banana crops.

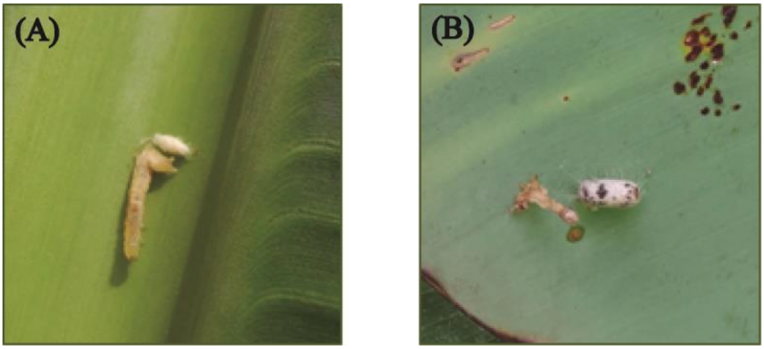

Figure 8. *Chrysodeixis chalcites* natural enemies: *C. chalcites* larva parasitized by (A) *Cotesia* sp.; (B) *Hyposoter rufiventris*.

3.6.2. Monitoring

Once the pest and its natural enemies are identified, the next step is the monitoring of the individuals present in the crop to estimate the incidence, density of population and damage, which allow a decision to be made regarding the moment to apply control interventions against the pest.

Different monitoring strategies can be carried out, such as the use of pheromone traps (Camacho-Pérez, 2006, Hernández-Santana, 2007, Del Pino et al., 2015) to determine first flight together with visual inspections to assess damage.

The use of large tricolor type traps is recommended to detect the presence of adult males (Fig. 9A), which include a high quality sexual pheromone and an insecticide (Fig. 9B).

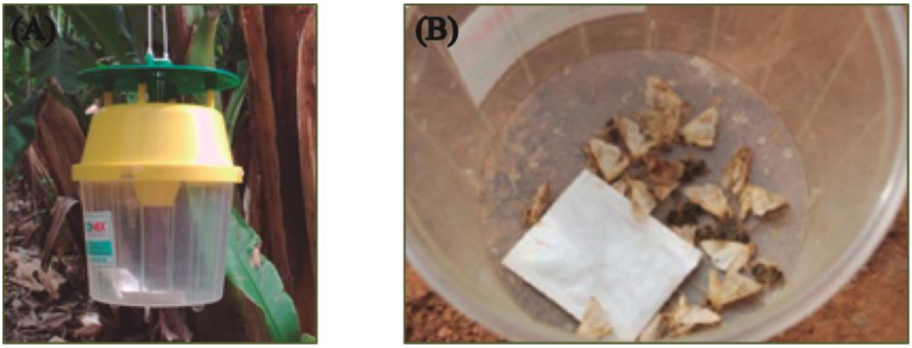

Figure 9. *Chrysodeixis chalcites* monitoring with pheromone traps: (A) tricolor type trap and (B) adult males killed with insecticide.

Traps placed outside the greenhouses had greater catches than inside, possibly due to the lack of drafts that disperse the pheromone inside the greenhouse (Camacho-Pérez, 2006, Hernández-Santana, 2007, Del Pino et al., 2015). After the first catches of adults in the traps it is advisable to carry out visual inspection for eggs and larvae on the plants.

C. chalcites incidence estimations in banana crops are recommended by visual inspection of young leaves, over the entire crop area, with special attention to the edges, sampling at least 10 plants on surfaces smaller than 0.5 ha, 2% of plants on surfaces between 0.5 and 5 ha and 1% of plants in areas greater than 5 ha (Martín-Gil et al., 2016).

3.6.3. Economic injury level and treatment thresholds

The moment at which action should be taken against the pest is very important in IPM. For this it is necessary to know what damage the crop can tolerate without suffering economic losses.

Often, in field conditions it is difficult to quantify lesions, so the number of insects is used as a lesion indicator. Thus, the economic injury level (EIL) is defined as the minimum population density that can cause economic losses (Pedigo, 1996; Pedigo et al., 1986; Pérez-Moreno, 2000; Shepard et al., 2009).

The economic threshold (ET) or treatment threshold (TT) is defined as the pest density at which management action should be taken to prevent an increasing pest population that might reach the EIL. Therefore ET or TT are often lower than the EIL (or sometimes equal) to allow control measures to take effect before the TT damage level is reached (Fig. 10).

TT calculation is complex, there are parameters such as the physical damage units per insect that are obtained as a result of laborious field trials. In addition, this threshold can vary for the same crop and pest from one year to another. Because of this, it is the component of the IPM that has been least developed to date, and it is the practical level that should be used to make decisions, that is, to treat or not to treat (Pérez-Moreno, 2000).

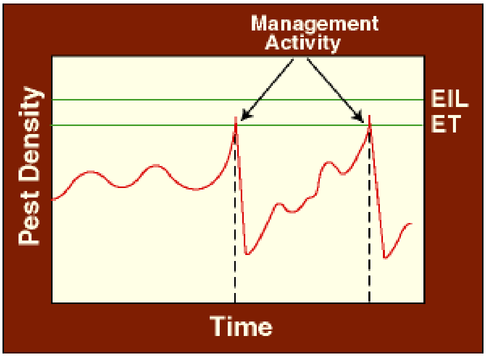

Figure 10. Relationship between the economic threshold (ET) and the economic injury level (EIL). When the pest density reaches the economic threshold (ET) a pest control intervention is applied. Adapted from Pedigo (1996).

Currently, in the absence of thresholds or intervention criteria for each phenological stage of *C. chalcites* in the banana crop, it is recommended to perform treatments when more than 5 adults are detected per pheromone trap, when more than 10% of young plants are affected, or when banana bunch damage is detected (Martín-Gil et al., 2016).

3.6.4. Control methods

IPM contemplates the use of different measures for the control of pests with different orders of priority, starting with preventive or cultural measures, then physical-mechanical, biological, biotechnical and, as the last option, chemical measures (Fig. 11).

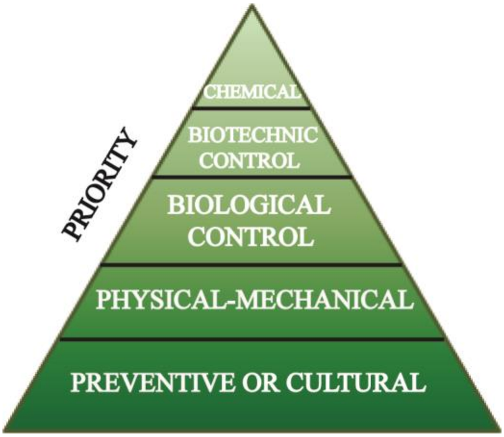

Figure 11. Pyramid with different pest control measures in order of priority.

3.6.4.1. Preventive or cultural measures

Cultural practices are a set of measures that are recommended in order to avoid or make the entry and infestation by the pest in the crop unfavorable (Lorenzo-Rodríguez, 2016). In this sense, to avoid the entry of *C. chalcites* adults in banana crops checking the integrity of greenhouse covers is recommended.

To avoid infestation by the pest several measures are recommended such as the elimination of weeds both inside and outside the greenhouse, as they are refuges for the caterpillars, or elimination of the last leaf produced before development of the bunch and the bracts that surround the bunch to avoid damage in the fruit and to achieve better coverage with insecticidal treatments (Del Pino et al., 2011). It is also possible to use trap plants at the edges or between the crop rows, which serve as reservoirs of natural enemies of the caterpillar, as well as pest host plants such as cabbage, maize or geranium, that keep the larvae away from the crop, and for which control measures can be targeted at these plants (Perera and Molina, 2007; Cabrera et al., 2009).

3.6.4.2. Physical-mechanical measures

Several physical and mechanical measures are also used to control the pest. The bagging of the banana bunch can act as a barrier that prevents the entry of *C. chalcites* larvae. However, prior to bagging, insecticide treatment must be carried out to ensure that the fruit is free of larvae (Camacho-Pérez, 2006; Del Pino et al., 2011). Treatment of the plants with pressurized water to remove the larvae can be effective (López-Cepero et al., 2014). The use of light traps and double doors in the greenhouses (Martín-Gil et al., 2016) can also be effective to avoid pest entry into the greenhouses.

3.6.4.3. Biological control

Biological control techniques are based mainly on the use of natural enemies and entomopathogenic organisms.

3.6.4.3.1. Natural enemies

C. chalcites presents numerous natural enemies (see 3.6.1.), but to date only the egg parasitoids *Trichogramma* have been used as biological control agents. Five species have been found in Canary Islands' banana crops, *T. achaeae* Nagaraja and Nagarkatti, 1970, *T. bourarachae* Pintureau and Babault, 1988, *T. euproctidis* Girault, 1911; *T. evanescens* Westwood, 1833 and *T. canariensis* Del Pino and Polaszek, 2013 (Del Pino et al., 2015; Polaszek et al., 2012;). *T. achaeae* is the most frequent and widely distributed species in the archipelago with a maximum percentage of natural parasitism of 87% (Fig. 12A) (Del Pino et al., 2015). Weekly introductions of between 500,000 and 750,000 individuals per hectare are recommended during *C.*

chalcites adult flying periods in spring and autumn (Del Pino et al., 2015). However, the commercial availability of *T. achaeae* is insufficient to control *C. chalcites* effectively (Del Pino et al., 2011), hence the need to use alternative methods.

3.6.4.3.2. Entomopathogenic organisms

Several entomopathogenic organisms have been described that affect *C. chalcites*, such as the microsporidian *Nosema manierae* Toguebaye and Bouix (Protozoa: Microspora) (Toguebaye and Bouix 1983) or the nematode *Steinernema carpocapsae* Weiser, 1955 (Nematoda: Steinermatidae) (Brødsgaard and Albajes, 1999). However, the main entomopathogenic organism currently used for *C. chalcites* control is the bacterium *Bacillus thuringiensis* (Bt) (Fig. 12B), commonly used in several regions all over the world to protect tomato crops (Vacante et al., 2001) or horticultural and ornamental crops (Broza and Sneh, 1994). In Canary Islands' banana crops, Bt var. kurstaki is the only microbial agent authorized (Méndez-Hernández et al., 2017), however, due to its wide use and the low number of active materials authorized for this crop, resistance has been described that diminishes the effectiveness of this product (Del Pino et al., 2011; Simón et al., 2015).

C. chalcites larvae are also naturally affected by an Alphabaculovirus (Fam. *Baculoviridae*) known as Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV) (Fig. 12C). Isolates of this NPV have been found in Almeria, Spain (ChchNPV-SP1) (Murillo et al., 2000) and in the Netherlands (ChchNPV-NL) (van Oers et al., 2004), while in Canary Islands' banana crops five native isolates were identified (ChchNPV-TF1 to ChchNPV-TF5) (Bernal et al., 2013). Previous studies indicated that ChchNPV-TF1 presented suitable insecticidal characteristics both in the laboratory (Bernal et al., 2013) and in small-scale greenhouse conditions (Simón et al., 2015). To date, its effectiveness under open-field conditions or in commercial plantations has not been demonstrated.

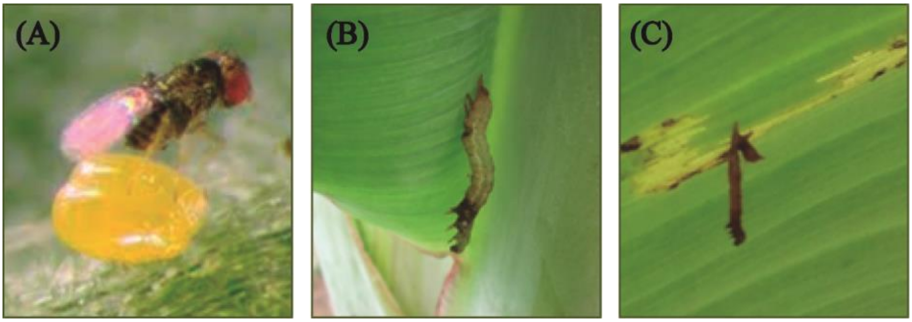

Figure 12. *Chrysodeixis chalcites* biological control: (A) *C. chalcites* egg being parasitized by *Trichogramma achaeae*; *C. chalcites* larval cadaver resulting from an infection with: (B) *Bacillus thuringiensis* and (C) nucleopolyhedrovirus.

3.6.4.4. Biotechnological control

Biotechnological control is based on the alteration of the physiological and communication processes of the pest species. There are different strategies of biotechnological control for *C. chalcites*, among them the use of sex attraction pheromones, mineral and plant products, soaps and oils (Del Pino et al., 2011; López-Cepero et al., 2014).

Traps with sex pheromones can be used for control measures (mass trapping), as the mating of adults is reduced (Martín-Gil et al., 2016).

3.6.4.5. Chemical control

Chemical control should be the last measure to be used in IPM programs. Currently there are a small number of chemical pesticides authorized in banana crops for the control of *C. chalcites*: lambda cyhalothrin 2.5% WG and 10% SC, chlorpyrifos 48% EC, indoxacarb 30% WG and spinosad 48% SC (Méndez-Hernández et al., 2017). Normally, a single product is used during the entire crop cycle, favoring the appearance of resistance (Cory and Myers, 2009; Horowitz et al., 1998) and resulting in the need for multiple insecticide applications. Moreover, continued use generates residues on the bananas that hamper their commercialization (Carvalho, 2006;

Hernández-Borges et al., 2009). Therefore, to avoid these problems it is necessary to find alternative and sustainable methods to alternate with the currently used products.

Among the sustainable methods described above, ChchNPV appears to be an attractive alternative due to its safety, compatibility with natural enemies, and the possibility of producing it on a commercial scale.

4. BACULOVIRUSES

Baculoviruses are arthropod-specific viruses that have been isolated from about 700 insect species belonging mostly to the orders Lepidoptera, Hymenoptera and Diptera (Caballero and Williams, 2008; Herniou et al., 2003). From an anthropocentric point of view, they are beneficial due to their potential for controlling insect pests. The signs of infection were first described 2,000 years ago in China in silkworm cultures (Benz, 1986), although it was not until the years 1950-1975 that baculoviruses were considered as biological insecticides (Ignoffo, 1981; Steinhaus, 1956). The first baculovirus-based insecticide was obtained in the United States from the Helicoverpa zea baculovirus, but failed commercially for several reasons (Ignoffo and Couch 1981). However, due to the numerous problems generated by the use of chemical insecticides, there have been numerous subsequent efforts to increase our understanding of their biology and ecology in order to develop them as bioinsecticides (Szewczyk et al., 2006).

4.1. Morphology, taxonomy and classification

Baculoviruses have a single, double-stranded, circular and supercoiled genome, of 80 to 180 kb in size. To date, more than 60 baculovirus genomes have been fully sequenced, and encode between 90 and 180 genes (Rohrmann, 2013). The elementary units of baculoviruses are the nucleocapsids, with a rod-shaped form and a diameter of 40-60 nm and 230-385 nm length. The nucleocapsids are enveloped by a lipoprotein bilayer membrane forming the virions, which are the morphological units that produce viral infection (Federici, 1986; Herniou et al., 2012; Rohrmann, 2013).

Two types of virions are formed during the infection cycle; occlusion derived virions (ODVs) and budded virions (BVs). These two types of virions have identical genomic material and similar nucleocapsid structure, but differ in their function and envelopes composition. ODVs act during primary infection that occurs in midgut epithelial cells of the host, while BVs are responsible for secondary infection (Fig. 13). ODVs are formed in the nucleus of cells infected by the virus and contain several nucleocapsids wrapped in a lipid bilayer synthesized *de novo* (Rohrmann, 2013; Stoltz, 1973). In the late stage of the infection cycle, ODVs are surrounded by a matrix protein (polyhedrin or granulin) forming the occlusion bodies (OBs) (Fig. 13), which are responsible for horizontal transmission and are adapted for survival in the environment (Rohrmann, 2013). In contrast, BVs are responsible for the systemic spread of disease through cell-to-cell transmission within each host insect.

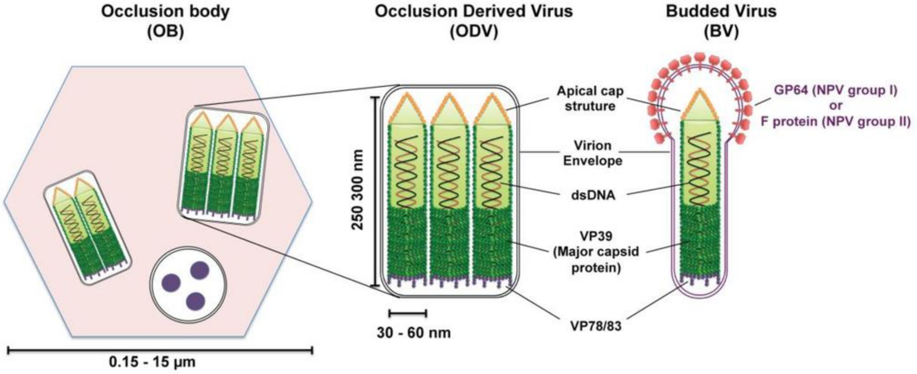

Figure 13. Structure of baculovirus occlusion bodies (OB) and two types of virions: occluded derived virion (ODV) and budded virion (BV). From Au et al. (2013).

Formerly, baculoviruses were classified based on OB morphology as nucleopolyhedroviruses (NPV) or granuloviruses (GV). NPVs produce large polyhedral-shaped OBs (0.15 to 15 µm), that may have a single nucleocapsid (single NPVs, SNPVs) or multiple nucleocapsids (multiple NPVs, MNPVs) within each ODV (Fig. 14). In contrast GVs have a small granular-shaped structure (400-600 nm) formed by a protein matrix of granulin with one ODV containing a single nucleocapsid (Fig. 14) (Funk et al., 1997).

Molecular techniques, such as restriction endonuclease profiles or DNA sequencing have allowed better differentiation of baculoviruses, and since 2006 the family *Baculoviridae* has been divided into four genera: *Alphabaculovirus* (lepidopteran-specific NPVs), *Betabaculovirus* (lepidopteran-specific GVs), *Gammabaculovirus* (hymenopteran-specific NPVs) and *Deltabaculovirus* (dipteran-specific NPVs) (Jehle et al., 2006; King et al., 2012). In its last report the International Committee on Taxonomy of Viruses (ICTV) recognized 66 baculovirus species, 40 Alphabaculovirus; 23 Betabaculovirus, 1 Deltabaculovirus and 2 Gammabaculovirus (ICTV, 2016), although this information is continuously updated.

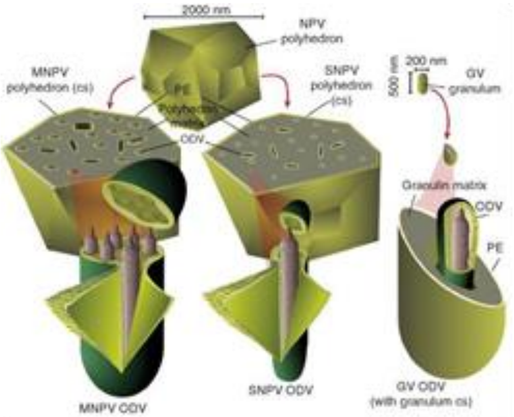

Figure 14. Structure of *Alphabaculovirus* and *Betabaculovirus* occlusion bodies (OBs) and their occlusion-derived virions (ODVs). The two different ODV phenotypes within the *Alphabaculovirus* genus are also shown: multiple (MNPVs) and single (SNPVs) nucleopolyhedroviruses. From Slack and Arif (2007).

4.2. Infection cycle and pathogenesis

The alphabaculovirus infection cycle stars when a susceptible larva ingests OBs, which are found on the host plant leaf (Fig. 15A). The polyhedrin matrix of OBs is solubilized due to the alkaline pH of insect midgut and then ODVs are released (Fig.15B). ODVs cross the peritrophic membrane that protects the epithelial cells thanks to viral enhancins and host cell proteinases (Slavicek and Popham, 2005; Wang and Granados, 1997). The ODV membrane, helped by proteins such as *per os* infectivity factors (PIFs), fuses with the epithelial cell membrane (Horton and Burand, 1993; Rohrmann, 2013) and the nucleocapsids are released into the cytoplasm where primary infection takes place (Fig. 15C). The nucleocapsids then pass to the cell nucleus through the nuclear pores (van Loo et al., 2001) and there the replication of viral DNA takes place, forming new nucleocapsids. Some nucleocapsids may bud out the basolateral side of the cell to continue viral spread (Fig. 15D) (Slack and Arif, 2007).

Newly formed BVs pass into the hemocoele cavity along the trachea avoiding the basal lamina (Slavicek and Popham, 2005; Wang and Granados, 1997), propagating the secondary infection to other susceptible tissues such as muscle, fat body, hemocytes and epithelial cells (Fig. 15E-F) (Engelhard et al., 1994; Flipsen et al., 1995). A part of the newly formed nucleocapsids remain in the cell nucleus and acquire a membrane created *de novo* to form ODVs. The ODVs are surrounded by the polyhedrin matrix to form OBs (Wood et al., 1994). At the end of secondary infection, the nuclear and the plasma membranes breakdown, liberating the OBs and other cellular contents into the hemocoele. Subsequently, the larval cuticle is degraded due to the action of viral chitinase and cathepsin enzymes, resulting in the release of OBs into the environment that serve as inoculum for new host insects (Fig. 15G) (Federici, 1997).

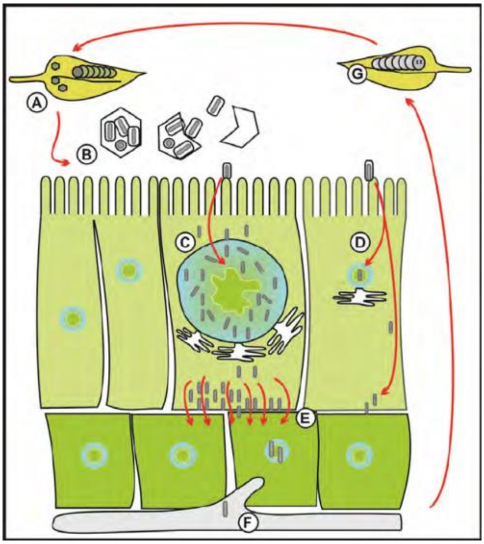

Figure 15. The alphabaculovirus infection cycle. Sequential steps NPV multiplication during primary and secondary infection are numbered by letters. (A) Larva ingests baculovirus-contaminated leaves. (B) The virions (ODVs) are released and bind to midgut epithelial cell. (C) In the cytoplasm the nucleocapsids are transported to the nucleus, where the viral DNA is released and the BVs are generated. (D) Nucleocapsids can pass through the cell to the hemocoele. (E) Viral particles enter susceptible cells and initiate a replication cycle to produce a new nucleocapsids. (F) OBs are generated in each infected cell. (G) Cell lysis occurs, larval integument breaks down and OBs are released to be eaten by other larvae for horizontal transmission. From Rodríguez et al. (2012).

The external signs and symptoms of viral infection appear days after ingestion of OBs and consist of changes in behavior such as loss of appetite and activity and also changes in appearance of the integument (Granados and Williams, 1986). Just before death, the viral genes *egt* and *ptp* induce the larvae to climb to the upper part of the host plant (Katsuma et al., 2012; van Houte et al., 2012), where they hang by their last abdominal pseudopods. This favors viral dispersion when the larva breaks down due to viral enzymes and physical agents (Federici, 1997). Different biotic and abiotic factors can promote the dispersion of OBs and facilitate horizontal transmission. Crop practices (Moscardi, 1999) as well as natural enemies (Caballero et al., 1991; Cossentine, 2009; Vasconcelos et al., 1996) can disperse the virus.

4.3. Baculovirus diversity

Baculoviruses present a great diversity both between the different viral species, known as interspecific diversity (Fig. 16A), and within a single viral species, named intraspecific diversity (Fig. 16B) (Muñoz and Caballero, 2001).

4.3.1. Interspecific diversity

Baculoviruses present a great natural diversity, since they affect a large number of host insects (Caballero and Williams, 2008), with the genus *Alphabaculovirus* being the most widely distributed (Jehle et al., 2006). Knowledge of the natural diversity of baculoviruses helps the development of baculovirus-based insecticides that have the best insecticidal properties (Caballero et al., 2014; Muñoz and Caballero, 2001; Figueiredo et al., 2009). This diversity is due to the different genes present in each baculovirus species, the genomic organization, the degrees of homology between common genes, the structure of the intergenic regions or even small deletions or insertions (Serrano et al., 2013; Simón et al., 2012; Thézé et al., 2014). The use of DNA-based techniques, such as restriction endonuclease analysis (REN) (Fig. 16) and genome sequencing, has been able to define these differences at the molecular level (Jehle et al., 2006, Miele et al., 2011). Phylogenetic analyses based on specific DNA sequences have also aided the classification of different virus species.

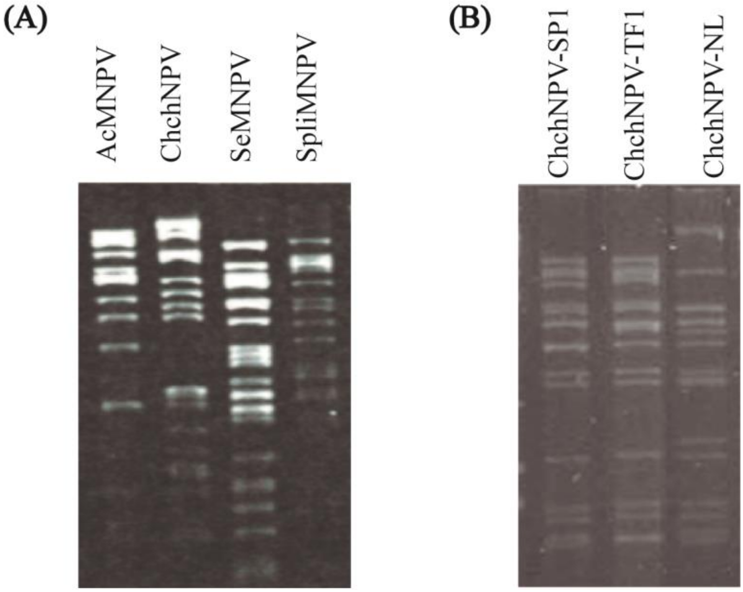

Figure 16. (A) *Eco*RI restriction endonuclease profiles of the genomic DNA of different alphabaculoviruses: Autographa californica MNPV (AcMNPV); Chrysodeixis chalcites NPV (ChchNPV), Spodoptera exigua MNPV (SeMNPV) and Spodoptera littoralis MNPV (SpliMNPV) (adapted from Murillo et al., 2000), (B) *Bgl*II restriction endonuclease profiles of the genomic DNA of different geographical isolates of ChchNPV: ChchNPV-SP1, ChchNPV-TF1 and ChchNPV-NL (adapted from Bernal et al., 2013).

4.3.2. Intraspecific diversity

Different geographical isolates of the same baculovirus species present great intraspecific variation, both at the genotypic and phenotypic levels (Erlandson, 2009; Harrison et al., 2014). Closely related isolates can be differentiated by their insecticidal properties such as pathogenicity and speed of kill (Cory et al., 2005; Harrison et al., 2012). This diversity may be due to host-pathogen adaptation processes and coevolution. Previous studies demonstrated that indigenous baculoviruses are usually more infective to local insect populations than isolates from other geographical origins (Cory and Myers, 2003; Erlandson, 2009; Barrera et al., 2011). This natural genetic diversity helps when selecting the most suitable components to produce a lethal infection in the host insect (Bernal et al., 2013; Caballero et al., 2014;

Caballero and Williams, 2008). Therefore, the study of baculovirus intraspecific variability within a geographic region where a control program is going to be performed is necessary to select the isolate with the best insecticidal characteristics.

Baculovirus isolates also show intrapopulation diversity, since isolates from a mixture of infected larvae or from a single infected larva consist of a mixture of different genotypes (Cory et al., 2005; Redman et al., 2010). The determination of these genotypic variants is generally performed using restriction endonuclease (REN) profiles of wild-type isolates in which submolar bands appear, indicating the presence of these genotype mixtures, or by DNA sequencing detecting nucleotide polymorphisms (Chen et al., 2001; Erlandson, 2009; Thézé et al., 2014). Genotypic variability may be due to various mechanisms, such as DNA deletions or insertions (Cory et al., 2005; Muñoz et al., 1998; Thézé et al., 2014), gene duplication, mutations, or insertions of transposons (Erlandson, 2009; Li et al., 2013). Very similar genotypes do not usually present large phenotypic differences, however in some cases small differences in the genome of these genotypes may influence morphological and biological functions such as pathogenicity, virulence and production of OBs (Cory et al., 2005; Harrison et al., 2012; Rowley et al., 2011).

Thus, determining the intraspecific diversity of a particular baculovirus is of special interest when selecting the most suitable active material to be developed as a bioinsecticide (Caballero et al., 2014; Moscardi, 1999; Bernal et al., 2013).

4.4. Baculoviruses as bioinsecticides

Baculoviruses have many advantages as bioinsecticides and have been used as biological control agents against agricultural and forestry pests for many decades (Eberle et al., 2012; Inceoglu et al., 2006; Szewczyk et al, 2009;

Vincent et al., 2007). These advantages include high specificity, as they only infect only one or two insect species (Caballero et al., 2009; Rodgers, 1993), this makes them safe for humans, animals and other organisms present in the crop (Ahmad et al., 2011; Gröner, 1986). In addition, they are able to persist in the environment and are able to infect new insects and can be applied by traditional means, which favors their commercial development (Moscardi, 1999).

Currently about 60 baculovirus-based products are used for the control of different insect pests worldwide (Eberle et al., 2012; Moscardi et al., 2011). There are several examples of the successful use of baculoviruses in pest control, especially the case of *Anticarsia gemmatalis* control with its native nucleopolyhedrovirus (AgMNPV) in Brazil's soybean crops for several decades, over an area of about 2 million hectares (Moscardi, 1999). Also noteworthy is the case of *Helicoverpa armigera* control in cotton crops in China with its nucleopolyhedrovirus (HearNPV), with this country being the world's main producer of baculoviruses (Yang et al., 2012; Zhang et al., 1995). *H. armigera* is also effectively controlled with HearNPV in Australia, India and South Africa (Moore et al., 2004; Moscardi et al., 2011). In Europe, the granulovirus of *Cydia pomonella* (CpGV) has been used for the control of this pest in pome fruit crops (Lacey et al., 2008; Vincent et al., 2007), although in this case resistance has been developed that can be combated with the use of new isolates or more effective genotypes (Asser-Kaiser et al., 2007; Eberle et al., 2008; Undorf-Spahn et al., 2012). Finally, an alphabaculovirus (SeMNPV) has also been used in Spain to control pests such as *Spodoptera exigua* in the horticultural greenhouses of south-east Spain (Caballero et al., 2009).

Some of the characteristics of baculoviruses that favor their development as bioinsecticides are high virulence, high transmission capacity, high persistence in the field, ease of mass production at low cost, safety for humans, animals and the environment (Ibarra and Del Rincón Castro, 2001).

However, they also present several limitations. One of the main ones is the slow speed of kill of the target insect compared to chemical insecticides. Chemicals are capable of killing larvae in a few hours, while baculoviruses kill larvae 3 to 20 days after OB ingestion (Caballero et al., 1992, Shapiro, 1986). Before the larva dies it continues feeding on the crop and causing damage, therefore, efforts have been made to identify active material with an improved speed of kill (Caballero et al., 2014).

Finally, after testing the efficacy of NPVs under laboratory conditions, efficacy should be determined under natural field conditions (Grzywacz et al., 2008; Gupta et al., 2010), as this efficacy may vary according to crop, abiotic and biotic conditions (Bianchi et al., 2000; Grant and Bouwer, 2009).

The efficacy of baculoviruses under field conditions can be affected by environmental conditions, mainly by ultraviolet radiation, which decreases OB persistence (Ignoffo, 1992). Therefore virus formulations may include solar protectants. The use of additives such as wetting agents, stickers or humectants to improve the application and coverage of OBs on plants is also common. In addition, factors such as temperature, pest density or crop phenology may also influence the efficacy of the virus.

Another key feature for the successful use of a baculovirus as a bioinsecticide is to establish an appropriate timing for applications depending on the persistence of OBs in the crop and the insect's feeding habits, among other factors. The dose of OBs in each application is also important as it directly influences the application costs. Because of this, it is recommended to use the lowest dose that achieves adequate control (Grzywacz, 2008).

4.5. C. chalcites nucleopolyhedrovirus as a bioinsecticide

Among the different ChchNPV isolates obtained in the Canary Islands, the ChchNPV-TF1 isolate stands out as of particular interest. This isolate was the most prevalent and widespread variant on the islands.

Moreover, ChchNPV-TF1 was the most pathogenic and also the fastest killing variant under laboratory conditions (Bernal et al., 2013). For this reason it was selected to examine its effectiveness at protecting young banana plants from *C. chalcites* damage under greenhouse conditions in a small-scale trial (Simón et al., 2015), which showed that this isolate was as effective as conventional insecticides. Moreover, previous studies showed that a specific mixture of ChchNPV genotypes presented increased pathogenicity and virulence, which was the subject of a European Patent (Caballero et al., 2014).

Finally, ChchNPV-TF1 efficacy was only proved at a small-scale with young banana plants under greenhouse conditions but not in open-field conditions or at a large-scale under the conditions of commercial banana crops. Therefore, such information could be of special value for the inclusion of this new biological control agent in IPM programs.

5. SCOPE OF INVESTIGATION

The aim of this thesis was to evaluate aspects required for the development of an integrated *C. chalcites* management program in Canary Islands' banana crops using a native ChchNPV isolate as a biological insecticide.

First, different parameters were estimated that might facilitate the calculation of thresholds for pest control decision-making. For this, the incidence and feeding damage caused by *C. chalcites* in banana crops were evaluated by performing surveys in the different plantations across the islands, in which foliar and fruit damage was evaluated. Production losses and economic losses due to direct damage to fruit were estimated by sampling in banana processing facilities located on four islands, and the costs derived from *C. chalcites* control were estimated from interviews conducted with field technicians and banana growers **(Chapter II)**. Two main parameters, treatment cost and production losses, were obtained, which are essential for the treatment threshold calculations. The results of this chapter are expected to be useful for implementing IPM programs.

Another important point in IPM is to prioritize the use of biological control agents instead of chemical pesticides. Among the biological control agents against this pest ChchNPV-TF1 has proved useful in laboratory and in small-scale greenhouse trials. However, ~65% of the banana production is produced in open field conditions in the Canary Islands, and therefore, it is necessary to evaluate the insecticidal efficacy in open-field conditions before using it in IPM. Consequently, the insecticidal efficacy of ChchNPV-TF1 in *C. chalcites* control was evaluated in young banana plants growing in pots not only in greenhouse but also in open-field plots **(Chapter III)**. However, as genetic diversity in pathogen populations has a marked influence on major phenotypic traits, the incidence and natural diversity of ChchNPV was determined in Canary Islands' banana crops, with the aim of selecting the most appropriate variant. For this purpose, *C. chalcites* larvae were sampled from all producing banana islands and mortality was recorded under laboratory conditions. Subsequently, viral isolates were identified by REN analysis. The optimal viral concentration and effectiveness of ChchNPV-TF1 in controlling *C. chalcites* damage to young banana plants in small-scale plots was measured under greenhouse and open-field conditions.

Finally, in **Chapter IV** the efficacy of this virus was determined in commercial mesh-built greenhouses, under the usual conditions of this crop. This chapter showed that ChchNPV-TF1 applications were as effective as conventional treatments at reducing larval density and fruit damage in commercial banana plantations in the Canary Islands.

In conclusion, the results of this thesis will be the basis for establishing *C. chalcites* damage thresholds in banana crops in the Canary

Islands, as well as for the use of ChchNPV-TF1 as a bioinsecticide in IPM of this pest.

6. REFERENCES

Ahmad, I., Khan, M.S.A., Khan, F., Singh, M., 2011. Microbial applications in agriculture and the environment: a broad perspective, in: Ahmad, I., Ahmad, F., Pitchel, J. (Eds.), Microbes and microbial technology. Springer, London, UK, pp. 1-27.

Amate, J., Barranco, P., Cabello, T., 1998a. Ciclo vital de *Chrysodeixis chalcites* (E.) (Lepidóptera: Noctuidae) en condiciones controladas. Boletín de Sanidad Vegetal Plagas, 24, 425-428.

Amate, J., Barranco, P., Cabello, T., 1998b. Identificación de larvas de las principales especies de Noctuidos plaga de España (Lepidóptera: Noctuidae). Boletín de Sanidad Vegetal Plagas 24,101-106.

Anon, 1984. Agricultural services of the Ministry of Agriculture and Natural Resources Institute, Reduit, Mauritius, pp.77.

ASPROCAN, 2016. 2015 annual statistics, Asociación de Organizaciones de Productores de Plátanos de Canarias. http://platanodecanarias.net/wp-content/uploads/2016/10/ESTADISTICAS_2015.pdf (accessed 14.5.17).

Asser-Kaiser, S., Fritsch, E., Undorf-Spahn, K., Kienle, J., Eberle, K.E., Gund, N.A., Reineke, A., Zebitz, C.P.W., Heckel, D.G., Huber, J., Jehle, J.A., 2007. Rapid emerge of baculvorirus resistance in codling moth due to dominant, sex-linked inheritace. Science 317, 1916-1918.

Au, S., Wu, W., Panté, N., 2013. Baculovirus nuclear import: open, nuclear pore complex (NPC) sesame. Viruses 5, 1885-1900.

Avidov, Z., Harpaz, I., 1969. Plant pests of Israel. Israel Universities Press, Jerusalem.

Bacallado-Aránega, J.J., 1972. Contribución al conocimiento de la faunula lepidopterológica de la Isla del Hierro (Lep. Heterocera). Vieraea. Volumen 2.

Barrera, G., Simón, O., Villamizar, L., Williams, T., Caballero, P., 2011. *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: Genetic and phenotypic comparison of field isolates from Colombia. Biological Control 58, 113-120.

Benz, G.A., 1986. Introduction: Historical perspectives, in: Granados, R.R., Federici, B.A. (Eds.), The biology of baculoviruses: Biological properties and molecular biology. CRCPress, Florida, pp. 1-35.

Bernal, A., Williams, T., Hernández-Suárez, E., Carnero, A., Caballero, P., Simón, O., 2013. A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: The basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banana crops. Biological Control 67, 101-110.

Bianchi, F.J.J.A., Snoeijing, I., van der Werf, W., Mans, R.M.W., Smiths, P.H., Vlak, J.M., 2000. Biological activity of SeMNPV, AcMNPV, and theree AcMNPV deletion mutants against *Spodoptera exigua* larvae (Lepidpotera: Noctuidae). Journal of Invertebrate Pathology 75, 28-35.

Bretherton, R.F., 1983. The Incidence of Migrant Lepidoptera in the British Isles, in: Heath, J., Emmet, A.M. (Eds), The moths and butterflies of Great Britain and Ireland: Volume 10, Noctuidae (Part II) and Agaristidae. Harley Books, Colchester, UK, pp. 222-224.

Brødsgaard, H.F., Albajes, R., 1999. Insects and mite pests, in: Albajes, R., Guillino, M.L.,van Lenteren, J.C., Elad, Y.K. (Eds), Integrated pest and disease management in greenhouse crops. Academic Press, Netherlands, pp 48-60.

Broza, M., Sneh, B., 1994. *Bacillus thuringiensis* spp. kurstaki as an effective control agent of lepidopteran pests in tomato fields in Israel. Journal of Economic Entomology 87, 923-928.

Caballero, P., Bernal, A., Simón, O., Carnero, A., Hernández-Suárez, E., Williams, T., 2014. Novel genotypes of *Chrysodeixis chalcites* single nucleopolyhedrovirus (ChchSNPV), a procedure for its production and use as a biological control agent.European Patent PCT-760.

Caballero, P., Murillo, R., Muñoz, D., Williams, T., 2009. El nucleopoliedrovirus de *Spodoptera exigua* (Lepidoptera; Noctuidae) como bioplaguicida: análisis de avances recientes en España. Revista Colombiana de Entomología 35, 105-115.

Caballero, P., Vargas-Osuna, E., Santiago-Alvarez, C., 1991. Parasitization of granulosis-virus infected and noninfected *Agrotis segetum* larvae and the virus transmission by three hymenopteran parasitoids. Entomologia Experimentalis et Applicata 58, 55–60.

Caballero, P., Williams, T., 2008. Virus entomopatógenos, in: Jacas, J.A., Urbaneja, A. (Eds.), Control Biológico de Plagas Agrícolas. Phytoma, Valencia, Spain, pp. 121-135.

Caballero, P., Zuidema, D., Santiago-Álvarez, C., Vlak, J.M., 1992. Biochemical and biological characterization of four isolates of *Spodoptera exigua* nuclear polyhedrosis virus. Biocontrol Science and Technology 2, 145-157.

Cabello, T., González, M.P., Justicia, L., Belda, J.E., 1996. Plagas de noctuidos (Lep.; Noctuidae) y su fenología en cultivos en invernaderos. Informaciones Técnicas 39/96. Dirección General de Investigación y Formación Agraria. Consejería de Agricultura y Pesca. Junta de Andalucía.

Cabello, T., 2009. Control biológico de Noctuidos y otros Lepidópteros, in: Jacas, J., Urbaneja, A. (Eds.), Control biológico de plagas. Phytoma, Valencia, Spain, pp. 279-306.

Cabello, T., Belda, J., 1994. Noctuidos plaga (Lepidoptera: Noctuidae) en cultivos hortícolas en invernaderos. Sanidad Vegetal en la Horticultura Protegida. Consejería de Agricultura y Pesca, Sevilla, Spain, pp. 179-211.

CABI, 2007. Crop Protection Compendium. Commonwealth Agricultural Bureau International (CABI), Wallingford, UK. http://www.cabi.org/cpc/advanceddatasheetsearch/?q=Chrysodeixis+chalcites&page=1&s0=20

CABI, 2014. Crop Protection Compendium International. Wallingford, UK. www.cabicompendium.org/cpc

Cabrera, J., Galán-Saúco, V., 2005. Evaluation of the banana cultivars Zelig, Grande Naine and Gruesa under different environmental conditions in the Canary Islands. Fruits 60, 1-13.

Cabrera, R., Lorenzo, C.D., Prendes, C., Martín, T., 2009. Evaluación de la eficacia de productos naturales contra *Chrysodeixis chalcites* y uso de plantas atrayentes para su control en el cultivo de platanera. Diseño de trampeo masivo para *Cosmopolites sordidus*. Convenio Asprocan – Fundación Empresa Universidad de La Laguna, Spain.

Cáceres-Hernández, J.J., Martín-Rodríguez, G., González-Gómez, J.I., 2013. La relevancia del plátano en la economía canaria. Departamento de Economía de las Instituciones, Estadística Económica y Econometría. Universidad de La Laguna., Spain.

Camacho-Pérez, J., 2006**.** Estudio del impacto sobre cultivo de platanera, de especies del Orden Lepidóptera. Trabajo Fin de Carrera. Escuela Técnica Superior de Ingeniería Agraria. Universidad de La Laguna, Spain.

Carvalho, F.P., 2006. Agriculture, pesticides, food security and food safety. Environmental Science and Policy 9, 685-692.

Cayrol, R.A., 1972. Famille des Noctuidae, in: Balachowsky, A.S. (Ed.) Entomologie appliquée a l'agriculture. Lépidoptères. Tome II. Volume *2*. Masson et Cie. París, 1255-1520.

Chen, X., IJkel, W.F.J., Tarchini, R., Sun, X., Sandbrink, H., Wang, H., Peters, S., Zuidema, D., Lankhorst, R.K., Vlak, J.M., Hu, Z., 2001. The sequence of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus genome. Journal of General Virology 82, 241-257.

CIE, 1977. Distribution maps of pests, Series A, Agricultural. Map No. 376 *Chrysodeixis chalcites*. CAB International. Wallingford, UK.

Cloyd, R.A., 2012. Indirect effects of pesticides on natural enemies, in: Soundararajan, R.P. (Ed.), Pesticides-Advances in chemical and botanical pesticides, InTech, Rijeka, Croatia, pp. 127-150.

Cloyd, R. A., Bethke, J. A., 2011. Impact of neonicotinoid insecticides in natural enemies in greenhouse and interiorscape environments. Pest Management Science 67, 3-9.

Cory, J.S., Green, B.M., Paul, R.K., Hunter-Fujita, F., 2005. Genotypic and phenotypic diversity of a baculovirus population within an individual insect host. Journal of Invertebrate Pathology 89, 101-111.

Cory, J.S., Myers, J.H., 2003. The ecology and evolution of insect baculoviruses. Annual Review of Ecology, Evolution and Systematics 34, 239-272.

Cory, J.S., Myers, J.H., 2009. Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis. Journal of Animal Ecology 78, 646-655.

Cossentine, J.E., 2009. The parasitoid factor in the virulence and spread of lepidopteran baculovirus. Virologica Sinica 24, 305-314.

De Liñan, C., 1998. Entomología Agroforestal. Insectos y ácaros que dañan montes, cultivos y jardines. Agrotécnicas S.L., Madrid.

Del Pino, M., 2011. Biología, ecología y control de *Chrysodeixis chalcites* (Esper, 1789) (Lepidoptera: Noctuidae) en cultivos de platanera de Canarias. Tesis Doctoral. Universidad de La Laguna, Spain.

Del Pino, M., Carnero, A., Hernández-Suárez, E, Cabello, T., 2011. La lagarta o bicho camello, *Chrysodeixis chalcites* (Esper, 1789), una plaga emergente en los cultivos de platanera de Canarias. Phytoma 225, 21-16.

Del Pino, M., Carnero, A., Hernández-Suárez, E., 2012. Curvas de vuelo de *Chrysodeixis chalcites* en cultivos de platanera de Canarias. http://biomusa.net/es/documentos/8-hd-curvas-de-vuelo-de-chrysodeixis-chalcites-en-cultivos-de-platanera-de-canarias-3.

Del Pino, M., Carnero, A., Hernández-Suárez, E, Cabello, T., 2015. Bases para la gestión integrada de *Chrysodeixis chalcites* (Lep.:Noctuidae) en cultivos de platanera de Canarias. Phytoma 271, 42-47.

Domínguez, E., López-Cepero, J., Nogueroles, C., 2012. Identificación y control de plagas y enfermedades, in: Nogueroles, C. (Ed.), Calidad y sostenibilidad en el cultivo de la platanera en Canarias. ASPROCAN, Tenerife, Spain, pp.145-170.

Eberle, K.E., Asser-Kaiser, S., Sayed, S.M., Nguyen, H.T., Jehle, J.A., 2008. Overcoming the resistance of codling moth against conventional *Cydia pomonella* granulovirus (CpGV-M) by a new isolate CpGV-I12. Journal of Invertebrate Pathology 98, 293-298.

Eberle, K.E., Jehle, J.A., Huber, J., 2012. Microbial control of crop pest using insect viruses, in: Abrol, D.P., Shankar, U. (Eds.), Integrated pest management: principles and practice. CAB International, Oxfordshire, UK, pp. 281-298.

Engelhard, E.K., Kam-Morgan, L.N.W., Washburn, J.O., Volk, L.E., 1994. The insect tracheal system: a conduit for the systemic spread of *Autographa californica* M nuclear polyhedrosis virus. Proceedings of the National Academy of Sciences USA 91, 3224–3227.

EPPO, 2013. Database on quarantine pests, PQR (v5.1). European and Mediterranean Plant Protection Organization, Paris, France.

Erlandson, M.A., 2009. Genetic variation in field populations of baculoviruses: mechanisms for generating variation and its potential role in baculovirus epizootiology. Virologica Sinica 24, 458-469.

FAOSTAT, 2016. Food and agriculture data, http://www.fao.org/faostat/en/#data/QC

Federici, B.A., 1986. Ultrastructure of baculoviruses, in: Granados, R.R., Federici, B.A. (Eds.), The biology of baculoviruses. Boca Raton, Florida, USA, pp. 61-88.

Federici, B.A., 1997. Baculovirus pathogenesis, in: Miller, L.K. (Ed.), The baculoviruses. Plenum Press, New York, USA, pp. 33-59.

Figueiredo, E., Muñoz, D., Murillo, R., Mexia, A., Caballero, P., 2009. Diversity of Iberian nucleopolyhedrovirus wild-type isolates infecting *Helicoverpa armigera* (Lepidoptera: Noctuidae). Biological Control 50, 43-49.

Flipsen, J.T., Martens, J.W., van Oers, M.M., Vlak, J.M., van Lent, J.W., 1995. Passage of *Autographa californica* nuclear polyhedrosis virus through the midgut epithelium of *Spodoptera exigua* larvae. Virology 208, 328-335.

Funk, C.J., Braunagel, S.C., Rohrmann, G.F., 1997. Baculovirus structure, in: Miller, L.K. (Ed.), The baculoviruses. Plenum Press, New York, USA, pp.7-32.

Galán-Saúco, V., Samarin, J.G., Carbonell, E.A., 1984. Estudio de la práctica del deshijado y la fenología de la platanera (*Musa acuminata* Colla AAA, cultivar pequeña enana) en la isla de Tenerife. III. Fenología de la planta en la isla de Tenerife. Fruits 39, 595-605.

Galán-Saúco, V., 1992. Los frutales tropicales en los subtrópicos. II. Plátano (Banano), Mundi-Prensa, Madrid, Spain.

García, R, Ortega, G., Pérez, J.M., 1992, Insectos de Canarias. Cabildo Insular de Gran Canaria. Las Palmas de Gran Canaria, Spain.

Gaumont, R., Moreau, R., 1961. Observations on the bionomics of *Plusia chalcites* Esp. (Lepidoptera: Noctuidae). Revue de Zoologie Agricole et Appliquée 60, 31-36.

González-Concepción, C., Gil-Fariña, M.C., Pestano-Gabino, C., 2008. Multivariate modelling of the Canary Islands banana output. The role of farmer income expectation. Journal of Social Sciences 4, 88-97.

Goodey, B., 1991. *Chrysodeixis chalcites* - observations on the life cycle in captivity. Entomologists Record, 103, 111-118.

Granados, R.R., Williams, K.A., 1986. *In vivo* infection and replication of baculoviruses, in: Granados, R.R., Federici, B.A. (Eds.), The Biology of Baculoviruses. CRC Press, Boca Raton, Florida, USA, pp. 89-127.

Grant, M., Bouwer, G., 2009. Variation in the time-mortality responses of laboratory and field populations of *Helicoverpa armigera* infected with nucleopolyhdedrovirus. Journal of Applied Entomology 133, 647-650.

Gröner, A., 1986. Specificity and safety of baculoviruses, in: Granados, R.R., Federici, B.A. (Eds.), The biology of baculoviruses, Vol. 1. CRC Press, Boca Raton, Florida, USA, pp.177-202.

Grzywacz, D., Mushobozi, W.L., Parnell, M., Jolliffe, F., Wilson, K.., 2008. Evaluation of *Spodoptera exempta* nucleopolyhedrovirus (SexNPV) for the field control of African armyworm *Spodoptera exempta* in Tanzania. Crop Protection 27, 17-24.

Gupta, R.K., Raina, J.C., Arora, R.K., Bali, K., 2010. Selection and field effectiveness of nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hübner). International Journal of Virology 3, 45-59.

Hachler, M., Jermini, M., Brunetti, R., 1998. Two new harmful noctuids on tomatoes in glasshouse in South and Western Switzerland. Revue Suisse de Viticulture, d'Arboriculture et d'Horticulture 30, 281-285.

Harakly, F.A., Farag, S.S., 1975. Biological studies on the tomato looper *Chrysodeixis chalcytes* (Esper) in Egypt. Bulletin de la Societe Entomologique d'Egypte, 59, 295-299.

Harrison, R.L., Popham, H.J.R., Breitenbach, J.E., Rowley, D.L., 2012. Genetic variation and virulence of *Autographa californica* multiple nucleopolyhedrovirus and *Trichoplusia ni* single nucleopolyhedrovirus isolates. Journal of Invertebrate Pathology 110, 33-47.

Harrison, R.L., Keena, M.A., Rowley, D.L., 2014. Classification, genetic variation and pathogenicity of *Lymantria dispar* nucleopolyhedrovirus isolates from Asia, Europe and North America. Journal of Invertebrate Pathology 116, 27-35.

Hernández-Borges, J., Cabrera Cabrera, J., Rodríguez-Delgado, M.A., Hernández-Suárez, E.M., Galán-Saúco, V., 2009. Analysis of pesticide residues in bananas harvested in the Canary Islands (Spain). Food Chemistry 113, 313-319.

Hernández-Santana, M.P., 2007. Seguimiento de *Chrysodeixis chalcites* y *Spodoptera littoralis* en platanera en la isla de El Hierro, búsqueda de entomófagos y entomopatógenos. Trabajo Fin de Carrera de Ingeniería Técnica Agrícola. Universidad de La Laguna, Spain.

Herniou, E.A., Olszewski, J.A., Cory, J.S., O'Reilly, D.R., 2003. The genome sequence and evolution of baculoviruses. Annual Review of Entomology 48, 211-34.

Herniou, E. A., Arif, B.M., Becnel, J.J., 2012. Family Baculoviridae, in: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus taxonomy, ninth report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, Netherlands, pp. 163-173.

Horowitz, A.R., Weintraub, P.G., Ishaaya, I., 1998. Status of pesticide resistance in arthropod pests in Israel. Phytoparasitica 26, 231-240.

Horton, H.M., Burand, J.P., 1993. Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. Journal of Virology 67, 1860-1868.

Ibarra, J.E., Del Rincón Castro, M.C., 2001. Capacidad insecticida de los baculovirus, in: Caballero, P., López-Ferber, M., Williams, T. (Eds.), Los baculovirus y sus aplicaciones como bioinsecticidas en el control biológico de plagas. Phytoma, Valencia, España, pp. 203-224.

ICTV, 2016. International Committee on Taxonomy of Viruses. http://www.ictvonline.org/virusTaxonomy.asp.

Ignoffo, C.M., 1981. Living microbial insecticides, in: Norris, J.R., Richmond, M.H. (Eds), Essays in Applied Microbiology. John Wiley, New York, pp. 2-31.

Ignoffo, C.M., Couch, T.L., 1981. The nuclear polyhedrosis virus of *Heliothis* species as a microbial insecticide, in: Burges, H.D. (Ed.), Microbial control of pests and plant diseases. Academic Press, London, pp. 330-362.

Ignoffo, C.M., 1992. Environmental factors affecting persistence of entomopathogens. Florida Entomologist 75, 516-525.

Inceoglu, A.B., Kamita, S.G., Hammock, B.D., 2006. Genetically modified baculovirus: a historical overview and future outlook. Advances in Virus Research 68, 323-360.

Inserra, S., Calabretta, C., 1985. Attack by noctuids: a recurring problem in greenhouse crops of the Ragusa coast. Tecnica Agricola 37, 283-297.

Ippolito, R., Parenzan, P., 1985. *Hoplodrina ambigua* D. & S. (Lepidoptera - Noctuidae) and other noctuids on artichoke in Apulia. Entomologica 20, 147-158.

Jannone, G., 1966. Results of phytosanitary inspections of foreign plants and plant products in the port of Genoa. On the presence of larvae of three species of Lepidoptera in consignments of bananas originating from the Canary Islands. Rivista di Agricoltura Subtropicale e Tropicale 60, 5-34.

Jehle, J.A., Blissard, G.W., Bonning, B.C., Cory, J.S., Herniou, E.A., Rohrmann, G.F., Theilmann, D.A., Thiem, S.M., Vlak, J.M., 2006. On the classification and nomenclature of baculoviruses: a proposal for revision. Archives of Virology 151, 1257-1266.

Katsuma, S., Koyano, Y., Kang, W., Kokusho, R., Kamita, S.G., Shimada, T., 2012. The baculovirus uses a captured host phosphatase to induce enhanced locomotory activity in host caterpillars. Plos Pathogens 8, e1002644.

King, A.M., Adams, M.J., Lefkowitz, E.J., Carstens, E.B., 2012. Virus taxonomy: Classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, San Diego, California, USA.

Lacey, L.A., Headrick, H.L., Arthurs, S.P., 2008. Effect of temperature on long-term storage of codling moth granulovirus formulations. Journal of Economic Entomology 101, 288-294.

Lempke, B.J., 1982. Interesting observations and catches of Lepidoptera in 1980. Entomologische Berichten 42,116-119.

Li, X., Burnight, E.R., Cooney, A.L., Brady, T., Sander, J.D., Staber, J., Wheelan, S.J., Joung, J.K., McCray, P.B., Bushman, F.D., Sinn, P.L., Craig, N.L., 2013. PiggyBac transposease tools for genome engineering. Proceedings of the National Academy of Sciences USA 25, 2279-2287.

Loginova, E., 1992. Some new pests of glasshouse crops in Bulgaria and their control by an IPM programme. Bull. OEPP/EPPO 22, 357-361.

López-Cepero, J., 2015. Análisis de residuos y gestión integrada de plagas en platanera. Phytoma 271,48-52.

López-Cepero, J., Puerta, M., Piedra Buena, A., 2014. Guía para la gestión integrada de plagas en platanera. Cuadernos divulgativos. COPLACA, 2.

Lorenzo, J.M., 2005. Seguimiento de la dinámica poblacional de *Dysmicoccus grassi* (Leonardi) (Hompotera: Pseudococcidae) y *Tetranichus urticae* Koch (Acari: Tetranychidae) en *Musa acuminata* Colla. Subgrupo Cavendish, cvs. Pequeña Enana y Gran Enana, al aire libre y en invernadero respectivamente**.** Tesis Doctoral Facultad de Biología. Universidad de La Laguna, Spain.

Lorenzo-Rodríguez, C. E., 2016**.** Determinación del nivel de ingesta de *Chrysodeixis chalcites* en platanera como base para la estimación de su Nivel Económico de Daño (NED) y umbral de tratamiento (UT). Trabajo Fin de Grado. Escuela Superior y Técnica de Ingeniería Agraria. Universidad de León, Spain.

MAPAMA, 2016. Superficies y producciones anuales de cultivo de acuerdo con el Reglamento (C) 543/2009. http://www.magrama. gob. es/estadística/pags/anuario/

2015-Avance/AE_2015_Avance.pdf.

Martín-Gil, A., González-Hernández, A., López-Leal, A., Espino-De Paz, A., Piedra-Buena, A., Taberner-Palou, A., Siguero-Montes, A., Ramos-Luis, A., Romero-Cuadrado, C., Domínguez-Palarea, E., Hernández-Suárez, E., López-Cepero, J., Reyes-Carlos, J.A., Arévalo-Jiménez, M.A., Gómez-Calmaestra, R., Perera-González, S., 2016. Guía de gestión integrada de plagas. Platanera. Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente (MAPAMA), Madrid, Spain.

Méndez-Hernández, C., Perera-González, S., Rodríguez-Serrano, M., 2017. Guía de lucha contra las plagas de la platanera. Tabla de productos fitosanitarios autorizados contra plagas y enfermedades en platanera. http://www.agrocabildo.org/publica/Publicaciones/subt_48_Anexo%20I.pdf.

Meszaros, Z., Tusnadi, C.K., 1994. Damage of a rare migrant noctuid moth (*Chrysodeixis chalcytes* Esper, Lep.: Noctuidae) in a glasshouse. Növényvédelem, 30, 83-84.

Miele, S.A.B., Garavaglia, M.J., Belaich, M.N., Ghiringhelli, P.D., 2011. Baculovirus: Molecular insights on their diversity and conservation. International Journal of Evolutionary Biology 2011, 379424. doi: 10.4061/2011/379424.

Moore, S.D., Pittaway, T., Bouwer, G., Fourie, J.G., 2004. Evaluation of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) for control of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on citrus in South Africa Biocontrol Science and Technology 14, 239-250.

Moscardi, F., 1999. Assessment of the application of baculoviruses for the control of Lepidoptera. Annual Review of Entomology 44, 257-289.

Moscardi, F., Souza, M.L., Castro, M.E.B., Moscardi, M., Szewczyk, B., 2011. Baculovirus pesticides: present state and future perspectives, in: Ahmad, I., Ahmad, F., Pichtel, J. (Eds.), Microbes and Microbial Technology. Springer, New York, USA, pp. 415-445.

Muñoz, D., Caballero, P., 2001. Diversidad natural de los baculovirus, in: Caballero, P., López-Ferber, M., Williams, T. (Eds.), Los baculovirus y sus aplicaciones como bioinsecticidas en el control biológico de plagas. Phytoma, Valencia, Spain, pp. 95-118.

Muñoz, D., Castillejo, J.I., Caballero, P., 1998. Naturally occurring deletion mutants are parasitic genotypes in a wild-type nucleopolyhedrovirus population of *Spodoptera exigua.* Applied and Environmental Microbiology 64, 4372-4377.

Murillo, H., Hunt, D.W.A., VanLaerhoven, S. L., 2013. First records of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae: Plusiinae) for east- central Canada. Canadian Entomologist 145, 338-342.

Murillo, R., Lipa, J.J., Muñoz, D., Amate, J., Barranco, P., Cabello, T., Caballero., P., 2000. Caracterización bioquímica de un nucleopoliedrovirus de *Chrysodeixis chalcites* autóctono de España. Boletin de Sanidad Vegetal Plagas 26, 637-644.

Passoa, S., 1995. *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae). PPQ Alert List Fact Sheet 35, USDA, APHIS.

Pedigo, L. P., 1996. Entomology and Pest Management. Second Edition. Prentice-Hall Pub., Englewood Cliffs, New Jersey, USA.

Pedigo, L. P., Hutchins, S. H., Higley, L. G., 1986. Economic injury levels in theory and practice. Annual Review of Entomology 31, 341-368.

Perera, S., Molina, M.J., 2002. Plagas y enfermedades de la platanera en Canarias y su control integrado, Coplaca (Cooperativa Platanera de Canarias), Tenerife, Spain.

Perera, S., Molina, M.J., 2007. Plagas y enfermedades en el cultivo ecológico de laplatanera, in: Nogueroles, C., Líbano, J. (Eds.), El cultivo ecológico de la platanera en Canarias. Gabinete de Proyectos Agroecológicos S.L., Tenerife, Spain, pp. 77-118.

Pérez-Moreno, I., 2000. Fundamentos teóricos del manejo integrado de plagas. Boletín Sociedad Española de Entomología Aplicada 27, 127-133.

Polaszek, A., Rugman-Jones, P.F., Stouthamer, R., Hernández-Suárez, E., Cabello, T., Del Pino-Pérez, M., 2012. Molecular and morphological diagnoses of five species of *Trichogramma*: biological control agents of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae) in the Canary Islands. BioControl 57, 21-35.

Rashid, F.F., Hammad, S.M., Hassan, S.M., 1971. The biology of *Autographa chalcites* L. in Alexandria region (Lepidoptera: Noctuidae). Bulletin de la Societe Entomologique d'Egypte, 55, 419-426.

Redman, E.M., Wilson, K., Grzywacz, D., Cory, J.S., 2010. High levels of genetic diversity in *Spodoptera exempta* NPV from Tanzania. Journal of Invertebrate Pathology 105, 190-193.

Regan, K., Ordosch, D., Glover, K.D., Tilmon, K.J., Szczepaniec, A., 2017. Effects of a pyrethroid and two neonicotinoid insecticides on population dynamics of key pest of soybean and abundance of their natural enemies. Crop Protection 98, 24-32.

Robinson, J.C., Galán-Saúco, V., 2010. Bananas and Plantations. CAB International, Wallingford, UK.

Rodgers, P.B., 1993. Potential of biopesticides in agriculture. Pesticide Science 39, 117-129.

Rodríguez, V.A., Belaich, M.N., Ghiringhelli, P.D., 2012. Baculoviruses: Members of Integrated Pest Management Strategies, in: Larramendy, M.L., Soloneski, S., (Eds.), Integrated Pest Management and Pest Control - Current and Future Tactics. InTech, Rijeka, Croatia, pp. 463-480.

Rohrmann, G.F., 2013. Baculovirus Molecular Biology. National Library of Medicine, National Center for Biotechnology Information, Bethesda, USA.

Rowley, D.L., Popham, H.J.R., Harrison, R.L., 2011. Genetic variation and virulence of nucleopolyhedroviruses isolated worldwide from the *heliothine* pests *Helicoverpa armigera*, *Helicoverpa zea*, and *Heliothis virescens*. Journal of Invertebrate Pathology 107, 112-126.

Serrano, A., Williams, T., Simón, O., López-Ferber, M., Caballero, P., Muñoz, D., 2013. Analogous population structures for two alphabaculoviruses highlight a functional role for deletion mutants. Applied and Environmental Microbiology 79, 1118-1125.

Seymour, P.R., Kilby, L.J., 1978. Insects and other invertebrates intercepted in check inspections of imported plant material in England and Wales during 1978. ADAS, Ministry of Agriculture, Fisheries and Food, Harpenden, UK.

Shapiro, M., 1986. *In vivo* production of baculoviruses, in: Granados, R.R., Federici, B.A. (Eds.), The biology of baculoviruses. CRC Press, Boca Raton, Florida, USA, pp. 31-62.

Shepard, B.M., Hammig, M.D., Carner, G.R., Ooi, P.A.C., Smith, J.P., Dilts, R., Rauf, A., 2009. Implementing integrated pest management in developing and developed countries, in: Peshin, R. (Ed.), Dissemination of IPM technology: Theory and practice. Springer, Netherlands, pp. 275-305.

Simón, O., Bernal, A., Williams, T., Carnero, A., Hernández-Suárez, A., Muñoz, D., Caballero, P., 2015. Efficacy of an alphabaculovirus-based biological insecticide for control of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) on tomato and banana crops. Pest Management Science 71, 1623-1630.

Simón, O., Palma, L., Williams, T., López-Ferber, M., Caballero, P., 2012. Analysis of a naturally-occurring deletion mutant of *Spodoptera frugiperda* multiple nucleopolyhedrovirus reveals *sf58* as a new *per os* infectivity factor of lepidopteran-infecting baculvoiruses. Journal of Invertebrate Pathology 109, 117-126.

Slack, J., Arif, B.M., 2007. The baculoviruses occlusion-derived virus: virion structure and function. Advances in Virus Research 69, 99-165.

Slavicek, J.M., Popham, H.J.R., 2005. The *Lymantria dispar* nucleopolyhedrovirus enhancins are components of occlusion-derived virus. Journal of Virology 79, 10578-10588.

Stanley, J., Chandrasekaran, S., Regupathy, A., 2009. Baseline toxicity of emamectin and spinosad to *Helicoverpa armigera* (Lepidoptera: Noctuidae) for resistance monitoring. Entomological Research 39, 321-325.

Steinhaus, E.A., 1956. Microbial control, the emergence of an idea. Hilgardia. 26**,** 107-160.

Stoltz, D. B., Pavan, C., Cunha, A.B.D., 1973. Nuclear polyhedrosis virus: A posible example of de novo intranuclear membrane morphogenesis. Journal of General Virology 19, 145-150.

Szewczyk, B., Hoyos-Carvajal, L., Paluszek, M., Skrzecz, W., Souza, M.L., 2006. Baculoviruses re-emerging biopesticides. Biotechnology Advances 24, 143-160.

Szewczyk, B., Rabalski, L., Krol, E., Sihler, W., Souza, M.L., 2009. Baculovirus biopesticides - a safe alternative to chemical protection of plants. Journal of Biopesticides, 2, 209-216.

Thézé, J., Cabodevilla, O., Palma, L., Willaims, T., Caballero, P., Herniou, .A. 2014. Genomic diversity in European *Spodoptera exigua* multiple nucleopolyhedrovirus isolates. Journal of General Virology 95, 2297-2309.

Toguebaye, B.S., Bouix, G., 1983. *Nosema manierae* n.sp. a microsporidian parasite of *Chilozacconius* (natural host) and *Heliothis armigera* (experimental host): life cycle and ultrastucture. Zeitschrift fur Parasitenkunde-Parasitology Research. 69, 191-205.

Undorf-Spahn, K., Fritsch, E. Huber, J., Kienzle, J., Zebitz, C.P.W., Jehle, J.A., 2012. High stability and no fitness costs of the resistance of codling moth to *Cydia pomonella* granulovirus (CpGV-M). Journal of Invertebrate Pathology 111, 136-142.

Uygun, N., Ozgur, F., 1980. Identification of pests of greenhouse vegetables in the Icel and Adana regions, and the effects of endosulfan smoke tablets and pirimicarb on *Myzus persicae* (Sulz.). Turkish Journal of Plant Protection 4, 185-192.

Vacante, V., Benuzzi, M., Palmeri, V., Brafa, G., 2001. Experimental trials of microbiological control of the Turkey moth (*Chrysodeixis chalcites* (Esper)) in Sicilian greenhouse crops. Informatore Fitopatologico 51, 73-76.

van Houte, S., Ros, V.I.D., Mastenbroek, T.G., Vendrig, N.J., Hoover, K., Spitzen, J., van Oers, M.M., 2012. Protein tyrosine phosphatase-induced hyperactivity is a conserved strategy of a subset of baculoviruses to manipulate Lepidopteran host behavior. Plos One 7, e46933.

van der Linden, A., 1996. Control of caterpillars in integrated pest management. Integrated Control in Glasshouses. IOBC wprs Bulletin, 19, 91-94.

van Loo, N.D., Fortunati, E., Ehlert, E., Rabelink, M., Grosveld, F., Scholte, B.J., 2001. Baculovirus infection of nondividing mammalian cells: Mechanisms of entry and nuclear transport of capsids. Journal of Virology 75, 961-970.

van Oers, M.M., Herniou, E.A., Usmany, M., Messelink, G.J., Vlak, J.M., 2004. Identification and characterization of a DNA photolyase-containing baculovirus from *Chrysodeixis chalcites*. Virology 330, 460-470.

van de Veire, M., 1993. First observations in glasshouse sweet peppers in Belgium and laboratory rearing of the parasitic wasp *Eulophus pennicornis* (Hym.: Eulophidae). Entomophaga, 38, 61-62.

Vasconcelos, S.D., Williams, T., Hails, R. S., Cory, J.S., 1996. Prey selection and baculovirus dissemination by carabid predators of Lepidoptera. Ecological Entomology 21, 98-104.

Vincent, C., Andermatt, M., Valéro, J., 2007. Madex and Virosoft CP4, viral biopesticides for codling moth control, in: Vincent, C., Goethel, M.S., Lazarovits, G., (Eds.), Biological Control: A global perspective. CAB International, Oxfordshire, UK, pp. 336-343.

Vilardebo, A., Guérout, R., 1964. *Plusia chalcites* Esp. (Lépidoptére Noctuidae) nuisible au bananier dans les Régions cotéres de l’ouest africain. Fruits 19, 195-208.

de Vos, R., Rutten, A.L.M., 1995. Migrating Lepidoptera in 1992 (fifty-third report). Entomologische Berichten 55, 37-46.

Wang, P., Granados, R.R., 1997. An intestinal mucin is the target substrate for a baculovirus enhancin. Proceedings of the National Academy of Science USA 94, 6977-6982.

Williams, T., Valle, J., Viñuela, E., 2003. Is the naturally derived insecticide Spinosad compatible with insect natural enemies? Biocontrol Science and Technology 13, 459-475.

Wood, H.A., Hughes, P.R., Shelton, A., 1994. Field studies of the cooclusion strategy with a genetically altered isolate of the *Autographa californica* nuclear polyhedrosis virus. Environmental Entomology 23, 211-219.

Woods, R., 2011. The moths of selected sites in the Tees Valley. The results of a 2011 study of five sites owned by Northumbrian Water Ltd. Industry Nature Conservation Association (INCA). pp. 78.

Yang, M.M., Li, M.L., Zhang, Y.A., Wang, Y.Z., Qu, L.J., Wang, Q.H., Ding, J.Y., 2012. Baculoviruses and insect pests control in China. African Journal of Microbiology Research 6, 214-218.

Zandigiacomo, P., 1990. The principal pests of soybean in north-eastern Italy in 1989. Informatore Fitopatologico 40, 55-58.

Zhang, B.C., 1994. Index of Economically Important Lepidoptera. CAB International. Wallingford, pp. 599.

Zhang, G.Y., Sun, X.L., Zhang, Z.X., Zhang, Z.F., Wan, F.F., 1995. Production and effectiveness of the new formulation of *Helicoverpa* virus pesticide-emulsifiable suspension. Virologica Sinica 10, 242-247.

Zimmerman, E.C., 1958. *Plusia (Autographa) chalcites* (Esper), in: Zimmerman, E.C. (Ed.), Insects of Hawaii. A Manual of the Insects of the Hawaiian Islands, including an enumeration of the species and notes on their origin, distribution, hosts, parasites, etc. Volume 7. Macrolepidoptera. The University of Hawaii Press; Honolulu, pp. 370-372.

CHAPTER II

Chrysodeixis chalcites, a pest of banana crops on the Canary Islands: incidence, economic losses and current control measures

ABSTRACT

Chrysodeixis chalcites is an emergent pest in bananas grown on the Canary Islands. Feeding damage to leaves and fruit and the control measures targeted at this pest were evaluated over a two-year period (2013-2014). The prevalence of infestations (42-100%) on the islands was similar during the two years of the study. Mean foliar damage (1.5-7.3% depending on island) and fruit damage (1.0-5.7%) detected in field surveys varied significantly across islands, plantation aspect and season. Fruit damage was not correlated with foliar damage ($P>0.05$). The weight of *C. chalcites* damaged bananas varied significantly (0.2-4.2% of harvested fruit) across islands, particularly in the spring. Overall 3,155 tonnes bananas/yr are likely discarded due to *C. chalcites* damage, representing 1.5% of annual production or 2.68 million €/yr. The most frequently used pesticide was indoxacarb, usually applied on three occasions per crop cycle, for which the cost of control measures would average 240 €/ha per crop cycle. The direct damage that *C. chalcites* causes to banana fruit results in significant economic losses in addition to the direct costs of pesticide based control measures. Effective and sustainable control strategies are required against this pest.

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1. INTRODUCTION

Banana (*Musa acuminata* Colla) represents one of the most important agricultural goods with an annual trade valued at nine billion dollars worldwide (Arias et al., 2003; Hallam, 1995; Raynolds, 2003). Banana is the main crop of the Canary Islands, representing 30% of total agricultural production (González-Concepción et al., 2008; Robinson and Galán-Saúco, 2010). In 2015, this crop was grown over an area of 8,975 hectares (i.e., 23% of the total cultivated area of the archipelago), with a total production of 381,983 tonnes, representing 0.3% of the world's banana production. The island of Tenerife is the largest producer of bananas (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 the Iberian Peninsula (91%), and only a small fraction is exported to Western Europe (0.1%), whereas the remainder (8.9%) is consumed locally (ASPROCAN, 2016; MAPAMA, 2016). Banana production is also of great social importance in the Canary Islands as it employs over 7,000 people, representing up to 10% of jobs on some of the islands. Many other sectors also provide services to, or depend on, banana production, including the tourism sector as banana plantations have become a destination for tourist visits (Bianchi, 2004; González-Concepción et al., 2008).

Bananas are cultivated in mesh-built greenhouses on the warmer southern slopes and in the open-field on the cooler, northern areas of these islands. As such, crops grown under mesh tend to be more prone to phytosanitary problems (Del Pino et al., 2011; Galán-Saúco, 1992; Robinson and Galán-Saúco, 2010). The noctuid moth, *Chrysodeixis chalcites* (Esper, 1789) was previously a minor pest that caused feeding damage leaves which did not usually justify control measures (Del Pino et al., 2011). However, since 2000, this insect has shown marked changes in its feeding habits for reasons that remain unknown. Specifically, larvae have begun to feed on the skin of the banana fruit causing marked aesthetic damage that completely eliminates the commercial value of the fruit (Del Pino et al., 2011; Perera and Molina, 2007). As a result of this unusual behavior, *C. chalcites* is now the lepidopteran pest that requires the greatest number of insecticide treatments and control costs appear to be increasing over time (Del Pino et al., 2011; Domínguez et al., 2012).

Although *C. chalcites* larvae are known to seriously affect the saleable yield of banana (Del Pino et al., 2011; Perera and Molina, 2007), accurate estimates of pest-induced losses are not available. Determining the production losses and economic impact caused by this pest in banana crops on the Canary Islands is necessary for pest control decision-making. Pest population assessment is one of the fundamental aspects used to determine the frequency of pest control interventions in Integrated Pest Management (IPM) programs. Since 2014 an IPM approach is mandatory in all crop production systems in Spain (Royal Decree 1311/2012, which incorporates European Directive 2009/128/EEC that established a framework for the sustainable use of pesticides in the European Union). Effective decision-making in IPM also relies on understanding the relationships between pest numbers, plant responses to injury and the resultant economic losses (Higley and Pedigo, 1993; Pedigo, 1996).

The objective of the present study was to evaluate the incidence and feeding damage inflicted by *C. chalcites* as well as to estimate the production losses and economic impact of this pest on banana crops in the Canary Islands. We also aimed to estimate losses arising from direct damage to fruit and indirect costs involved in the purchase and application of insecticides targeted at this insect. The results of the present study are part of a wider approach to define *C. chalcites* damage thresholds in banana crops and the potential use of novel biological insecticides that are currently under development (Bernal et al., 2013; Simón et al., 2015).

2. MATERIALS AND METHODS

2.1. Evaluation of *C. chalcites* incidence and feeding damage

To evaluate the incidence and damage due to *C. chalcites*, surveys were conducted in commercial banana plantations both in open-field and mesh-built greenhouses, on the five main banana-producing islands of the archipelago: Tenerife, La Palma, Gran Canaria, La Gomera and El Hierro.

A total of 81 surveys were conducted over a period of two years, during the spring, summer and autumn of 2013 and 2014 (Fig. 1; Table 1).

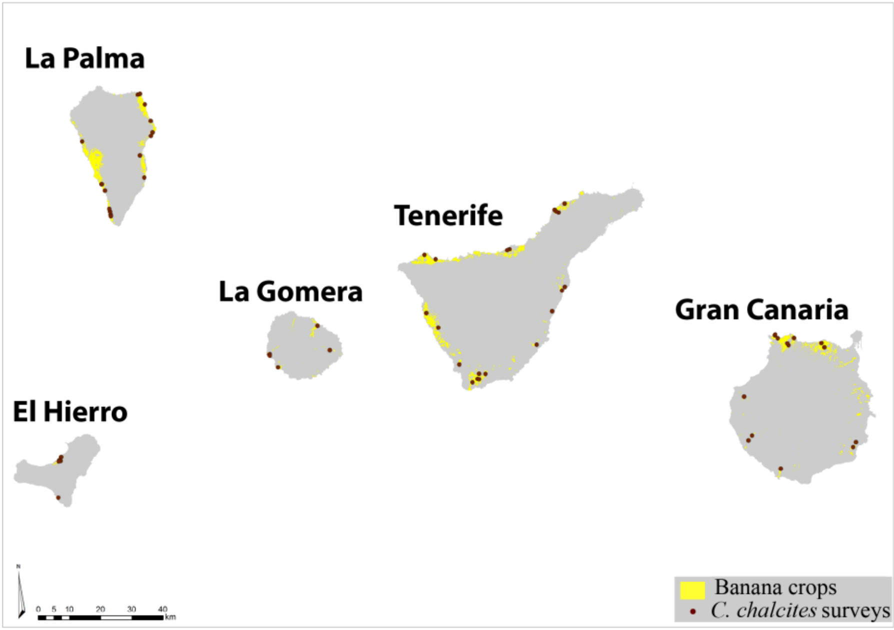

Figure 1. Five main banana-producing islands of Canary archipelago with banana crop areas and surveys realized in them.

Surveys were not performed during the winter (November–February), as previous studies demonstrated that the pest was absent or present in very low densities (Del Pino et al., 2011; Perera and Molina, 2007). Damage assessments were performed on thirty randomly selected plants following a zigzag pattern within each plantation. For this, each location was classified as infested or not by *C. chalcites* based on direct observation of feeding damage on leaves and fruit. The aspect of each banana plot (North- or South-facing)

Table 1. Sampled localities with coordinates, number of plots inspected in each location and number of surveys conducted in each plot during spring (March to May), summer (June to August) and autumn (September to November) in 2013 and 2014.

Locality	Coordinates		Plots (Surveys)					
			2013			2014		
	Latitude (N)	Longitude (W)	Spring	Summer	Autumn	Spring	Summer	Autumn
Tenerife								
Arona	28° 01′ 47″	16° 39′ 07″	-	3(3)	2(2)	5(5)	2(2)	-
Adeje	28° 04′ 19″	16° 43′ 02″	-	1(1)	-	1(1)	-	-
Guía Isora	28° 13′ 08″	16° 49′ 40″	-	-	1(1)	-	-	-
Fasnia	28° 13′ 45″	16° 24′ 57″	-	-	-	1(1)	-	-
Güimar	28° 17′ 59″	16° 22′ 31″	-	-	-	1(1)	-	1(1)
Los Silos	28º 22' 30"	16º 48' 01"	-	-	-	-	-	1(1)
Buenavista	28° 23′ 12″	16° 50′ 15″	-	-	-	1(1)	-	-
Puerto Cruz	28° 24′ 24″	16° 33′ 30″	-	-	2(2)	-	-	-
Valle Guerra	28° 31′ 04″	16° 24′ 25″	-	1(1)	1(1)	-	1(1)	-
Tejina	28° 32′ 24″	16° 22′ 44″	-	-	-	-	1(1)	-
La Palma								
Fuencaliente	28° 29′ 38″	17° 52′ 07″	1(1)	2(2)	-	-	-	2(2)
El Remo	28° 33′ 18″	17° 53′ 15″	-	-	-	-	1(1)	1(1)
Charo Verde	28° 34′ 26″	17° 53′ 55″	1(1)	1(1)	-	-	-	-
Mazo	28° 35′ 43″	17° 45′ 33″	-	-	-	1(1)	-	-
Breña Alta	28° 39′ 34″	17° 46′ 32″	-	-	-	-	-	1(1)
Tijarafe	28° 41′ 45″	17° 57′ 58″	-	-	-	1(1)	-	-
Puntallana	28° 42′ 59″	17° 44′ 26″	-	-	-	3(3)	-	-
San Andrés	28° 48′ 25″	17° 45′ 47″	-	-	-	-	1(1)	-
Barlovento	28° 50′ 16″	17° 46′ 48″	-	-	-	-	2(2)	-
Gran Canaria								
Arguineguín	27° 46′ 41″	15° 39′ 57″	-	-	-	2(2)	-	-
Vecindario	27° 50′ 28″	15° 25′ 51″	1(1)	-	1(1)	-	2(2)	1(1)
Veneguera	27° 52′ 23″	15° 45′ 37″	-	-	2(2)	-	-	-
Aldea	27° 59′ 13″	15° 47′ 12″	-	-	1(1)	-	-	-
Arucas	28° 07″ 51″	15° 31′ 34″	1(1)	-	-	-	-	1(1)
Guía	28° 09′ 27″	15° 37′ 31″	1(1)	-	-	-	-	-
Gáldar	28° 10′ 01″	15° 41′ 13″	1(1)	-	4(4)	-	-	-
La Gomera								
La Dama	28° 03′ 17″	17° 18′ 33″	1(1)	-	-	-	-	-
Valle Gran Rey	28° 05′ 33″	17° 20′ 13″	-	-	-	-	3(3)	-
San Sebastián	28° 06′ 26″	17° 08′ 29″	-	-	-	-	1(1)	-
Hermigua	28º 10′ 37″	17º 10′ 59″	-	-	-	-	1(1)	-
El Hierro								
Tacorón	27° 39′ 50″	18° 01′ 03″	-	-	-	1(1)	-	-
Frontera	27° 46′ 52″	18° 00′ 29″	-	-	4(4)	4(4)	3(3)	-

was also noted. Taking into account the number of banana plots with damage and the total number of plots analyzed, the prevalence of *C. chalcites* infestation for each island and aspect was calculated.

Foliar damage was evaluated in young banana plants (immature plants of less than 1 m height) (Fig. 2A), using a visual scale consisting in five categories: Category 0: no damage, Category 1: 5-20% leaf damage (percentage of leaf area showing damage), Category 2: 21-40% leaf damage, Category 3: 41-60% leaf damage and Category 4: >60% leaf damage (Del Pino M, unpublished data) (Fig. 2B). Damage to fruit, in contrast, was evaluated in adult plants (Fig. 2A) by determining the percentage of damaged banana handles respect to the total present on each bunch. A small fruit perforation characteristic of *C. chalcites* feeding in any finger (fruit) of the handle was considered as the entire handle affected (as the handle would be discarded during processing and packing), after the area encompassing these damaged handles was estimated respect to the total and assigned to one of the following categories: Category 0: no damage, Category 1: 2-10% of the handles of the bunch damaged, Category 2: 11-25% of the handles, Category 3: 26-50% of the handles, Category 4: 51-75% of the handles, and Category 5: 76-100% of the handles (Fig. 2C).

Thereafter, average percentage of foliar and fruit damage was calculated using the formula described by Townsend-Heuberger (1943):

$$\left(\frac{\sum(\mathrm{nxv})}{\mathrm{V\,x\,N}}\ \mathrm{x}\,100\right)$$

Where n is the number of sample units in each category, v the value of each category (0, 1, 2, 3, 4 or 5), V the value of the highest category and N the total number of sample units.

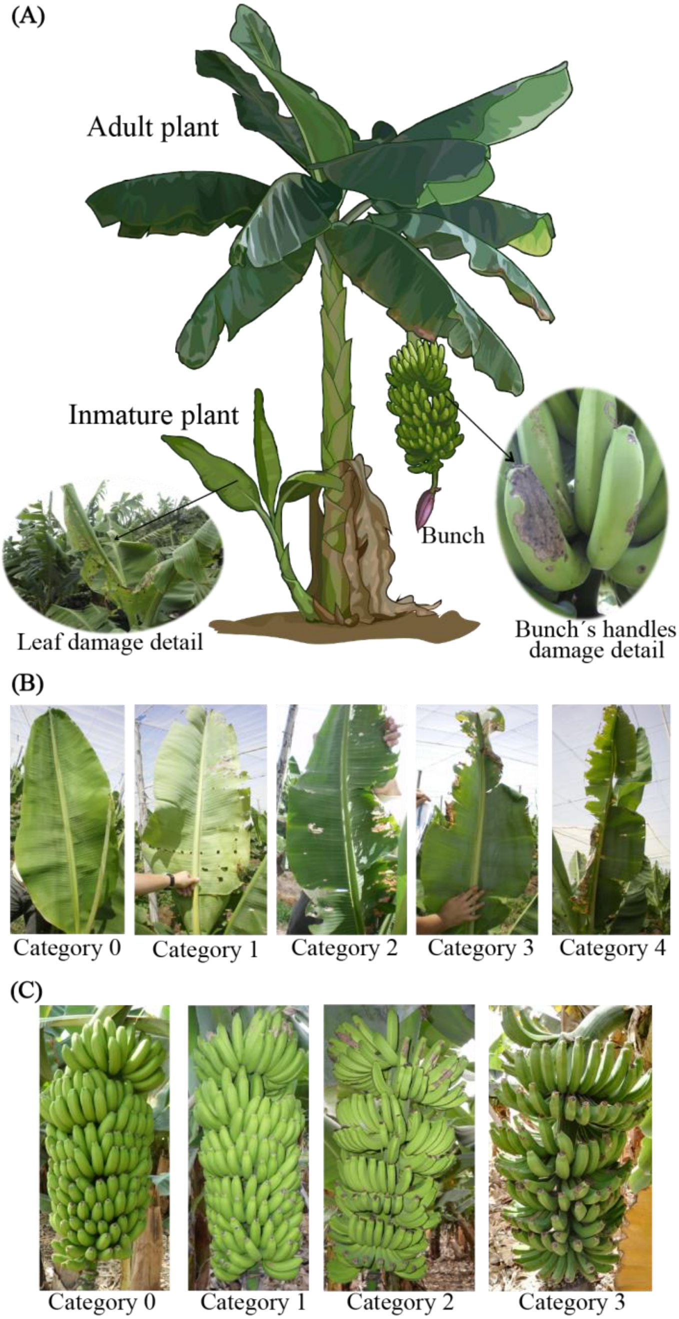

Figure 2. (A) Scheme of immature banana plant with detail of leaf damage by *C. chalcites* and adult banana plant with bunch and detail of bunch handles damage. (B) Visual scale of leaf damage by *C. chalcites* and (C) visual scale of fruit damage by *C. chalcites.*

The data obtained on the island of La Gomera were not included in the analyses, as few surveys were performed due to the low incidence observed by the field technicians, and because just one survey was performed in 2013, which was negative for *C. chalcites* infestation.

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

Direct economic losses were estimated by sampling in banana processing facilities. A processing facility was selected on each island that received bananas from different areas (Fig. 3). The processing facilities were located at San Miguel de Abona (Tenerife, 28° 6′ 11″ N, 16° 36′ 42″ W), Los Llanos de Aridane (La Palma, 28° 39′ 56″ N, 17° 54′ 32″ W), Gáldar (Gran Canaria, 28° 9′ 29″ N, 15° 39′ 37″ W) and Frontera (El Hierro, 27° 46′ 26″ N, 18° 0′ 40″ W). The packaging plant on Tenerife received bananas from 9 banana-growing zones across 5 municipalities. On La Palma, the packaging plant received bananas from 7 zones (3 municipalities). The packaging plant on Gran Canaria received bananas from 5 municipalities and that on El Hierro received fruit from one municipality (Fig. 3). Each zone was sampled on at least three occasions.

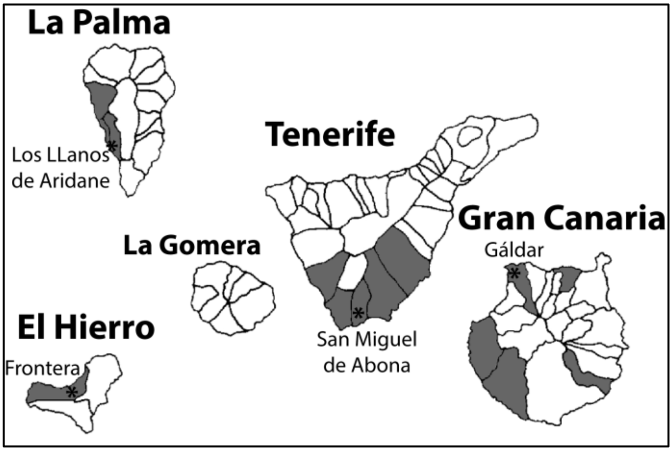

Figure 3. Location of banana processing facilities sampled in the islands and banana-growing zones where them received bananas.

A total of 30 surveys, from November 2014 to September 2015, were performed in the different banana packaging plants: 13 in Tenerife (samples taken from 26 different batches of fruit arriving from local municipalities mentioned above), 10 in La Palma (27 samples), 4 in Gran Canaria (20 samples) and 3 in El Hierro (20 samples). Each survey lasted 6 hours during the morning processing and packaging activity in the plant (8.00–14.00 hrs).

Economic losses were estimated by weighing the fruit discarded due to *C. chalcites* damage. The total weight of discarded fruit was then obtained in relation to the total weight of fruit processed during the 6 h sampling period in each processing plant.

To extrapolate the losses across each island and their economic impact, the number of hectares cultivated in each island was considered, as well as the market price for bananas in 2015, which depends on their quality (ASPROCAN, 2016). The average annual banana production was 174,792 tonnes on Tenerife, 131,585 tonnes on La Palma, 78,935 tonnes on Gran Canaria, and 2,856 tonnes on El Hierro. The average prices during the sampling period were 0.97 €/kg for premium quality, 0.81 €/kg for first class and 0.51€/kg for second class fruit. As it was not possible to determine which quality category damaged bananas would have been assigned to in the absence of *C. chalcites*-induced feeding damage, the losses for each category were based on the average production in each category in the study period, namely 47% in the premium category, 41% for first class and 12% for second class categories.

2.3. Estimated costs of control

The costs derived from *C. chalcites* control in banana were estimated following established methodology (Bielza and Lacasa, 1998). Insecticidal cost involves the costs associated with the purchase of the insecticidal product (product price, application rate, and frequency of treatments) and the implementation costs (labour and time spent).

To calculate the insecticide cost, the prices of the phytosanitary products were obtained from local distributors. The application rates (dose/hl) were those recommended by the product label in banana cultivation, and the frequency of treatments was obtained from interviews described below.

As the implementation costs are variable, different surveys were carried out on the islands by interviewing field technicians and banana growers. A total of 40 interviews were conducted in different areas of the islands, 15 in Tenerife, 10 in La Palma, 9 in Gran Canaria and 6 in El Hierro. The main information sought through this method was focused on the area treated, the type of production system (open-air or mesh-greenhouse), the part of the plant to be treated, the application system, the volume of each treatment, the labour costs (€/hour and €/ha), the dose applied and frequency of treatments.

2.4. Statistical analysis

Data on the prevalence of infestation across years or seasons were fitted to generalized linear models with a binomial error structure specified in GLIM (Numerical Algorithms Group 1993). The significance of changes in model deviance were assessed with reference to χ^2 statistics (Crawley, 1993). Data on the prevalence of infestation in plots with a northern-facing or a southern-facing aspect were subjected to t-tests whereas data that could not be normalized by transformation were subjected to Mann-Whitney U tests.

The percentages of foliar and fruit damage were normalized by arcsine transformation and subjected to analysis of variance (ANOVA) and Tukey's test ($P<0.05$) of average percentages by islands and by seasons, whereas comparisons of year and aspect were performed by t-tests. Data that could not be normalized through transformation were subjected to the non-parametric Kruskall-Wallis and Dunn's test ($P<0.05$) or the Mann-Whitney U test. Spearman Rank Correlation was used to assess the correlation between foliar and fruit damage.

Finally, to estimate the economic impact of *C. chalcites* on islands the percentage of discarded banana (by weight) was normalized by arcsine transformation and analyzed by ANOVA and Tukey's test ($P<0.05$). These analyses were performed using the Statistix v.10 software package (Tallahassee, FL, USA).

3. RESULTS

3.1. *C. chalcites* incidence and feeding damage

The incidence of *C. chalcites* infestation on the different islands was similar during the two years of the study ($\chi^2=2.51$; d.f.=1; $P=0.11$) ranging from 42-50% of plots infested on Gran Canaria to 100% of plots infested on El Hierro (Fig 4A). The incidence of *C. chalcites* in banana plots was similar among plots with a northern-facing or a southern-facing aspect ($t=0.41$, d.f.=6; $P=0.70$), with the exception of Gran Canaria on which southern-facing slopes were more frequently infested than northern-facing slopes (Fisher's exact test, $P=0.025$) (Fig. 4B). The percentage of *C. chalcites* incidence in function of the seasons did not differ significantly across the islands ($\chi^2=5.24$, d.f.=2, $P=0.07$), even for Gran Canaria (Fisher's exact test $P=0.072$) (Fig. 4C).

Foliar damage was similar during both years of sampling on Tenerife, La Palma, Gran Canaria and El Hierro (Fig. 5A). Fruit damage was also similar in both years on the islands of Tenerife, La Palma, Gran Canaria and El Hierro (Fig. 5B). Therefore, since no significant differences were observed in *C. chalcites* incidence and damage during the two sampling years, the data from both years were pooled for subsequent analyses.

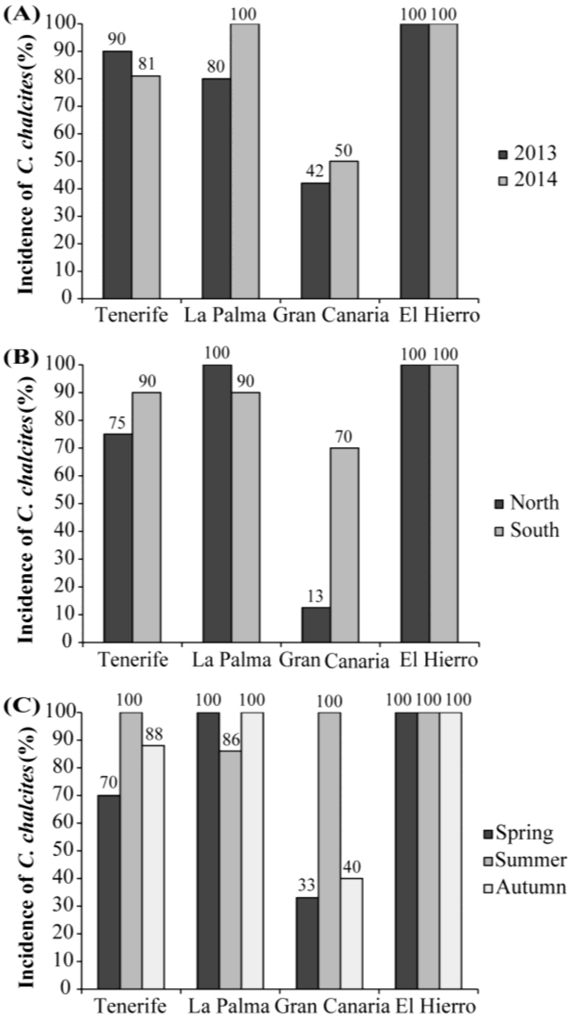

Figure 4. *C. chalcites* incidence (A) during the two sampling years, (B) according to northern-facing or southern-facing aspect or (C) in function of the seasons on the different islands.

Thus, mean foliar damage evaluated in field surveys over the two-year period was significantly higher in El Hierro (7.3%), La Palma (7.1%) and Tenerife (5.4%) than in Gran Canaria (1.5%) ($F_{3,71}$=5.85, P=0.001). Similarly, fruit damage in field surveys was significantly higher in El Hierro (5.7%) and Tenerife (4.8%) than in Gran Canaria (1%) ($F_{3,71}$=5.12, P=0.003), whereas La Palma (3.3%) presented an intermediate prevalence of fruit damage (Fig. 5C).

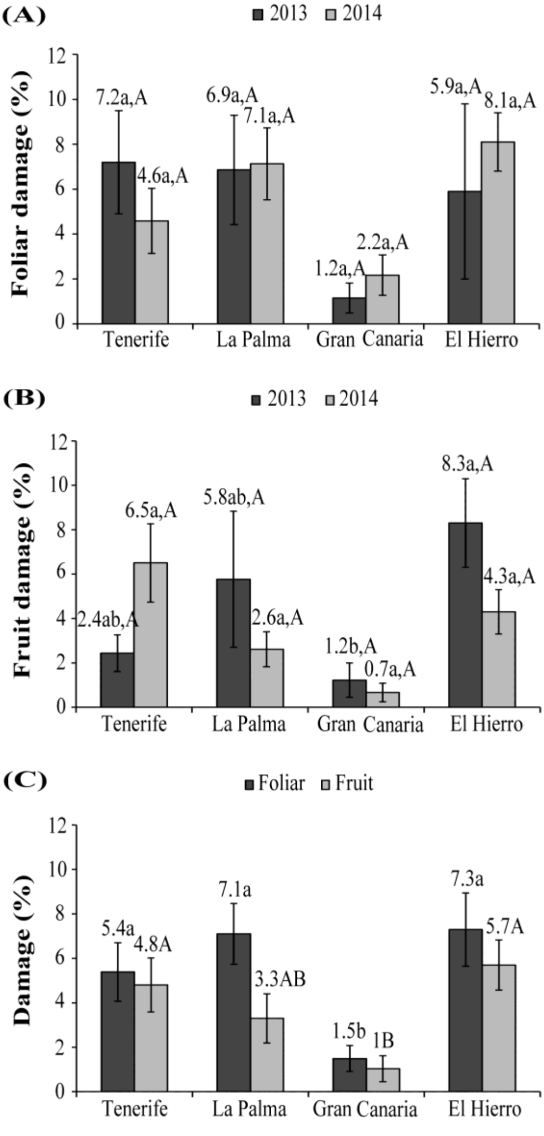

Figure 5. Percentage of (A) foliar and (B) fruit damage produced by *C. chalcites* in banana crops in the different islands during the two year sampling period. (C) Mean percentages of foliar and fruit damage after pooling the data from both years. Values followed by identical letters did not differ significantly (ANOVA followed by Tukey´s test (P>0.05) for comparison between islands and t-tests at P>0.05 for comparison of values within each island). Lower case letters are for comparison of values between islands, whereas upper case letters for comparisons within each island in (A) and (B). Lower case letters refer to the foliar values and upper case letters fruit values in (C). Vertical lines indicate the standard error.

Foliar and fruit damage was classified according to crop aspect (North and South-facing slopes) (Fig. 6A and 6B). Foliar damage was higher in the southern-facing crops in Gran Canaria (Mann-Whitney U, P=0.03), while on the islands of Tenerife (t=0.4, d.f.=25, P=0.69) and La Palma (t=0.6, d.f.=16, P=0.56), no differences were observed for plots that differed in aspect. The effect of slope was not analyzed for El Hierro as just one observation was performed on southern-facing crops. Feeding damage was significantly higher on northern-facing slopes on El Hierro and La Palma than in Gran Canaria ($F_{3,32}$=5.51, P=0.004), while the damage in Tenerife was intermediate. In contrast, in the southern-facing crops foliar damage did not differ significantly between islands ($F_{3,35}$=1.76, P=0.18) (Fig. 6A).

Regarding fruit damage, the prevalence of damaged fruit on Tenerife was significantly higher in the southern-facing crops compared to those on northern-facing slopes (Mann-Whitney U, P=0.02), whereas on La Palma (t=0.08; d.f.=16; P=0.94), and Gran Canaria (Mann-Whitney U, P=0.49), damage was not significantly influenced by plantation aspect. When comparing the influence of plot aspect on fruit damage, crops grown on the northern-facing slopes of El Hierro were significantly more damaged than on Tenerife, whereas damage on La Palma was intermediate ($F_{2,25}$=6.25, P=0.006). In contrast, fruit damage on southern-facing slopes did not differ significantly between the islands ($F_{3,35}$=1.72; P=0.18) (Fig. 6B).

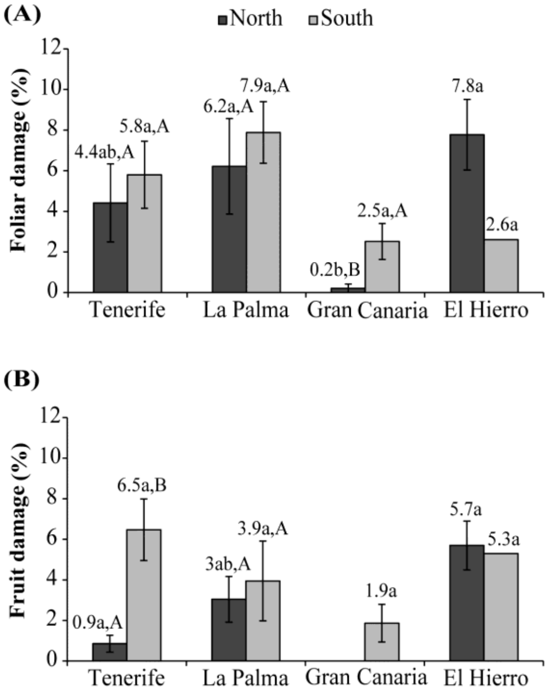

Figure 6. Percentage of (A) foliar and (B) fruit damage produced by *C. chalcites* in banana crops according to crops aspect; northern-facing or southern-facing. Values followed by identical letters did not differ significantly (t-tests at P>0.05 or Mann-Whitney U for comparison of values within each island and ANOVA followed by Tukey´s tests at P>0.05 for comparison between islands). Lower case letters refer to values between islands and upper case letters for comparisons within each island. Vertical lines indicate the standard error.

Foliar and fruit damage was also evaluated in the different seasons of the year, as there are several flights of the pest during the year with peaks in spring and autumn. Foliar damage in field surveys did not differ significantly between seasons on each island (Tenerife, $F_{2,24}$=1.95, P=0.16; La Palma, $F_{2,15}$=0.91, P=0.42; Gran Canaria, $F_{2,15}$=1.60, P=0.23 and El Hierro, $F_{2,9}$=0.61, P=0.57) (Fig. 7A). When comparing islands within each season, in spring El Hierro had significantly higher foliar damage than Tenerife or Gran Canaria ($F_{3,24}$=4.97; P=0.008), whereas La Palma presented intermediate damage. In summer ($F_{3,17}$=0.20; P=0.89) and autumn ($F_{3,22}$=2.23; P=0.11) foliar damage was similar among all islands (Fig. 7A). In contrast, fruit damage tended to be highest in spring but this effect was only significant on El Hierro where fruit damage was higher in spring (6.1%) and autumn (8.3%) than in summer (1.3%) ($F_{2,9}$=4.62, P=0.04). When considering each season, no significant differences were observed between the islands in spring ($F_{3,24}$=2.66; P=0.07) and summer ($F_{3,17}$=0.10; P=0.96), whereas in autumn fruit damage was significantly higher on El Hierro than on La Palma or Gran Canaria

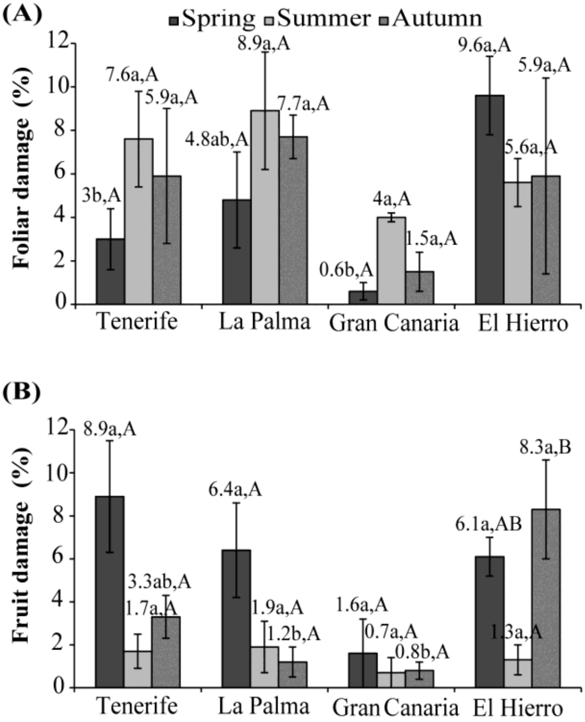

Figure 7. Percentage of (A) foliar and (B) fruit damage produced by *C. chalcites* in banana crops in the different seasons of the year. Values followed by identical letters did not differ significantly (ANOVA followed by Tukey´s test (P>0.05) for foliar damage and no parametric Kruskal-Wallis followed by Dunn's test (P>0.05) for fruit damage). Lower case letters indicate comparisons of values between islands and upper case letters for comparisons within each island. Vertical lines indicate the standard error.

($F_{3,22}$=7.07, P=0.002), and fruit damage on Tenerife was intermediate (Fig. 7B). No significant correlation was found between foliar damage and fruit damage (r=0.142; Spearman P>0.05).

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

The weight of bananas that could not be sold due to *C. chalcites* feeding damage was significantly higher on El Hierro (mean weight±SE: 31.5±8.8 kg damaged bananas in each packing facility, representing 4.2% of lost fruit with respect to the total weight of bananas processed during each 6 h sampling period) and La Palma (27.1±13.1 kg, 1.8%) compared to Gran Canaria (10.7±7.9 kg, 0.4%) and Tenerife (8.7±5.5 kg, 0.2%) ($F_{3,89}$=23.99, P<0.001) (Fig. 8A). Across the seasons, significantly more bananas were discarded in spring in La Palma ($F_{2,24}$=4.70, P=0.02) and Tenerife ($F_{2,23}$=6.77, P=0.01) than during the other seasons. In El Hierro high losses also occurred in spring, but it was not possible to compare across the other seasons due to low numbers of observations. Considering the spring period alone, significantly greater weights of bananas were discarded on El Hierro and La Palma compared to those discarded on Gran Canaria ($F_{3,43}$=7.25, P<0.01) (Fig. 8B).

Extrapolating this discarded percentage to the total banana production per year on each island, the total weight of discard bananas in a year was obtained. Therefore, on Tenerife 350 tonnes of *C. chalcites* damaged fruit would be expected to be discarded from a total production of 174,792 tonnes. Similarly, on La Palma *C. chalcites* damaged fruit was estimated to account for 2,369 tonnes from a total production of 131,585 tonnes. On Gran Canaria 316 tonnes would be discarded from a total of 78,935 tonnes and on El Hierro, 120 tonnes would be discarded from a total production of 2,856 tonnes. Therefore, across the four main banana-producing Canary Islands 3,155 tonnes of bananas per year are likely to be discarded due to *C. chalcites* damage.

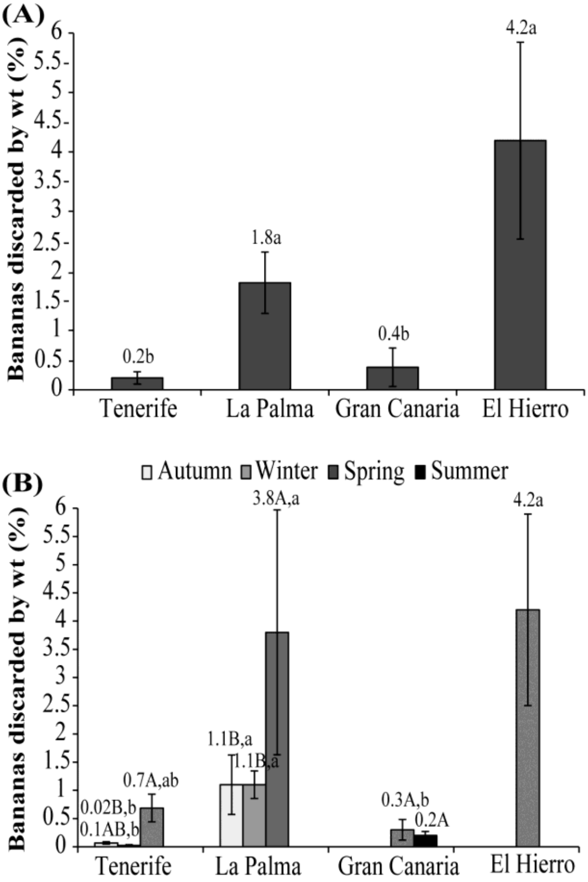

Figure 8. (A) Mean percentage of bananas by weight (wt) that could not be sold due to *C. chalcites* damage with respect to the total weight of fruit processed in the different processing plants on the islands and (B) percentage of crop losses by weight in the different seasons of the year. Values followed by identical letters did not differ significantly (no parametric Kruskal-Wallis followed by Dunn´s test (P>0.05) for (A) and ANOVA followed by Tukey´s test (P>0.05) for (B)). Lower case letters are for comparison of values between islands and upper case letters for comparisons within each island. Vertical lines indicate the standard error.

Taking into account the market price established for this period and the average proportion of fruit that meet quality categories a total of 2.68 million euros could be lost, of which 1.44 million euros were due to losses in premium class fruit, 1.05 million euros in first class and 193,000 euros in second class fruit (Table 2). By island, the highest losses were estimated on La

Palma (2,011,755 €) followed by Tenerife (297,220 €), Gran Canaria (268,347 €) and El Hierro (101,904 €). Taking into account that the value of total annual production in 2015 was 182 million euros (MAPAMA, 2016), *C. chalcites* fruit feeding damage appeared to represent a loss of approximately 1.5% of the overall annual production of saleable fruit.

Table 2. Economic losses per year (€) and losses related to the cultivated hectares (€/Ha) in the different islands. For calculations, the average prices for 2015 of green bananas for each category, premium quality (0.97 €/Kg), first class (0.81 €/Kg) and second class (0.51 €/Kg) fruit, were taken into account.

Economic losses (€/year) in each island	Total	Premium	First	Second
Tenerife	297,220	159,565	116,235	21,420
La Palma	2,011,755	1,080,027	789,745	144,983
Gran Canaria	268,347	144,235	104,944	19,339
El Hierro	101,904	54,708	39,852	7,344
Total	2,679,226	1,438,535	1,050,776	193,086
Economic losses (€/ha) per each island				
Tenerife (4,095 Ha)	73	39	29	5
La Palma (3,033 Ha)	663	356	259	48
Gran Canaria (1,766 Ha)	152	82	59	11
El Hierro (61.2 Ha)	1,665	894	651	120
Total	2,553	1,071	998	184

In relation to the average cultivated area that was 8,975 ha in 2015, *C. chalcites* damage would represent approximately 306 €/ha on average. Losses across the islands varied from 73 €/ha to 1,665 €/ha (Table 2). Interestingly, there was a negative relationship between *C. chalcites* induced damage and cultivated area. The most serious losses per hectare basis were calculated for El Hierro (1,665 €/ha), which is the island that has the smallest cultivated area (61.2 ha). On the other islands estimated losses varied from 663 €/ha over 3,033 ha on La Palma, 152 €/ha over 1,766 ha on Gran Canaria to 73 €/ha over the 4,095 ha of cultivated area on Tenerife.

3.3. Estimating costs of *C. chalcites* control

The results of the interviews with field technicians and growers are summarized in Table 3. Regarding the treatment target or part of the plant to be treated, from a total 40 surveys performed in 26 cases (65%) the whole plant was treated, in 8 cases (20%) the treatment was directed at the banana fruits and in 6 cases (15%) small immature plants (<2 m height) were treated. The volume of treatment depended on plant phenology, so that the mean application volume was 3,773±1,114 l/ha (mean±SE) for young plants and 2,474±246 l/ha for adult plants, probably because young plants are grown at higher densities than mature plants. In contrast, treatments targeted specifically at developing fruit involved a mean application volume of 765±54 l/ha. Treatment of young plants also involved higher labor costs (mean 92±10 €/ha) than whole plants (53±13 €/ha), or applications targeted at the fruit (36±2 €/ha).

Another finding that arose from the interviews was the different products used for *C. chalcites* control. Indoxacarb was most frequently used for *C. chalcites* control (29 cases, 72%), followed by chlorpyrifos (13 cases, 32%), *Bacillus thuringiensis* var. kurstaki (Bt) (8 cases, 20%) and the pyrethroid lambda-cyhalothrin (2 cases, 5%), while the botanical insecticide azadirachtin was used just once (2.5%). The cost of treatments was lowest in the indoxacarb (1.09 €/hl) and Bt (1.33 €/hl) treatments and highest in the azadarachtin treatment (15.14 €/hl), whereas the chlorpyrifos and lambda-cyhalothin treatments were of intermediate cost at 2.51 €/hl and 2.63 €/hl, respectively (Table 3). The overall cost of treatment for each insecticide was calculated from the price per hectolitre multiplied by the volume of treatment (insecticide cost) and added to the labor cost (Table 3).

In most cases (77%) insecticide treatments were performed with only one product, whereas in 23% of cases two products were used in mixtures. More than half of the insecticide treatments (58%) were performed when *C.*

Table 3. Plant protection products used in *C. Chalcites* control, frequency of use, price, dose, price per 100 liters (hl), insecticide cost and total cost of treatment, based on interviews of 40 field technicians and growers performed on the Canary Islands.

Insecticide	Frequency	Price (€) (1)	Dose(2)	Price (€/hl)	Insecticide cost (€/ha)*			Total cost (€/ha)**		
		(mean ± SE)		(mean ± SE)	Young	Whole	Fruit	Young	Whole	Fruit
Indoxacarb 30% WG 250 g (Steward)	72.5%	68 ± 6.0	4 g/hl	1.09 ± 0.10	41	27	8	133	80	44
Chlorpirifos 48% SC 1 L (Dursban)	32.5%	13 ± 0.6	200 ml/hl	2.51 ± 0.12	95	62	19	187	115	55
Bacillus thuringiensis *kurstaki* 32% WG 1kg (Dipel)	20%	27 ± 0.9	50 g/hl	1.33 ± 0.04	50	33	10	142	86	46
Lambda-cyhalothrin 2.5 WG 1 kg (Karate king)	5%	33 ± 5.0	80 g/hl	2.63 ± 0.36	99	65	20	191	118	56
Azadirachtin 3.2% SC (Align)	2.5%	101 ± 9.0	150 ml/hl	15.14 ± 1.32	572	375	116	664	428	152

(1) Price of product obtained from distributors.
(2) Dose recommended by the product label.
* Insecticide cost is related with the volume of treatment used, for young plant was 3,773 ± 1114 l/ha , for whole plant 2,474 ± 246 l/ha and for the bunch 765 ± 54 l/ha.
** Total cost represented the summarized of the insecticide cost and the labor cost, which was estimated for young plant 92 ± 10/€/ha, for whole plant 53 ± 13 €/ha and for the bunch 36 ± 2 €/ha.

chalcites was detected by the presence of larvae or damage to leaves or fruit that varied according to island and location. The frequency of treatments varied between 0 and more than 3 applications per year, with three treatments per cropping cycle being the most common (36%) (data not shown).

Considering that the most frequently used pesticide was indoxacarb, that an average of three treatments were usually required during the crop cycle and that the main treatment target was the whole plant, the total cost of *C. chalcites* control during a cycle of banana cultivation in the Canary Islands would likely average 240 €/ha. The value of average production per hectare (based on an average production of 44 tonnes/ha), taking into account the different quality categories and their market prices, would be 37,365 €. Therefore the average cost of control of *C. chalcites* with indoxacarb represents approximately 0.64% of the overall crop value, taking into account that young plants do not mature and begin to produce fruit until approximately 9 months of age.

4. DISCUSSION

Estimation of agricultural losses is key for the rational management of *C. chalcites* and for evaluation of the effectiveness of current plant protection practices in banana crops in the Canary Islands. Our study revealed that control of *C. chalcites* mainly involves applications of indoxacarb, chlorpyrifos or the biological insecticide, Bt. The costs associated with insecticide purchase and application should be included in IPM-based decision-making, particularly when considering the economic impact of the pest in banana crops. Therefore, the present study aimed to determine the incidence, damage levels, production losses due to direct damage and the treatment costs related to *C. chalcites* control.

Feeding damage by *C. chalcites* was most severe on the islands of El Hierro, La Palma and Tenerife, whereas *C. chalcites* was of minor importance on Gran Canaria (Del Pino et al., 2011; Domínguez et al., 2012). Although previously considered a minor pest that mostly fed on leaves, by 2002 fruit damage by *C. chalcites* had become generalized in El Hierro crops. This was followed by fruit damage reported in banana plantations located in the south of La Palma, and later in southern Tenerife, and finally in 2012 in Gran Canaria (Del Pino et al., 2011; Perera and Molina, 2007). One of the factors that may have contributed to the resurgence of *C. chalcites* as an emerging pest could be the elimination of authorized insecticides in banana crops, from 24 active compounds in 2006 to 12 compounds in 2014 (López-Cepero, 2015). Nonetheless, the reasons behind the changes in feeding behavior directed towards fruit are unclear and, given the highly mobile nature of this pest, seem unlikely to reflect behavioral changes in an isolated local pest population.

The prevalence of *C. chalcites* infestations and feeding damage on southern slopes is likely influenced by the cropping system established on each slope, as mesh-built greenhouses are more common on southern-facing slopes that are warmer and dryer than the northern-facing slopes. The mesh greenhouses also protect banana crops from onshore winds (Galán-Saúco, 1992; Robinson and Galán-Saúco, 2010). In addition, temperatures are higher on the southern-facing slopes (Table 2S), which likely favor the development of *C. chalcites*. Differences in the maximum and minimum temperatures between slopes can reach up to five degrees, especially in Gran Canaria. These differences in temperature can influence the duration of the biological cycle of the pest, shortening it in case of the higher temperatures (Barrionuevo et al., 2012; Danks, 2000; Mironidis and Savopoulou-Soultani, 2008), which is probably why more generations of *C. chalcites* can occur on the crops grown on southern facing slopes during the year.

In banana crops grown under mesh on southern-facing slopes, *C. chalcites* generations follow one another throughout the year with no diapause (Del Pino et al., 2011; Domínguez et al., 2012; García et al., 1992; Perera and

Molina, 2007), unlike the generational patterns observed in glasshouses in northern Europe (van de Veire, 1993; Vos and Rutten, 1995). However, *C. chalcites* has two predominant flights during the year, one in spring (May-June) and the other in autumn (September-October) (Bernal et al., 2013; Del Pino et al., 2011). Adults are able to migrate over great distances from northern Europe to southern Africa (García et al., 1992). For example, Spitzer and Jaros (2004) observed a mass flight of immigrant adults on the southeast coast of Tenerife in spring. According to the results presented in this study, no large differences occurred in foliar and fruit damage according to the seasons. Larvae of *C. chalcites* feed preferentially on young leaves, and particularly the young leaves of young plants. Larvae may attack the banana crop from transplanting until fruit maturity, but the most sensitive growing periods are from transplanting to the first foliar stages and flowering and fruiting, which occur during the main peak flight of the pest in the spring (Del Pino et al., 2011; Galán-Saúco, 1992; Robinson and Galán-Saúco, 2010). In young plants, larvae produce damage by perforating leaves (Del Pino et al., 2011; Domínguez et al., 2012; Perera and Molina, 2007; Simón et al., 2015; Vilardebo and Guérout, 1964). Planting of young banana plants is usually done during summer periods and although the adult flight is less prevalent compared with the spring, the presence of individuals might have consequences for plantations of young plants, as at this moment the crop is highly attractive to the pest. However, in the present study the foliar damage produced by *C. chalcites* in young plants is unlikely to have had a substantial effect on final crop production, as major defoliation would be needed to cause crop losses. 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. Damage to fruit in the spring is particular important as it coincides with the development of the fruit after flowing 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 and Galán-Saúco, 2010).

Therefore, monitoring and controlling *C. chalcites* during the spring season would be of particular value for the effective protection of developing fruit. Crop protection strategies, based on preventing quantitative crop losses rather than pest outbreaks represent a promising approach to rationalize pesticide use. However, in the present study no correlation was found between foliar damage and fruit damage in banana crops in the Canary Islands. Therefore, foliar damage observed at a given moment is unlikely to be a useful predictor of the impact of *C. chalcites* on marketable yield, and so, alternative predictors should be evaluated. Useful predictors of future losses in yield have been observed in many other systems including stem borers in maize (Ajala and Saxena, 1994) and rice (Muralidharan and Pasalu, 2006), pests and diseases in coffee (Cerda et al., 2017), the presence of parasitic plants in carrot and pea crops (Bernhard et al., 1998), among many others.

Observations on banana damage registered in packaging plants agreed with the results obtained through field surveys on fruit damage. For example, on El Hierro where 5.7% of fruit damage was observed in field surveys, losses of fruit in packaging plants was of similar magnitude (4.2%), whereas in Gran Canaria (0.4%) and Tenerife (0.2%) fewer losses were registered due to direct damage to fruit. However, although on Tenerife 4.8% of fruit damage was registered in field just 0.2% of fruit was discarded, which suggests that workers at this processing facility used different criteria for rejecting superficially damaged fruit. Previous studies with *C. chalcites* in banana crops in the Canary Islands estimated average losses of banana production at 9.4%

of the total bunch (Del Pino et al., 2011; Domínguez et al., 2012). However, these estimates were performed based on small numbers of plants in controlled conditions, therefore losses may have been overstated. In the present study, fruit loss estimates were between 0.2% and 4.2%. It may also be that some packaging plants lower the quality category depending on the grade of *C. chalcites* damage, which carries an associated reduction in value but does not necessarily prevent the sale of the damaged bananas. It was not possible to estimate *C. chalcites* related changes in fruit quality classifications as only the weight of discarded fruit was measured. The same level of pest damage can cause different yield losses because quality standards differ between different banana growing producer associations. These quality standards for packaging bananas consider both the presence of insects on the fruit and superficial appearance. A similar situation was observed on tomato crops in southern Spain, where damage levels tolerated for processing tomatoes largely depend on criteria established by the tomato processing industry and vary according to final use of goods (e.g., juice, past, canning, etc.), cropping years, annual production and especially by market conditions (Torres-Vila et al., 2003). Taking into account the percentage of the discarded bananas and the area cultivated on the different islands, we estimated that on average *C. chalcites* is likely to be responsible for annual losses of approximately 2.68 million euros despite the adoption of control measures.

Control measures targeted at *C. chalcites* undoubtedly contribute to the costs of production. According to the results obtained in this study, three main insecticides are used commonly in banana crops; indoxacarb, chlorpyrifos and *Bacillus thuringiensis*. Indoxacarb and *Bacillus thuringiensis* are biorational products, whereas chlorpyrifos is a broad-spectrum organophosphate pesticide. These insecticides are commonly used in other lepidopteran-crop systems (Broza and Sneh, 1994; Moore et al., 2004). Our results also highlighted that the volumes used to apply treatments were highly variable, as were the labour costs to treat one hectare of crop. This is mainly due to the treatment system used and the time spent in applying treatments. For example in some areas of the islands, such as La Palma, bananas are grown in small, highly fragmented plots, where tractor mounted spray machinery is not used. This results in an increased volume and time required to treat the crop. Indeed, 82% of banana crops are grown on small farms of less than 1 ha. Therefore, low levels of mechanization tend to increase the overall cost of production.

On El Hierro and La Palma, losses due to *C. chalcites* damage to fruit exceed the cost of insecticide purchase and application, whereas on Tenerife and Gran Canaria the losses due to *C. chalcites* damage were lower than the control costs. It is necessary to design IPM programs that consider different aspects that could improve the ratio of production costs to crop yields, particularly those related to treatment thresholds. Establishing treatment thresholds is complex, but necessary to make decisions in IPM systems. Banana growers and field technicians currently use observations on feeding damage or evidence of larval infestations in their plantations as the main criteria for implementing control measures against *C. chalcites,* although the cost-benefit relationship of insecticide treatments remains to be determined across a range of pest densities and phenological stages of plant development and fruiting. Finally, continuous use of a single dominant insecticide to control *C. chalcites*, as occurs on the Canary Islands, is likely to favour the development of resistance. As such, new insecticides are required for use in rotation with the handful of authorized compounds. Among these the nucleopolyhedrovirus of *C. chalcites* (ChchNPV) has been proven to be a highly effective control agent (Bernal et al., 2013; Simón et al., 2015). Experiments are in progress to determine the efficacy of this virus-based insecticide in commercial banana crops in comparison with the other authorized products.

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6. REFERENCES

Ajala, S.O., Saxena, K.N., 1994. Interrelationship among *Chilo partellus* (Swinhoe) damage parameters and their contribution to grain yield reduction in maize (*Zea mays* L.). Applied Entomology and Zoology 29, 469-479.

Arias, P., Dankers, C., Liu, P., Pilkauskas, P., 2003. The World Banana Economy (1985-2002), FAO Food and Agriculture Organization of the United Nations, Rome.

ASPROCAN, 2016. 2015 annual statistics, Asociación de Organizaciones de Productores de Plátanos de Canarias. http://platanodecanarias.net/wp-content/uploads/2016/10/ESTADISTICAS_2015.pdf (accessed 12.4.17)

Barrionuevo, M.J., Murúa, M.G., Goane, L., Meagher, R., Navarro, F., 2012. Life table studies of *Rachiplusia nu* (Guenée) and *Chrysodeixis* (=Pseudoplusia) *includens* (Walker) (Lepidoptera: Noctuidae) on artificial diet. Florida Entomology 95, 944-951.

Bernal, A., Williams, T., Hernández-Suárez, E., Carnero, A., Caballero, P., Simón, O., 2013. A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: The basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banana crops. Biological Control 67, 101-110.

Bernhard, R.H., Jensen, J.E., Andreasen, C., 1998. Prediction of yield loss caused by *Orobanche* spp. in carrot and pea crops based on the soil seedbank. Weed Research 38, 191-197.

Bianchi, R.V., 2004. Tourism restructuring and the politics of sustainability: a critical view from the European periphery (The Canary Islands). Jornal of Sustainable Tourism 12, 495-529.

Bielza, P., Lacasa, A., 1998. Cálculo del umbral económico de daño de trips del trigo, *Haplothrips tritici* (Kurdjumov). Boletin de Sanidad Vegetal Plagas 24, 239-250.

Broza, M., Sneh, B., 1994. *Bacillus thuringiensis* ssp. kurstaki as an effective control agent of lepidopteran pests in tomato fields in Israel. Journal of Economic Entomology 87, 923-928.

Cerda, R., Avelino, J., Gary, C., Tixier, P., Lechevallier, E., Allinne, C., 2017. Primary and secondary yield losses caused by pests and diseases: assessment and modelling in coffee. PloS ONE 12, e0169133.

Crawley, M.J., 1993. GLIM for ecologists, Blackwell Science, Oxford.

Danks, H.V., 2000. Measuring and reporting life-cycle duration in insect and arachnids. European Journal of Entomology 97, 285-303.

Del Pino, M., Carnero, A., Hernández-Suárez, E., Cabello, T., 2011. La lagarta o bicho camello, *Chrysodeixis chalcites* (Esper, 1789), una plaga emergente en los cultivos de platanera de Canarias. Phytoma 225, 21-16.

Domínguez, E., López-Cepero, J., Nogueroles, C., 2012. Identificación y control de plagas y enfermedades, in: Nogueroles, C. (Ed.), Calidad y sostenibilidad en el cultivo de la platanera en Canarias. ASPROCAN, Tenerife, pp.145-170.

Galán-Saúco, V., 1992. Los frutales tropicales en los subtropicos, II. Plátano (Banano), Mundi-Prensa, Madrid.

García, R., Ortega, G., Pérez, J.M., 1992. Insectos de Canarias. Cabildo Insular de Gran Canaria, Las Palmas de Gran Canaria.

González-Concepción, C., Gil-Fariña, M.C., Pestano-Gabino, C., 2008. Multivariate modelling of the Canary Islands banana output. The role of farmer income expectation. Journal of Social Sciences 4, 88-97.

Hallam, D., 1995. The world banana economy, in: Gowen, S. (Ed.), Bananas and Plantains. Chapman Hall, London, pp. 509-533.

Higley, L.G., Pedigo, L.P., 1993. Economic injury level concepts and their use in sustaining environmental quality. Agriculture, Ecosystems and Environment 46, 1-4.

López-Cepero, J., 2015. Análisis de residuos y gestión integrada de plagas en platanera. Phytoma 271, 48-52.

MAPAMA, 2016. Statistical yearbook, breakthrough 2015. http://www.mapama.gob.es/estadistica/pags/anuario/2015-Avance/AE_2015_Avance.pdf (accessed 12.4.17)

Mironidis, G.K., Savopoulou-Soultani, M., 2008. Development, survivorship, and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) under constant and alternating temperatures. Environmental Entomology 37, 16-28.

Moore, S.D., Pittaway, T., Bouwer, G., Fourie, J.G., 2004. Evaluation of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) for control of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on citrus in South Africa. Biocontrol Science and Technology 14, 239-250.

Muralidharan, K., Pasalu, I.C., 2006. Assessments of crop losses in rice ecosystems due to stem borer damage (Lepidoptera: Pyralidae). Crop Protection 25, 409-417.

Pedigo, L.P., 1996. Entomology and Pest Management, Prentice-Hall Pub, Englewood Cliffs, NJ.

Perera, S., Molina, M.J., 2007. Plagas y enfermedades en el cultivo ecológico de la platanera, in: Nogueroles, C., Líbano, J. (Eds.), El cultivo ecológico de la platanera en Canarias. Gabinete de Proyectos Agroecológicos SL, Tenerife, pp. 77-118.

Raynolds, L.T., 2003. The Global Banana Trade, in: Striffler, S., Moberg, M. (Eds.), Banana Wars: Power, Production, and History in the Americas. Duke University, Durham, pp. 23-47.

Robinson, J.C., Galán-Saúco, V., 2010. Bananas and Plantations, CAB International, Wallingford, UK, pp. 13-17.

Simón, O., Bernal, A., Williams, T., Carnero, A., Hernández-Suárez, A., Muñoz, D., Caballero, P., 2015. Efficacy of an alphabaculovirus-based biological insecticide for control of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) on tomato and banana crops. Pest Management Science 71, 1623-1630.

Spitzer, K., Jaros, J., 2004. Mass migration of *Chrysodeixis chalcites* (Esper, 1789) in Tenerife, Canary Islands (Lepidoptera: Noctuidae). SHILAP Revista de Lepidopterología 32, 19-22.

Torres-Vila, L.M., Rodríguez-Molina, M.C., Lacasa-Plasencia, A., 2003. Impact of *Helicoverpa armigera* larval density and crop phenology on yield and quality losses in processing tomato: developing fruit count-based damage thresholds for IPM decision-making. Crop Protection 22, 521-532.

Townsend, G.R., Heuberger, J.W., 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter 27, 340-343.

Turner, D.W., Hunt, N., 1987. Planting date and defoliation influence the time of harvest of bananas. Scientia Horticulturae 32, 233-248.

van de Veire, M., 1993. First observations in glasshouse sweet peppers in Belgium and laboratory rearing of the parasitic wasp *Eulophus pennicornis* (Hym.: Eulophidae). Entomophaga 38, 62-63.

Vilardebo, A., Guérout, R., 1964. *Plusia chalcytes* Esp. (Lépidoptère Noctuidae) nuisible au bananier dans les régions cotières de L´ouest Africain. Fruits 19, 195-208.

Vos, R., Rutten, A.L.M., 1995. Migrating Lepidoptera in 1992 (fifty-third report). Entomologische Berichten 55, 37-46.

CHAPTER III

Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV): natural occurrence and efficacy as a biological insecticide on young banana plants in greenhouse and open-field conditions on the Canary Islands

ABSTRACT

Chrysodeixis chalcites, an important pest of banana crops on the Canary Islands, is usually controlled by chemical insecticides. The present study aimed to evaluate the effectiveness of the most prevalent isolate of the Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV, *Baculoviridae*). Overall the prevalence of ChchNPV 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. On Tenerife, infected larvae were present at 11 out of 17 plantations sampled. The prevalence of infection in larvae bananas grown under greenhouse structures was significantly higher (3%) than in open-field sites (1.4%). The ChchNPV-TF1 isolate was the most abundant and widespread of four genetic variants of the virus. Application of 1.0×10^9 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. These results should prove useful for the registration of a ChchNPV-based insecticide for integrated management of this pest in banana crops on the Canary Islands.

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1. INTRODUCTION

Banana is the main crop on the Canary Island archipelago, covering an area of about 9,000 hectares and a total production of 381,983 tonnes in 2015 (MAPAMA, 2016). The tomato looper, *Chysodeixis chalcites* (Esper, 1789) is a noctuid pest of bananas in the Canary Islands (Del Pino et al., 2011; Domínguez et al., 2012). Larvae often feed on banana fruit, causing damage to the epidermis that greatly reduces the market value of damaged fruit (Dominguez et al., 2012). Control of this pest mainly involves the use of pesticides (Del Pino et al., 2011; Domínguez et al., 2012; Perera and Molina, 2007). However, the low number of active compounds authorized for this crop in the European Union, and the repeated use of these products during the crop cycle (Del Pino et al., 2011; Bernal et al., 2013) favors the development of resistance (Horowitz et al., 1998), further reducing the effectiveness of the currently approved pesticides. In addition, since 2014 integrated pest management (IPM) is mandatory in crops grown in Spain (Royal decree 1311/2012, which incorporates Directive 2009/128/EEC). IPM is an ecologically based pest control strategy that favors natural mechanisms of pest control with minimal disruption from broad-spectrum pesticides (Flint and van den Bosch, 1981; Pedigo, 1996).

Lepidopteran nucleopolyhedroviruses (genus *Alphabaculovirus*, *Baculoviridae*) are virulent and selective pathogens with an established track-record as effective biological insecticides (Eberle et al., 2012). A single-nucleocapsid nucleopolyhedrovirus isolated from a single infected *C. chalcites* larva collected in banana crops in southern Tenerife was characterized and named ChchNPV-TF1 (Bernal et al., 2013). Laboratory and small-scale greenhouse trials on young banana plants indicated that the high pathogenicity and fast speed of kill of this isolate favored its development as a biological insecticide for the control of this pest (Bernal et al., 2013; Simón et al., 2015). Studies on other lepidopteran-nucleopolyhedrovirus pathosystems indicate that genetic diversity in the pathogen population has a marked influence on major phenotypic traits including pathogenicity, measured in terms of concentration-mortality metrics, speed of kill and production of progeny virus particles in each infected insect (Arrizubieta et al., 2014; Erlandson, 2009; Cory et al., 2005). As these characteristics are of major importance to the efficacy of virus-based biological insecticides, the genetic identity of the virus is an issue of key importance when selecting the active material for production, formulation and field testing of virus-based products.

The objective of the present study was to estimate the genetic diversity of ChchNPV variants present in *C. chalcites* populations on the Canary Islands and to evaluate the insecticidal efficacy of ChchNPV-TF1, which was the most prevalent variant. Efficacy trials were performed in comparison with two frequently used insecticides in young banana plants grown in small-scale greenhouse and open-field conditions

2. MATERIALS AND METHODS

2.1. Insects

The *C. chalcites* colony, used for artificial infestation of plants in greenhouse and open-field trials, was established with larvae collected in banana crops in southern Tenerife. This 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, wheatgerm and yeast (Cabello and Hernández, 1988). Adults were fed *ad libitum* with 10% v/v honey solution.

2.2. Prevalence and diversity of ChchNPV in *C. chalcites* populations in banana crops.

During field surveys conducted in banana crops on the Canary Islands over a two-year period, from December 2012 to December 2014, plantations at 11 localities in Tenerife, 10 in La Palma, 7 in Gran Canaria, 4 in La Gomera and 2 in El Hierro were surveyed with the aim of determining the natural prevalence of ChchNPV infection (Table 1).

Table 1. Detailed information on ChchNPV surveys indicating the localities prospected, the crop system (OF: open-field and GH: mesh greenhouses), the number of surveys performed, the number of larvae collected and the larvae that died from virus infection and finally the ChchNPV isolates identified.

Canary Islands banana crops sample			Coordinates		Crop	Surveys	Larvae	Isolates (n)	ChchNPV isolates
Island	Locality	Area	Lat. (N)	Long. (W)	system	(n)	(n)	(% prevalence)	(number of larvae infected)
Tenerife	Fasnia	Fasnia	28° 13' 45"	16° 24' 57"	OF/GH	1	91	0 (0)	-
	Güimar	Puertito Güimar	28° 17' 21"	16° 23' 04"	GH	2	83	1 (1.2)	TF1 (1)
	Granadilla	San Isidro	28° 02' 33"	16° 33' 33"	GH	1	26	1 (3.9)	TF1 (1)
	Arona	El Cordero	28° 02' 43"	16° 37' 51"	GH	2	223	6 (2.7)	TF1 (5)
		El Fraile	28° 01' 50"	16° 39' 23"	GH	4	261	7 (2.7)	TF1 (6)
		Las Galletas	28° 01' 13"	16° 40' 24"	GH	2	102	1 (0.9)	TF1 (1)
		Valle Grande	28° 01' 47"	16° 39' 07"	GH	2	199	0 (0)	-
		Parque La Reina	28° 02' 43"	16° 39' 08"	GH	3	204	0 (0)	-
		Guaza	28° 01' 35"	16° 39' 11"	GH	1	34	1 (2.9)	TF3 (1)
	Adeje	Caldera del Rey	28° 04' 19"	16° 43' 02"	OF/GH	4	463	22 (4.8)	TF1 (12), TF2 (8)
	Guía Isora	Abama	28° 10' 37"	16° 47' 18"	OF	1	61	0 (0)	-
		Alcalá- Aurora	28° 13' 08"	16° 49' 40"	OF	1	81	0 (0)	-
	Buenavista	La Laja	28° 23' 12"	16° 50' 15"	GH	1	48	1 (2.1)	TF2 (1)
	Los Silos	Caleta	28° 23' 07"	16° 49' 39"	OF	1	56	2 (3.6)	TF1 (1), TF2 (1)
	Puerto Cruz	La Vera	28° 24' 24"	16° 33' 30"	OF	2	171	2 (1.2)	-
	Valle Guerra	Catesa	28° 31' 20"	16° 24' 43"	GH	3	367	12 (3.3)	TF1 (10)
	Tejina	Tejina Costa	28° 32' 24"	16° 22' 44"	GH	1	66	0 (0)	-
Total							2536	56(2.2)	TF1 (37), TF2 (10), TF3 (1)
La Palma	Fuencaliente	La Ballena	28° 29' 11"	17° 52' 14"	OF	3	167	3 (1.8)	TF1 (1)
	Los Llanos	El Remo	28° 33' 18"	17° 53' 15"	GH	3	128	1 (0.8)	TF1 (1)
		Charco verde	28° 34' 26"	17° 53' 55"	OF/GH	2	43	0 (0)	-
		Todoque	28° 36' 50"	17° 54' 20"	OF/GH	2	123	0 (0)	-
	Tijarafe	Tijarafe	28° 41' 45"	17° 57' 58"	GH	1	28	0 (0)	-
	Puntallana	Cabrera	28° 45' 36"	17° 44' 34"	OF	1	42	0 (0)	-
		Martín Luis	28° 42' 59"	17° 44' 26"	OF/GH	1	100	1 (1)	TF1 (1)
	Barlovento	Faro	28° 50' 16"	17° 46' 48"	OF	1	55	0 (0)	-
	San Andrés	Charco Azul	28° 48' 25"	17° 45' 47"	OF	1	21	0 (0)	-
	Mazo	El Pocito	28° 35' 43"	17° 45' 33"	OF	1	54	3 (5.6)	TF1 (3)
	Breña Alta	El Socorro	28° 39' 34"	17° 46' 32	OF	1	54	1 (1.9)	TF2 (1)
Total							815	9 (1.1)	TF1 (6), TF2 (1)

Table 1. Continued

Canary Islands banana crops sample			Coordinates		Crop	Surveys	Larvae	Isolates (n)	ChchNPV isolates
Island	Locality	Area	Lat. (N)	Long. (W)	system	(n)	(n)	(% prevalence)	(number of larvae infected)
Gran Canaria	Vecindario	Pozo Izquierdo	27° 50’ 28”	15° 25’ 51”	GH	5	264	0 (0)	-
	Mogán	Arguineguín	27º 46’ 41”	15º 39’ 57”	OF	1	52	0 (0)	-
		Veneguera	27° 52’ 23”	15° 45’ 37”	OF	1	16	0 (0)	-
	La Aldea	La Aldea	27° 59’ 13”	15° 47’ 12”	OF/GH	1	36	0 (0)	-
	Gáldar	Gáldar Costa	28° 09’ 25”	15° 40’ 42”	OF/GH	1	72	0 (0)	-
		Gáldar	28° 08’ 11”	15° 38’ 36”	OF/GH	4	86	0 (0)	-
	Arucas	Bañaderos	28° 08’ 35”	15° 32’ 09”	OF	2	39	0 (0)	-
Total							565	0 (0)	-
La Gomera	San Sebastián	Chejelipes	28º 06’ 26”	17º 08’ 29	GH	1	38	0 (0)	-
	Hermigua	Hermigua	28º 10’ 37”	17º 10’ 59”	OF	1	21	0 (0)	-
	Vallehermoso	La Dama	28° 03’ 17”	17° 18’ 33”	GH	1	67	0 (0)	-
	ValleGranRey	Las Malezas	28º 05’ 33”	17º 20’ 13”	OF	1	63	1 (1.6)	TF3 (1)
Total							189	1 (0.5)	TF3 (1)
El Hierro	Frontera	El Matorral	27° 46’ 08”	18° 00’ 59”	GH	11	269	37 (13.8)	TF1 (33), TF3 (1), TF5 (3)
	El Pinar	Tacorón	27° 39’ 50”	18° 01’ 03”	GH	1	64	0	-
Total							333	37 (11.1)	TF1 (33), TF3 (1), TF5 (3)
Total overall							4,438	103 (2.3)	TF1 (76), TF2 (11), TF3 (3), TF5 (3)

Each location was classified as infested or not by *C. chalcites*, based on direct observation of feeding damage on leaves and fruit (Fuentes et al., 2017). Thereafter in each infested plantation, larvae were collected from plants that showed *C. chalcites* feeding damage, both mature and immature plants. The sampling effort was similar for each location and involved a 4-hour period collecting larvae, or until ~100 larvae had been collected at each site. Collections were performed intermittently over the two year period. As the density of infestation varied, the number of larvae collected at each site varied accordingly (Table 1).

Larvae were collected using a soft paintbrush, and individualized in 25 ml plastic pots containing diet. Larvae were maintained in a laboratory rearing chamber at 25 ± 2 °C, 70 ± 15% RH and 16:8 hours (L:D) photoperiod, until death or pupation. Larvae were inspected daily and those died with the typical signs of polyhedrosis disease were individually frozen at -20 ºC for subsequent analysis. All larvae that died were observed using an optical microscope (x1000) to determine the presence of OBs.

To determine the identity of isolates from virus-killed field-collected insects, restriction endonuclease analysis (REN) was performed. For this, viral occlusion bodies (OBs) were purified from cadavers by homogenization in 0.1% (wt/vol) sodium dodecyl sulfate (SDS) and were filtered through muslin to remove debris. The resulting suspension was centrifuged at 3,800 x *g* for 5 minutes, OBs were resuspended in 500 µl of 0.1% SDS and centrifuged again at 3,800 x *g* for 5 minutes. OBs were finally resuspended in sterile distilled water. For viral DNA extraction, 100 µl of purified OB suspension was mixed with 100 µl of 0.5M Na_2CO_3, 50 µl of 10% SDS and distilled water to a final volume of 500 µl, incubated at 60ºC for 10 minutes and centrifuged at 3,800 x *g* for 5 minutes. The resulting supernatant was treated with 25 µl of proteinase K (20 mg/ml) at 50ºC for 30 minutes and then treated twice with 500 µl of TE-saturated phenol and once with 500 µl chloroform.The aqueous phase was recovered and DNA was precipitated by adding 1/10 volume 3M sodium acetate at pH 5.2 and 2.5 volumes of 100% cold ethanol. The DNA pellet was washed with 70% cold ethanol and resuspended in 50-100 µl 0.1x TE (Tris HCl-EDTA) buffer. For REN analysis, 2 µg of viral DNA were incubated with 5 units of *Bgl*II (Takara) at 37ºC for 4 hours following the manufacturer's instructions. The reaction was stopped by adding 4 µl of loading buffer (0.25% w/v bromophenol blue, 40% w/v sucrose). Samples were loaded on 1% agarose gel and subjected to electrophoresis in TAE buffer (40 mM Tris-acetate, pH 8.0; 1 mM EDTA). The gel was stained using ethidium bromide, observed on a UV transilluminator and photographed using the GeneSnap Chemi-Doc package (BioRad, CA).

2.3. Production of ChchNPV-TF1 OBs

For field trials, ChchNPV-TF1 isolate OBs were produced by inoculating fifth and sixth instar laboratory-reared *C. chalcites* larvae with 5.00×10^7 OBs/ml or 9.02×10^8 OBs/ml, respectively (Bernal et al., 2013; Simón et al., 2015). OB inocula were suspended in 10% sucrose solution and 0.001% Fluorella blue food dye, and fed to larvae using the droplet feeding method (Hughes and Wood, 1981). Inoculated larvae were placed in 24 well tissue culture plates with semi-synthetic diet and incubated at 25ºC. Larvae were checked daily for signs of polyhedrosis disease and dead insects were collected and stored at -20ºC. OBs were collected by thawing infected insects, followed by homogenization, filtration through muslin and centrifugation at 3,800 x g for 5 minutes. The resulting pellet of OBs 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 OBs produced for field trials was confirmed by REN analysis using *Bgl*II (Bernal et al., 2013).

2.4. Determining the optimal ChchNPV-TF1 concentration

To characterize the concentration-response for ChchNPV-TF1 OBs under field conditions trials were performed in 2013 using young banana plants (*Musa acuminata*, var. Dwarf Cavendish) of approximately 3 months old with 7 leaves and ~ 50 cm height. Plants were grown in 21 cm diameter pots in the experimental plots of the Instituto Canario de Investigaciones Agrarias (ICIA) (Tenerife, Spain). Trials were conducted in a plastic greenhouse of 600 m^2 on the southern slope of Tenerife (28º 19' 2.8" N; 16º 22' 59" W) and in a 500 m^2 open-field plot on the northern slope of the same island (28º 31' 43.5" N; 16º 23' 13" W).

Trials involved ChchNPV OBs applied at four different concentrations: 10^7, 10^8, 10^9, 10^{10} OBs/l, and water as the control. All treatments included 0.1% (v/v) Agral (Agro S.A., Madrid, Spain) wetter-sticker and were applied using a 2 l compressed-air hand sprayer (SOLO® 402, Sindelfingen, Germany). The experimental design consisted of randomized plots with four replicates per treatment.

Experimental plots of 25 m^2 comprised four rows of five plants each at a distance of 1 m from the adjacent plant (total = 20), of which 12 were border plants and 8 were central plants. Plants were artificially infested with *C. chalcites* eggs placed on the underside of the three youngest leaves of each plant. The number of eggs put in each plant varied from 50–150. Seven days later, when larvae had reached the second instar, plots were sprayed with a 1 l volume of each treatment (equivalent to 400 l/ha, which is usual for small banana plants). All applications were made between 8.00-11.00 am.

The efficacy of the different OB concentrations was calculated using the formula described by Henderson-Tilton (1955):

$$\% \text{ Efficacy: } 1- (Lta \times Lcb / Lca \times Ltb) \times 100$$

Where Lta is the number of living larvae in plots after treatment, Ltb the number of living larvae in plots before treatment, Lca the number of living larvae in control plots after treatment and Lcb the number of living larvae in control plots before treatment.

In addition, the percentage of larval mortality at different time intervals after treatment was also determined. For this, 25 *C. chalcites* larvae were collected at random from the twelve border plants from each plot at time point 0 (immediately prior to the application of treatments), and at 1, 3, 5 and 7 days post-application. Larvae were reared individually in the laboratory in 25 ml plastic cups with semi-synthetic diet until death or pupation. Larvae that died with the characteristic signs of virus infection were stored at -20 ºC and REN analysis was performed subsequently to confirm that they had died due to ChchNPV-TF1 infection.

2.5. Insecticidal efficacy of ChchNPV-TF1 in greenhouse and open-field trials

ChchNPV efficacy was compared with two conventional treatments in greenhouse and open-field trials during 2013 and 2015 at the same sites used for the concentration-mortality trials. Similarly, 3 month old banana plants var. Dwarf Cavendish, ~50 cm height and with 7 leaves grown in pots were used in greenhouse and open-field trials.

The experiments involved four treatments: (i) indoxacarb 30% WG (Steward, DuPont, Paris, France) applied at 0.04 g/l; (ii) *Bacillus thuringiensis* var. kurstaki 32% WG (Dipel DF, Kenogard, Barcelona, Spain) applied at 0.5 g/l; (iii) ChchNPV-TF1 OBs applied at 10^9OBs/l and (iv) water control. All treatments included 0.1% (v/v) Agral wetter-sticker. Treatments were applied in a volume of 1 l using a 2 l capacity compressed-air hand sprayer (SOLO® 402, Sindelfingen, Germany). All applications were made between 8.00 and 11.00 am.

In 2013, greenhouse and open-field experimental plots comprised four rows of 5 plants distributed over an area of 25 m^2, as described for the concentration-mortality trials. In 2015, plots comprised 24 plants, distributed in 4 rows with 6 plants per row, with 16 border plants and 8 central plants. Plants were placed at 1 m intervals with a 1 m space between rows within each plot. Plots were separated by a 2 m high cloth curtain to avoid cross-contamination between treatments. In the greenhouse trial the pots were placed on the ground whereas in open-field pots were buried in the ground to avoid being blown over by the wind. In both years, greenhouse trials involved a fully randomized plot design with three replicates per treatment, while open-field trials were based on a Latin square design with four replicates per treatment.

Plants were artificially infested with eggs batches 7 days prior to treatments that were applied as described in the concentration-mortality trials. Similarly, insecticidal efficacy at 7 days post-application, and larval mortality in insects collected at 1, 3, 5 and 7 days post-application and reared in the laboratory until death or pupation, were measured as described in the concentration-mortality trials.

Foliar damage was also estimated. In 2013, damage was estimated by calculating the percentage of damage increase in all the leaves (at least 7) of the eight central plants by counting the initial number of foliar perforations characteristics of *C. chalcites* feeding damage (old damage) on each leaf in each treatment prior to insecticidal treatments, and the final number of perforations, using the formula:

$$\text{Damage increase (\%)} = (Da - Db/\ Da) \times 100$$

Where Db is the damage before treatment, and Da the damage after treatment.

In contrast in 2015, foliar damage was estimated using the ImageJ image processing software (Java and National Institutes of Health, USA). For this, in each plot one leaf per plant was randomly selected from each of the 8 central plants (total 8 leaves per plot). Leaves were collected at the end of the trial, scanned using a conventional scanner and analyzed using ImageJ software. The percentage of each leaf that had been consumed by *C. chalcites* was calculated based on the entire leaf area.

2.6. Statistical analyses

When necessary percentage values for foliar damage were normalized by arcsine transformation. The percentage of efficacy and the percentage of foliar damage increase were subjected to analysis of variance (ANOVA) and mean separation by Tukey's test (P<0.05), using the Statistix v.10 package (Analytical Software, Tallahassee, FL, USA). Mortality of larvae collected from treated and control plants and reared in the laboratory at sequential times post-application was subjected to repeated measures analysis of variance (ANOVA).

3. RESULTS

3.1. Prevalence and diversity of ChchNPV infection in *C. chalcites* populations

A total of 4,438 larvae were collected from greenhouse and open-field banana crops, across 30 different sites on the islands (Table 1). The majority of larvae were collected in Tenerife (57%), followed La Palma (18%), Gran Canaria (13%), El Hierro (8%,) and La Gomera (4%). Of these, 103 larvae (2.3%) developed the characteristic signs of lethal polyhedrosis disease during laboratory rearing. None of the larvae were observed with signs of polyhedrosis disease during the process of collecting insects in the field. Overall, 95 (2.1%) larvae died due to parasitism, mostly by larval endoparasitoids such as *Cotesia* spp. or *Hyposoter* spp. Parasitism was ~3-fold higher in larvae collected in open-field than in greenhouse conditions, but was not analyzed in detail.

Of the virus diseased larvae, 56 were collected on Tenerife, 37 on El Hierro, 9 on La Palma, a single larva on La Gomera and no larvae on Gran Canaria (Table 1). The prevalence of infection varied from 0-4.8% on Tenerife and was usually low (0-2%) on the other islands, except for a single site on El Hierro where 13.8% of larvae were infected (37 out of 269 insects collected). The number of sites at which infected larvae were collected varied significantly between islands, from 11 out of 17 sites on Tenerife to a minimum of 0 out of 7 sites on Gran Canaria (Fisher's exact, P=0.029).

The influence of the greenhouse structure on the prevalence of virus infection was determined by examining the number of infected and healthy larvae collected from each type of production system using the values given in Table 1. Sites at which both open-field and greenhouse structures were sampled were classified as greenhouse samples. The overall prevalence of infection in greenhouse structures was 3% (91 infected out of 3027 larvae sampled), which was twice the prevalence of infection in larvae collected in open-field sites (1.4%, 12 infected out of 846 larvae sampled) (χ^2=6.44, df=1, P=0.011, not including sites on Gran Canaria on which no infected larvae were collected). This relationship remained significant even when all mixed sites (open-field and greenhouse) were removed from the analysis (χ^2=6.63, df=1, P=0.010).

Of the 103 virus infected larvae, 93 isolates could be identified by their restriction profile (Table 1). The majority of isolates (N=76) were identified as ChchNPV-TF1, representing 82% of the identified isolates. ChchNPV-TF2 was the next most prevalent with 11 isolates (12%), followed by ChchNPV-TF3 and ChchNPV-TF5 profiles with 3 isolates each (3% each). The previously characterized ChchNPV-TF4 variant restriction profile was not observed in any of the infected larvae. The ChchNPV-TF1 isolate was present in the islands of Tenerife (37 isolates), El Hierro (33 isolates) and La Palma (6 isolates), while ChchNPV-TF2 was present on Tenerife (10 isolates), and La Palma (1 isolate). ChchNPV-TF3 was present at low prevalence on Tenerife, La Gomera and El Hierro (1 isolate on each island). Finally, ChchNPV-TF5 was only present on El Hierro (3 isolates). Variants ChchNPV-TF1, -TF2, -

TF3 and -TF5 were collected from insects in greenhouse production systems, whereas variants -TF1, -TF2 and -TF3 were present in insects from open-field sites. As ChchNPV-TF1 was the most abundant and widespread variant on the archipelago, a single ChchNPV-TF1 isolate from Caldera del Rey in Tenerife was selected for field assays, as the majority of ChchNPV-TF1 isolates were found in Tenerife, especially at the Caldera del Rey site.

3.2. Determining the optimal ChchNPV-TF1 concentration

In terms of efficacy, significant differences were observed between the different viral concentrations applied both in greenhouse ($F_{3,12}$=40.47, P<0.01) (Fig 1A) and open-field plots ($F_{2,9}$=13.19, P=0.002) (Fig 1B).

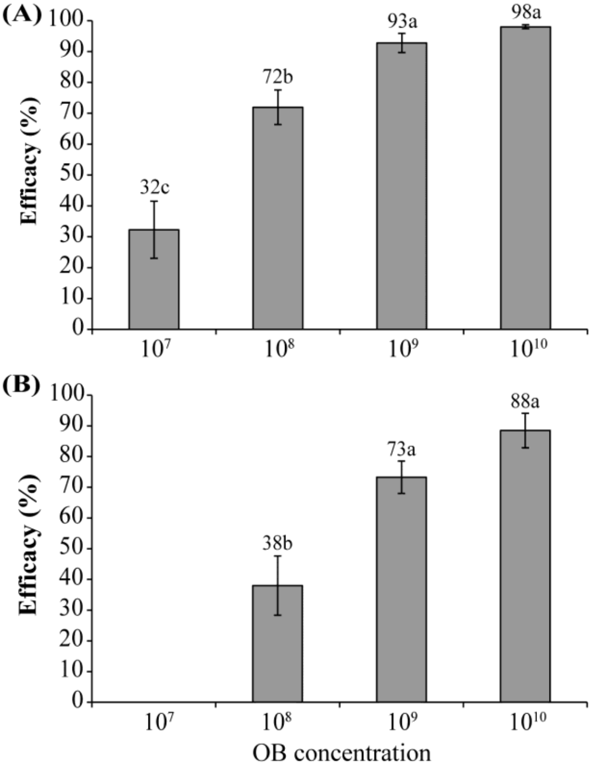

Figure 1. Percentage of insecticidal efficacy of different concentrations of ChchNPV-TF1 in (A) greenhouse and (B) open-field trials. Values followed by identical letters did not differ significantly (ANOVA, Tukey's test, P>0.05). Vertical lines indicate the standard error.

The two highest concentrations, 10^8 and 10^9 OBs/l, were similarly effective at controlling *C. chalcites* in both types of setting (Tukey's test, $p>0.05$), with up to 98% and 88% efficacy under greenhouse and open-field conditions, respectively. Application of 10^8 OB/l resulted in intermediate control efficacy (38-72% depending on setting), whereas 10^7 OB/l resulted in zero control efficacy of *C. chalcites* in the open-field and 32% control efficacy in greenhouse grown plants (Figs 1A and 1B).

In relation to the larval mortality produced at different time points after treatments, the different OB concentrations produced variable mortalities across the different time points both in greenhouse (Fig 2A) and open-field plots (Fig 2B). In the greenhouse trial, the three highest viral concentrations, 10^8, 10^9 and 10^{10} OBs/l, produced similar mortalities at different time points, between 87% to 100% (Tukey´s test, $P>0.05$), which were significantly higher than mortalities observed at the lowest concentration 10^7 OBs/l (Tukey´s test, $P<0.05$) which fluctuated around 50% during the 7 days of the trial. In contrast, in the open-field, the two highest concentrations produced similar mortalities of 86-100% (Tukey's test, $P>0.05$), whereas mortality in the lowest concentration treatment (10^7 OB/l) declined rapidly following a peak of 73% at day 1, and the 10^8 OB/l treatment fluctuated between 80 and 31% mortality during the trial (Fig 2B). DNA extracted from each group of dead larvae showed the same profile as the ChchNPV-TF1 variant, confirming that larvae died due to ChchNPV-TF1 infection.

According to these results, the concentration of 10^9 OBs/l was selected as the most suitable concentration for subsequent trials in greenhouses and open-field plots, since this concentration produced mortalities similar to those of the highest concentration tested.

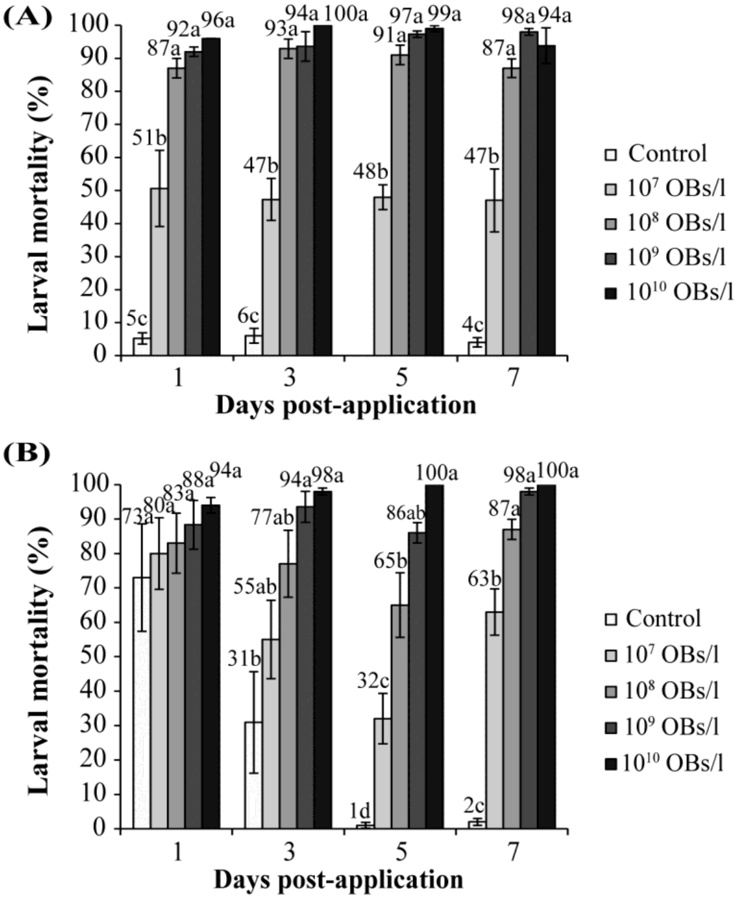

Figure 2. Percentage of larval mortality in insects collected at different times after treatment with different concentrations of ChchNPV-TF1 in (A) greenhouse and (B) open-field trials. Insects were reared in the laboratory until death or pupation. Values followed by identical letters did not differ significantly for comparisons of treatments within each time point (repeated-measures ANOVA, Tukey's test, P>0.05). Vertical lines indicate the standard error.

3.3. Insecticidal efficacy of ChchNPV-TF1 in greenhouse and open-field trials

Under greenhouse conditions foliar damage did not differ significantly between the different treatments and control plots in both years, with a 43-64% increase in foliar damage across all treatments and control in 2013 ($F_{3,66}$=2.37, P=0.08) (Fig 3A), compared to 0.6%-1.5% foliar damage in 2015

($F_{3,44}$=1.44, P=0.24) (Fig 3B). In open-field trials, in 2013 foliar damage was significantly lower in plots treated with the different insecticides compared with control plots ($F_{3,12}$=17.64, P<0.01) (Fig 3C). Similarly, in 2015 foliar damage was significantly lower in treated plots ($F_{3,60}$=5.78, P=0.001) (Fig 3D). In 2013 ChchNPV-TF1 treatment reduced foliar damage as did Bt (Tukey´s test, p>0.05), but less observed in the indoxacarb treatment (Tukey´s test, p<0.05) (Fig 3C). In contrast, in 2015 the ChchNPV-TF1 treatment was as effective as Bt and indoxacarb (Tukey´s test, p>0.05) (Fig 3D).

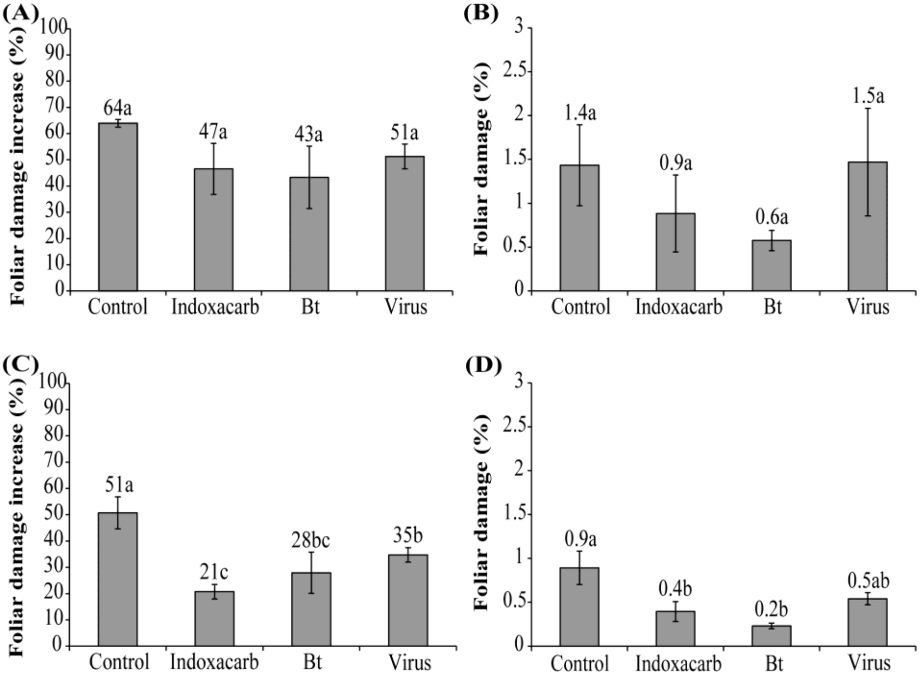

Figure 3. Percentage of foliar damage increase and foliar damage produced by *C. chalcites* in greenhouse in (A) 2013 trial and (B) 2015 trial, respectively, and percentage of foliar damage increase and foliar damage in open-field in (C) in 2013 trial and (D) in 2015 trial, respectively. Values followed by identical letters did not differ significantly (ANOVA, Tukey's test, P>0.05). Vertical lines indicate the standard error.

The insecticidal efficacy of ChchNPV-TF1 OBs in 2013 was similar to that of the other insecticides used in the greenhouse trial ($F_{2,6}$=2.63, P=0.15) (Fig 4A) and open-field trial ($F_{2,9}$=3.67, P=0.07) (Fig 4B). Similarly, in 2015 ChchNPV-TF1 was as effective as Bt and indoxacarb in open-field plots ($F_{2,9}$=2.79, P=0.11) (Fig 4C), whereas in greenhouses the ChchNPV-TF1 treatment was slightly less effective than indoxacarb but similar to that of Bt ($F_{2,6}$=21.33, P=0.002) (Fig 4D).

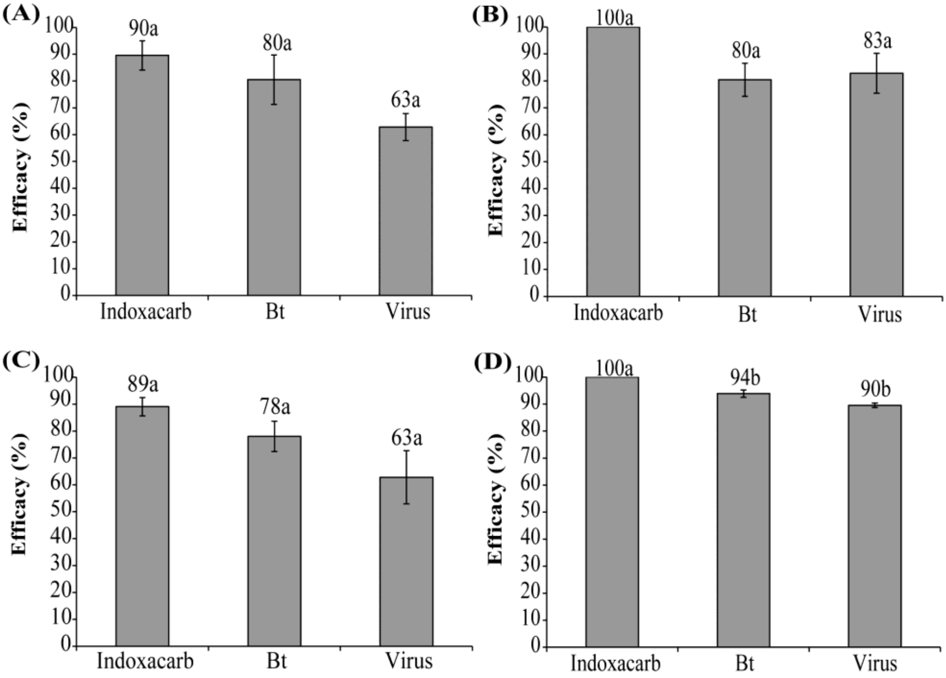

Figure 4. Percentage of insecticidal efficacy of different treatments in (A) greenhouse and (B) open-field trials in 2013 and in (C) open-field and (D) greenhouse trials in 2015. Values followed by identical letters did not differ significantly (ANOVA, Tukey's test, P>0.05). Vertical lines indicate the standard error.

ChchNPV-TF1 was the only treatment that resulted in a high prevalence of mortality in larvae collected over time. In 2013, control mortality was consistently low (1-8%) in insects collected over time from both greenhouse (Fig 5A) and open-field plots (Fig 5B). Insects from ChchNPV-

TF1 treated plots experienced 76-96% mortality during laboratory rearing in samples from the greenhouse trial (Fig 5A) and 95-100% in the open-field trial (Fig 5B). In contrast, insects from the indoxacarb and Bt treated plots had an intermediate prevalence of mortality during laboratory rearing, which tended to decrease over time, from 53-57% (day 1) to 13-16% (day 7) in larvae collected from the greenhouse plots (Fig 5A) and 41-52% (day 1) to 0-23% (day 7) in the open-field plots (Fig 5B). The low mortality observed in larvae collected in indoxacarb and Bt treatment was likely due to the rapid action of these insecticides in comparison with the virus, resulting in an initial peak of mortality that declined over time.

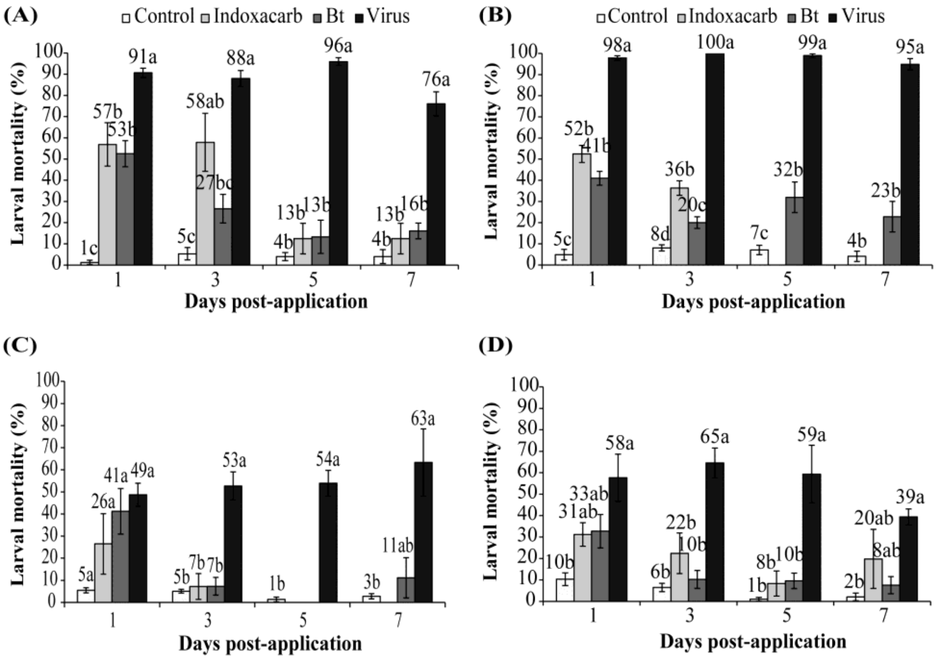

Figure 5. Percentage of larval mortality in laboratory-reared larvae collected at different time intervals after treatments in (A) greenhouse and (B) open-field trials in 2013 and in (C) greenhouse and (D) open-field trials in 2015. Values followed by identical letters did not differ significantly for comparisons of treatments within each time point (repeated-measures ANOVA, Tukey's test, P>0.05). Vertical lines indicate the standard error.

During the trials performed in 2015, insects from the control treatment experienced low mortality during laboratory rearing in the greenhouse (1-5%) and open-field trial (1-10%) (Figs 5C and 5D). Mortality during laboratory rearing varied in the ChchNPV treatment in the greenhouse trial (49-63% mortality) and the open-field trial (39-65% mortality), which was generally higher than mortalities observed in the other insecticide treatments. Laboratory-reared larvae from both indoxacarb and Bt treatments initially experienced intermediate prevalence of mortality in the greenhouse (26-41%) and open-field trail (31-33%), but mortality declined in samples taken during the trails both in the greenhouse (Fig 5C) and open-field plots (Fig 5D). At some time points near to the end of the trials (at day 5 and 7 post-application), no larvae were present on experimental plants treated with conventional insecticides, likely due to the insecticidal activity and rapid action of of Bt (Fig 5C) and indoxacarb (Figs 5B and 5C).

4. DISCUSSION

The present study aimed to evaluate the natural diversity of ChchNPV on the Canary Islands and the efficacy of this virus to control *C. chalcites* populations on young banana plants both in greenhouses and open-field trials. Overall, 2.3% of larvae collected from natural infestations of *C. chalcites* on banana plants died from ChchNPV infection during laboratory rearing, although cadavers with the characteristics signs of polyhedrosis disease were never observed in the field (except during field trials following application of the virus). In a smaller scale study in 2006 involving surveys of *C. chalcites* larvae on the islands of Tenerife, La Palma, Gran Canaria and El Hierro, overall 2.5% died due to lethal polyhedrosis disease (Bernal et al., 2013). This rather low prevalence of enzootic infection is similar to that reported in other nucleopolyhedrovirus pathosystems such as the nucleopolyhedrovirus of Spodoptera frugiperda (SfMNPV) on maize in Mexico (Ordoñez-García et al., 2015), Colombia (Gómez-Valderrama et al., 2010) or Brazil (Valiciente and Barreto, 1999). In contrast, high prevalence of infection and, on occasions, epizootics of lethal disease, have been detected in other nucleopolyhedrovirus-insect pathosystems, particularly in high density insect populations (Caballero et al., 1992; Cory and Myers, 2009; Graham et al., 2004).

Parasitism in laboratory-reared larvae was 2.1% overall, indicating a modest contribution of larval parasitoids to the control of *C. chalcites* populations at densities at which growers are likely to apply insecticidal control measures. Very similar results were obtained during a small scale survey performed in 2006 in which 2.3% of field-collected larvae died from parasitism (Bernal et al., 2013).

The majority of virus-infected *C. chalcites* larvae were collected on Tenerife (54%) and El Hierro (36%), in agreement with a previous study in which 69% and 22% of infected larvae were collected on Tenerife and El Hierro, respectively (Bernal et al., 2013). Although the highest numbers of larvae were collected on Tenerife (indicating a higher prevalence of infestation of banana crops by this pest on this island), there was no clear relationship between numbers of larvae collected and the prevalence of virus infection detected during laboratory rearing.

In contrast to parasitism, which was more accused in open-field collected larvae than in greenhouses (3:1), the prevalence of virus infection in greenhouse structures was twice that in open-field sites. This may in part be due to higher densities of infestation under greenhouse conditions, given that four-fold more larvae were collected during greenhouse compared to open-field sampling. However, a more likely explication resides in the protection from ultraviolet light provided by the greenhouse structure. Baculoviruses are rapidly inactivated by ultraviolet light (Ignoffo and Garcia, 1992) and plastic greenhouses effectively filter a large part of the ultraviolet spectrum (Costa et al., 2002; Lasa et al., 2007a). Although the effect of the mesh-built greenhouses used for banana production on incident ultraviolet light was not measured in the present study, it seems likely that reduced ultraviolet radiation on banana crops under plastic mesh could favor the conservation of ChchNPV OBs on foliage and in the upper layers of soil, resulting in a higher prevalence of virus infection in larvae compared to those feeding in open-field plantations.

In line with our previous study (Bernal et al., 2013) ChchNPV-TF1 was the most prevalent variant in the Canary Islands. The ChchNPV-TF2, ChchNPV-TF3 and ChchNPV-TF5 variants were isolated from larvae collected across the different islands, whereas previously we had only found these variants on southern Tenerife (Bernal et al., 2013). The prevalence of the ChchNPV-TF1 variant over that of other variants and its presence at numerous sites across the islands led us to use this variant in field trials. Previously we developed a specific mixture of ChchNPV genotypes with increased pathogenicity and virulence that has been the subject of a European Patent (Caballero et al., 2014). The stability of the specific genotype mixture was corroborated in the laboratory but not in field conditions, which may favour transmission of different proportions of certain genotypes resulting in changes to the overall phenotypic characteristics (Cory et al., 2005; Cory and Myers, 2009; Graham et al., 2004; Hodgson et al., 2002). Clearly this issue requires empirical testing but the ChchNPV-TF1 variant provided a useful model against which we will be able to evaluate the insecticidal efficacy of the specific genotype mixture in the future.

The insecticidal efficacy of the ChchNPV-TF1 variant was compared in greenhouse and open-field conditions, as ~65% of the banana production is produced in open-field conditions in the Canary Islands (MAPAMA, 2016) and we had not previously performed such a comparison. As greenhouse conditions tend to be more stable than those of open-field crops (Galán-Saúco et al., 1998; Robinson and Galán-Saúco, 2010), the harsher conditions of open-field crops might have implications for the quantities of viral OBs required for effective control or may affect the persistence of viral OB treatments on crop foliage (Grzywacz et al., 2008). Contrary to our expectations application of 10^9 OBs/l resulted in high mortality of *C. chalcites* larvae both in greenhouse and open-field banana plants. Previous studies had indicated that 10^9 OBs/l might be sufficient to protect banana plants from *C. chalcites* feeding damage under greenhouse conditions (Simón et al, 2015). This concentration of viral OBs is similar to that reported in other baculoviruses used as biological insecticides, such as those of nucleopolyhedroviruses applied for the control of *Spodoptera* spp. in greenhouse and field crops (Barrera et al., 2017, Grzywacz et al., 2008; Lasa et al., 2007b). As the leaf area and presence of fruit in the crop changes during growth the volume required to treat large plants tends to increase so that the overall quantity of OBs applied per hectare depends on the growth stage and crop phenology. For example, in the present study one liter of spray suspension at 10^9 OBs/l was used to treat 25 m^2 of young banana plants. As adult plants require volumes of up to 2,000 l/ha in commercial plantations (Fuentes et al., 2017), this would involve up to $2x10^{12}$ OBs/ha. This amount is within the range of OB applications typically used for commercial biological insecticides targeted at lepidopteran pests (Grzywacz et al., 2008).

The high natural larval mortality observed in greenhouse and open-field trials did not mask the efficacy of ChchNPV-TF1 as a biological insecticide. This natural mortality might be due to environmental factors such as wind or high temperatures recorded in greenhouses during the trials (up to 40ºC), as well as to the presence of predators or the movement of larvae from experimental plants in search of additional food resources (Ruberson et al., Biswas et al., 2003). Generally, ChchNPV-TF1 was as effective as conventional treatments reducing foliar damage and number of larvae under greenhouse and open-field conditions. We had previously observed that application of 10^8 OB/l of the ChchNPV-TF1 variant was as effective as indoxacarb and Bt treatments in controlling pest infestation and foliar damage under greenhouse conditions (Simón et al, 2015). Indeed, applications of 10^9 OBs/l were significantly more effective than the conventional treatments in the previous study. The reasons for this higher efficacy remain unclear. This may be due to variation in environmental conditions as previous field trails were performed in autumn, whereas those of the present study were performed during summer months during periods of high temperatures and strong sunlight that could have reduced OB persistence on plants. The inset colony used to infest experimental plants in the present study may also have been less susceptible to infection than colony used during the previous study, as strain variation in insect susceptibility to pathogens is a well-recognized phenomenon (Robertson et al., 1995).

Nonetheless in the present study, the insecticidal efficacy of ChchNPV was also clearly demonstrated under both greenhouse and open-field conditions. Indeed, under certain conditions baculoviruses can be as effective as chemical insecticides, although other characteristics such as their specificity, persistence in insect populations and their ability to control pests that are resistant to chemical insecticides make them uniquely valuable pest control agents in a range of situations (Eberle et al., 2012, Knox et al., 2015; Moscardi, 1999). However, the efficacy of the virus in protecting banana fruit from *C. chalcites* feeding damage remains to be tested. This is crucial because foliar feeding by this pest has little effect on banana yields whereas direct damage to bananas totally eliminates the commercial value of scarred fruit. Experiments are in progress to evaluate the efficacy of the virus in plantations of fruiting adult plants.

Application of ChchNPV-TF1 OBs resulted in an extended period of larval mortality in larvae collected and reared in the laboratory. Most larvae died 5-7 days after application of the virus (data not shown), but the fact that larvae collected at up to 7 days post-treatment continued to show high levels of lethal virus disease during laboratory rearing suggests that they had acquired a lethal dose of the virus several days after application of the virus. In contrast, indoxacarb and Bt treatments resulted in a rapid peak in mortality that declined over the 7-day sampling period, reflecting the different modes of action of these products compared to that of the virus. Similar results have been observed in samples taken over time in crops treated with baculoviruses and conventional insecticides for control of *Spodoptera exigua* in greenhouses (Lasa et al., 2007b; Belda et al, 2000; Kolodny-Hirsch et al., 1997) or *Helicoverpa armigera* (Cherry et al., 2000; Gupta et al., 2007). These results indicate a greater persistence of ChchNPV-TF1 OBs on the banana plant with respect to conventional treatments. Therefore, ChchNPV may provide an extended period of pest control, producing larval mortality for longer, compared to the other insecticides used in our study.

Currently, a low number of active substances are authorized for *C. chalcites* control in banana crops, with indoxacarb and Bt var. kurstaki being the most frequently used products (Fuentes et al., 2017). ChchNPV-TF1 provides an attractive alternative to Bt for *C. chalcites* control as a highly effective and highly specific insecticide that does not leave xenobiotic residues in fruit and is compatible with IPM systems that aim to conserve natural enemy populations (Pedigo, 1996; Biswas et al., 2003; Erlandson et al., 2007). ChchNPV-TF1 based products could be incorporated into integrated pest management programs, given the compatibility of this virus with biological and chemical control measures, thus reducing farmer dependence on synthetic insecticides and thereby reducing the likelihood of the development of insecticide resistance in the pest population. As a low number of substances are authorized and those are used repeatedly, the market for a ChchNPV-TF1-based product is well defined and could commercially viable if adopted by banana growers on the islands.

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6. REFERENCES

Arrizubieta, M., Williams, T., Caballero, P., Simón, O., 2014, Selection of a nucleopolyhedrovirus isolate from *Helicoverpa armigera* as the basis for a biological insecticide. Pest Management Science70, 967-976.

Barrera, G.P., Gómez-Valderrama, J.A., Villamizar-Rivero, L.F., 2017. Efficacy of microencapsulated nucleopolyhedroviruses from Colombia as biological insecticides against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Acta Agronomica 66, 267-274.

Belda, J.E., Mirasol, E., Escribano, A., Rapallo, S., Caballero, P., 2000. Eficacia de nucleopoliedrovirus (VPNse) en el control de *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) en pimiento en invernadero. Boletín de Sanidad Vegetal Plagas 26, 619-628.

Bernal, A., Williams, T., Hernández-Suárez, E., Carnero, A., Caballero, P., Simón, O., 2013. A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: The basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banana crops. Biological Control 67, 101-110.

Biswas, D., Narayanan, K., Chakraborty, M., 2003. Survey for natural enemies of *Galleria mellonella* and cross infectivity of its nucleopolyhedrovirus. Entomon 28, 179-183.

Caballero, P., Aldebis, H.K., Vargas-Osuna, E., Santiago-Álvarez, C., 1992. Epizootics caused by a nuclear polyhedrosis virus in populations of *Spodoptera exigua* in Southern Spain. Biocontrol Science Technology 2, 35-38.

Caballero, P., Bernal, A., Simón, O., Carnero, A., Hernández-Suárez, E., Williams, T., 2014. Novel genotypes of *Chrysodeixis chalcites* single nucleopolyhedrovirus (ChchSNPV), a procedure for its production and use as a biological control agent. European Patent PCT-760.

Cabello, T., Hernández, M.D., 1988. Actividad de alimentación de las larvas de *Agrotis segetum* (Denis y Schiffermüller) y *A. ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) y niveles de daños en maíz. Boletín de Sanidad Vegetal Plagas 14, 295-305.

Cherry, A.J., Rabindra, R.J., Parnell, M.A., Geetha, N., Kennedy, J.S., Grzywacz, D., 2000. Field evaluation of *Helicoverpa armigera* nucleopolyhedrovirus formulations for control of the chickpea pod-borer, *H. armigera* (Hübn), on chickpea (*Cicer arietinum* var. Shoba) in southern India. Crop Protection 19, 51-60.

Cory, J.S., Green, B.M., Paul, R.K., Hunter-Fujita, F., 2005. Genotypic and phenotypic diversity of a baculovirus population within an individual insect host. Journal of Invertebrate Pathology 89, 101-111.

Cory, J.S., Myers, J.H., 2009. Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis. Journal of Animal Ecology 78, 646-655.

Costa, H.S., Robb, K.L., Wilen, C.A., 2002. Field trials measuring the effects of ultraviolet-absorbing greenhouse plastic films on insect populations. Journal of Economic Entomology 95, 113–120.

Del Pino, M., Carnero, A., Hernández-Suárez, E., Cabello, T., 2011. La lagarta o bicho camello, *Chrysodeixis chalcites* (Esper, 1789), una plaga emergente en los cultivos de platanera de Canarias. Phytoma 225, 21-16.

Domínguez, E., López-Cepero, J., Nogueroles, C., 2012. Identificación y control de plagas y enfermedades, in: Nogueroles, C. (Ed.), Calidad y sostenibilidad en el cultivo de la platanera en Canarias. ASPROCAN, Tenerife, pp.145-170.

Eberle, K.E., Jehle, J.A., Huber, J., 2012. Microbial control of crop pest using insect viruses. in: Abrol, D.P., Shankar, U., (Eds). Integrated pest management: principles and practice. CAB International, Oxfordshire, pp. 281-298.

Erlandson, M.A., 2009. Genetic variation in field populations of baculoviruses: mechanisms for generating variation and its potential role in baculovirus epizootiology. Virologica Sinica 24, 458-469.

Erlandson, M., Newhouse, S., Moore, K., Janmaat, A., Myers, J., Theilmann, D., 2007.Characterization of baculovirus isolates from *Trichoplusia ni* populations from vegetable greenhouses. Biological Control 41, 256-263.

Flint, M.L., van den Bosch, R., 1981.Introduction to integrated pest management. New York: Plenum press.

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017. *Chrysodeixis chalcites*, a pest of banana crops on the Canary Islands: incidence, economic losses and current control measures. Crop Protection Submitted.

Galán-Saúco, V., Cabrera-Cabrera, J., Hernández-Delgado, P.M., Rodríguez-Pastor M.C., 1998. Comparison of protected and open-air cultivation of Grand Naine and Dwarf Cavendish banana. Acta Horticulturae 490: 247-259.

Gómez-Valderrama, J.A., Guevara-Agudelo, E.J., Barrera-Cubillos, G.P., Cotes-Prado, A.M., Villamizar-Rivero, L.F., 2010. Aislamiento, identificación y caracterización de nucleopoliedrovirus nativos de *Spodoptera frugiperda* en Colombia. Revista Facultad Nacional Agronomia de Medellín 63, 5511-5520.

Graham, R.I., Tyne, W.I., Possee, R.D., Sait, S.M., Hails, R., 2004. Genetically variable nucleopolyhedroviruses isolated from spatially separate populations of the winter moth *Operophtera brumata* (Lepidoptera: Geometridae) in Orkney. Journal of Invertebrate Pathology 87, 29-38.

Grzywacz, D., Mushobozi, W.L., Parnell, M., Jolliffe, F., Wilson, K., 2008. Evaluation of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) for the field control of African armyworm (*Spodoptera exempta*) in Tanzania. Crop Protection 27, 17-24.

Gupta, R.K., Raina, J.C., Arora, R.K., Bali, K., 2007. Selection and field effectiveness of nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hübner). International Journal of Virology 3, 45-59.

Henderson C.F., Tilton, E.W., 1955. Tests with acaricides against the brown wheat mite. Journal Economic Entomology 48, 157-161.

Hodgson, D.J, Vanbergen, A.J., Hartley, S.E., Hails, R.S., Cory, J.S., 2002. Differential selection of baculovirus genotypes mediated by different species of host food plant. Ecology Letters 5, 512-518.

Horowitz, A.R., Weintraub, P.G., Ishaaya, I., 1998. Status of pesticide resistance in arthropod pests in Israel. Phytoparasitica 26, 231-240.

Hughes P.R., Wood, H.A., 1981. A synchronous per oral technique for the bioassay of insect viruses. Journal of Invertebrate Pathology 37, 154-159.

Ignoffo, C.M., Garcia, C., 1992.Combinations of environmental factors and simulated sunlight affecting activity of inclusion bodies of the *Heliothis* (Lepidoptera: Noctuidae) nucleopolyhedrosis virus. Environmental Entomology 21,210-213.

Kolodny-Hirsch, D.M., Sitchawat, T., Jansiri, T., Chenrchaivachirakul, A., Ketunuti, U., 1997. Field Evaluation of a Commercial Formulation of the *Spodoptera exigua* (Lepidoptera: Noctuidae) Nuclear Polyhedrosis Virus for Control of Beet Armyworm on Vegetable Crops in Thailand. Biocontrol Science Technology 7, 475-488.

Knox, C., Moore, S.D., Luke, G.A., Hill, M.P., 2015. Baculovirus-based strategies for the management of insect pests: a focus on development and application in South Africa. Biocontrol Science and Technology 25, 1-20.

Lasa, R., Ruiz-Portero, C., Alcázar, M.D., Belda, J.E., Caballero, P., Williams, T., 2007a. Efficacy of optical brightener formulations of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as a biological insecticide in greenhouses in southern Spain. Biological Control 40, 89-96.

Lasa, R., Pagola, I., Ibáñez, I., Belda, J.E., Williams, T., Caballero, P., 2007b. Efficacy of *Spodoptera exigua* multiple nucleopolyhedrovirus as a biological insecticide for beet armyworm control in greenhouses of southern Spain. Biocontrol Science Technology 17, 221-232.

MAPAMA. Superficies y producciones anuales de cultivo de acuerdo con el Reglamento (CE) 543/2009. Ministerio de Agricultura y pesca, alimentación y medio ambiente. 2016. Available from: http://www.mapama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/ (accessed 12.4.17).

Moscardi, F., 1999. Assessment of the application of baculoviruses for the control of Lepidoptera. Annual Review of Entomology 44, 257-289.

Ordoñez-García, M., Ríos-Velasco, C., Berlanga-Reyes, D.I., Acosta-Muñiz, C.H., Salas-Marina, M.A., Cambero-Campos, O.J., 2015. Occurrence of natural enemies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Chihuahua, Mexico. Florida Entomology 98, 843-847.

Pedigo, L.P., 1996. Entomology and Pest Management. Prentice-Hall Pub. New Jersey.

Perera, S., Molina, M.J., 2007. Plagas y enfermedades en el cultivo ecológico de la platanera, in: Nogueroles, C., Líbano, J., (Eds.), El cultivo ecológico de la platanera en Canarias. Gabinete de Proyectos Agroecológicos SL, Tenerife, pp. 77-118.

Robertson J.L., Preisler, H.K., Ng, S.S., Hickle, L.A., Gelernter, W.D., 1995. Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. Journal of Economic Entomology 88, 1-10.

Robinson, J.C., Galán Saúco, V., 2010. Bananas and plantains, CABI, Wallingford, UK.

Ruberson, J.R., Herzog, G.A., Lambert, W.R., Lewis, W.J., 1994. Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: role of natural enemies Florida Entomology 77, 440-453.

Simón, O., Bernal, A., Williams, T., Carnero, A., Hernández-Suárez, A., Muñoz, D., Caballero, P., 2015. Efficacy of an alphabaculovirus-based biological insecticide for control of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) on tomato and banana crops. Pest Management Science 71, 1623-1630.

Valicente F.H., Barreto, M., 1999. Levantamento dos inimigos naturais da lagarta do cartucho do milho, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), na região de Cascavel, PR. Anais da Sociedade Entomológica do Brasil 28, 333-337.

CHAPTER IV

Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV): an effective biological control agent against *C. chalcites* damage in commercial banana crops on the Canary Islands

ABSTRACT

The effectiveness of a native Chrysodeixis chalcites nucleopolyhedrovirus isolate from Tenerife (ChchNPV-TF1), which proved useful to control *C. chalcites* damage in young banana plants at small-scale, was measured in commercial banana mesh-built greenhouses on the Canary Islands. In 2014 trials, foliar damage evolution was similar between both control strategies. Surprisingly, fruit damage was higher in Tenerife ChchNPV-TF1 treatment plot (4.8±1.9%) than in conventional plot (0.2±0.2%). While in Gran Canaria and La Palma a similar fruit damage was recorded in ChchNPV-TF1 treatment (1.1±0.7% and 0±0%, respectively) and conventional plots (2.9±1.2% and 0.3±0.3%, respectively). In Tenerife and Gran Canaria trials larval mortality was higher in ChchNPV-TF1 treatment plots (68±10% and 28±13%, respectively) than in conventional plots (17±2.9% and 5±3.5%, respectively). In contrast, in La Palma the low *C. chalcites* incidence did not enable us to determine larval mortality. A second trial was performed in Tenerife in 2015, in which treatments started several months before fruit development. As shown in 2014, foliar damage was similar in both control strategies and larval mortality was higher in ChchNPV-TF1 treatment plot. But in this case, fruit feeding damage was equalized between viral (4.0±1.7%) and conventional (1.9±1.1%) treatment plots. Therefore, ChchNPV-TF1 might provide an effective control against *C. chalcites* damage in commercial banana crops on Canary Islands, as long as viral treatments are applied before fruit development. Therefore, this new biological control agent proves useful for its inclusion in integrated management of *C. chalcites* in banana crops on the Canary Islands.

1. INTRODUCTION

Banana (*Musa acuminata* Colla) is the main crop of the Canary Islands, not only at surface (8,975 hectares) and production level (381,983 tons in 2015), but also at economic level, with an economic impact of 182 million euros (MAPAMA, 2016). The island of Tenerife is the largest producer of bananas (45% of the total production), followed by La Palma (33%) and Gran Canaria (20%), while the remaining 2% of the production occurs on the islands of La Gomera, El Hierro and Lanzarote (ASPROCAN, 2016).

Bananas are cultivated in mesh-built greenhouses on the warmer southern slopes and in the open-field on the cooler northern parts (González-Concepción et al., 2008). As such, crops grown under mesh tend to be more prone to phytosanitary problems (Galán-Saúco, 1992; Robinson and Galán-Saúco, 2010). Actually, one of the most harmful pests is *Chrysodeixis chalcites* (Esper, 1789) (Lepidoptera: Noctuidae). Despite treatment control, its incidence in banana crops could reach 100% in some islands (Fuentes et al., 2017a). Major damages are produced in leaves of immature plants, with no substantial effect on final crop production. Whereas direct feeding damage on fruits has a clear and important impact on the commercial values of this product, reaching the losses 3 million euros/year (Fuentes et al., 2017a). Damage to fruit during the spring is particularly important as it coincides with the development of the fruit (Del Pino et al., 2011; Fuentes et al., 2017a), and with the main peak flight of the pest (Del Pino et al., 2011). Therefore, monitoring and controlling *C. chalcites* during spring season becomes necessary.

Currently, *C. chalcites* control is based on the use of a single dominant insecticide among the few active substances authorized by the European Union in banana crops (Méndez-Hernández et al., 2017), being the most commonly used substances the chemical indoxacarb, followed by chlorpyrifos and the biological insecticide *Bacillus thuringiensis* var. kurstaki (Bt) (Fuentes et al., 2017a). But the continuous used of a single dominant insecticide led to *C. chalcites* developing resistance and to reduce the effectiveness of these products (Del Pino et al., 2011; Horowitz et al., 1998). As such, new insecticides are required to rotate with the low number of authorized compounds. Among these highlighted baculoviruses, being a clear alternative to the normally used pesticides (Knox et al., 2015).

Previous studies showed that the most widespread isolate of the nucleopolyhedrovirus of *C. chalcites* in Canary Islands (ChchNPV-TF1) (Bernal et al., 2013; Fuentes et al., 2017b), showed to be highly effective control agent reducing foliar damage and larval density in young banana plants grown in small-scale under greenhouse and open-field conditions (Bernal et al., 2013; Fuentes et al., 2017b; Simón et al., 2015). However, previous to its inclusion in integrated pest control programs it is necessary to assess its efficacy in protecting banana fruits from *C. chalcites* feeding damage.

Therefore, the aim of the present study was to evaluate the efficacy of ChchNPV-TF1 in comparison with conventional treatments, reducing fruit damage in commercial banana crops mesh-built greenhouses during a complete crop cycle.

2. MATERIALS AND METHODS

2.1. Production of ChchNPV-TF1 OBs

The *C. chalcites* colony used for ChchNPV-TF1 production was established with larvae collected in banana crops in Southern Tenerife, Spain. This colony was maintained in the Instituto Canario de Investigaciones Agrarias (ICIA), Tenerife, Spain, at 25±1ºC, 60-80% relative humidity and a photoperiod of 16:8 h (light:dark) on a semi-synthetic diet described by Cabello and Hernández (1988). Adults were fed with 10% (v/v) diluted honey.

ChchNPV-TF1 OBs were produced by inoculating fifth or sixth instar laboratory-reared larvae with $5.00x10^7$ OBs/ml or $9.02x10^8$ OBs/ml, respectively (Bernal et al., 2014), suspended in 10% sucrose solution and 0.001% Fluorella blue food dye, using the droplet feeding method (Hughes and Wood, 1981). Infected larvae were placed in transparent plastic boxes of 24 wells with semi-synthetic diet and incubated at 25ºC. Larvae were checked daily for signs of polyhedrosis disease and dead insects were collected and stored at -20ºC. OBs were purified by filtration through muslin and centrifugation at 3,800 x *g* for 5 minutes. Purified OBs were resuspended in sterile water and their concentration was determined by counting the samples in triplicate using an Improved Neubauer haemocytometer (Superior Marienfield, Laude-Koeningshofen, Alemania) under phase contrast microscopy at x 400. Purified OBs were stored at 4ºC until its use. The identity of multiplied OBs was confirmed by restriction endonuclease analysis using *Bgl*II, which previously was found to allow a correct identification of ChchNPV isolates (Bernal et al., 2013).

2.2. Experimental design in commercial mesh-built greenhouses in Canary Islands

The commercial banana crops were selected in those islands with high incidence of *C. chalcites* and/or wide cultivated area, and in those plantations with high incidence within each island (Fuentes et al., 2017a). Initially one trial per island was carried during spring-summer of 2014, to coincide with the most susceptible period for determining the quantity and quality of the harvested fruit in banana crops (Fuentes et al., 2017a), and with the first flight of *C. chalcites* in the islands (Del Pino et al., 2011; Fuentes et al., 2017a). Concretely, in Tenerife the trial was carried out from April to October 2014, while in Gran Canaria and La Palma from May to November and from May to October, respectively. A second trial was designed in Tenerife during the complete crop cycle, from October 2014 to September 2015 (2015 Trial), covering the second flight that occurs in autumn and the first flight in spring (Del Pino et al., 2011; Fuentes et al., 2017a).

The banana plantations in Tenerife were located in the area of El Fraile in the south of Tenerife. 2014 trial was carried out in a 1.17 ha mesh-built greenhouse (28º 0' 31" N 16º 41' 1" W), while 2015 trial in a 7.29 ha mesh-built greenhouse (28º 0' 14" N 16º 41' 4" W), both plots with ~1,850 plants/ha planting density. In Gran Canaria, the trial was developed in 1.18 ha mesh-built greenhouse in Vecindario in the south of the island (27º 50' 24" N 15º 25' 47" W), with ~1,800 plants/ha. Finally, in La Palma the trial was realized in two little mesh-built greenhouses located in El Remo, in the southwest of the island: one of 0.2 ha (28º 33' 18"N 17º 53' 12" W) and the other of 0.22 ha (28º 33' 17.7"N 17º 53' 12" W), both with ~1,600 plants/ha density. In Tenerife and Gran Canaria, greenhouses were divided in two similar zones for each treatment, while in La Palma each treatment was applied in a different mesh-built greenhouse.

ChchNPV-TF1 was supplied to technicians in 1-L bottles containing $1x10^{13}$ OBs/l. OBs were added to the spray tank at a rate of 1 ml of ChchNPV-TF1 suspension per 10 l of spray volume, being the effective ChchNPV-TF1 concentration of $1x10^{9}$ OBs/l of spray (Fuentes et al., 2017b). Following usual crop practices, technicians applied the product with a commercial wetter-sticker and an acid to reduce the final pH to approximately 6.5 in a volume of water sufficient to wet the crop entirely. In Tenerife trials 0.05% (v/v) Agral (Sygenta S.A., Madrid, Spain) was used as wetter-sticker, and no pH regulator was required since the water had an adequate pH. In Gran Canaria 0.05% (v/v) wetter-sticker Humectan plus (Agriphar Iberia, S.L.U., Sevilla, Spain) was used, and the ACM-Peache Plus as pH regulator (Agro Consulting del Mediterráneo S.L., Valencia), and in La Palma Adrex and Lower 7 as wetter-sticker and pH corrector (SAPEC Agro España, S.A.U., Valencia, Spain), respectively. In Tenerife trials treatments were applied using a McCormick

GM 50 tractor with a six nozzles atomizer of 400 litters tank (Tomix, Torres Vedras, Portugal), whereas in Gran Canaria a Dorado F 100 tractor with twelve nozzles atomizer of 600 litters tank. In contrast, in La Palma treatments were performed using hose with 1.5-2 mm nozzle gun. On the same days or in the days close to the NPV application, the conventional treatment was also carried out in the corresponding area of the commercial plantation, with products normally used to control *C. chalcites* in banana crops; indoxacarb, *Bacillus thuringiensis* var. kurstaki, chlorpyrifos or spinosad (Fuentes et al., 2017a). The application frequency was subjected to personal observations by technicians and the rest of cultural practices were carried out according to the usual practices of greenhouses.

In 2014 trials, in Tenerife seven applications were performed starting in May and with a monthly frequency between the first four treatments, and with 1-2 weeks frequency between the last three treatments (Table 1). While

Table 1. Treatment timing in commercial banana crops in the islands of Tenerife, Gran Canaria and La Palma, indicating the date of the treatment application, volume used in each application and the product used in conventional (Conv.) and baculovirus (BV) treatment plot.

Year	Island	Treatment date	Applied volume (l)	Treatment	
				Conv.	BV
2014	Tenerife	15/05/2014	600	Bt	ChchNPV-TF1
		12/06/2014	600	Indoxacarb	ChchNPV-TF1
		10/07/2014	600	Bt	ChchNPV-TF1
		12/08/2014	800	Spinosad	ChchNPV-TF1
		21/08/2014	800	Bt	ChchNPV-TF1
		28/08/2014	800	Bt	ChchNPV-TF1
		16/09/2014	800	Indoxacarb	ChchNPV-TF1
	Gran Canaria	18/06/2014	900	Bt	ChchNPV-TF1
		26/08/2014	800	Bt	ChchNPV-TF1
		25/09/2014	900	Bt	ChchNPV-TF1
	La Palma	22/07/2014	1,000	Bt	ChchNPV-TF1
		02/09/2014	900	Bt	ChchNPV-TF1
		02/10/2014	1,000	Bt	ChchNPV-TF1
2015	Tenerife	26/09/2014	2,400-2,800	Bt	ChchNPV-TF1
		10-11/10/14	2,400-2,800	Bt	ChchNPV-TF1
		25-27/10/2014	3,600	Indoxacarb	ChchNPV-TF1
		06/11/2014	3,600	-	ChchNPV-TF1
		23/12/2014	2,000	Bt	ChchNPV-TF1
		23/03/2015	2,000	Indoxacarb	ChchNPV-TF1
		05/06/2015	2,000	Bt	ChchNPV-TF1
		13/07/2015	2,000	Bt	ChchNPV-TF1
		02/09/2015	2,000	-	ChchNPV-TF1

in Gran Canaria and La Palma trials only three treatments were performed with an approximately one-month frequency (Table 1). Finally, in 2015 trial in Tenerife nine treatments were performed, the first four treatments every two weeks and the rest with one month and three months interval.

2.3. Evaluation of the effectiveness of ChchNPV-TF1

2.3.1. Foliar damage

Foliar damage was evaluated by visual scale in 28-32 randomly selected plants in conventional and ChchNPV-TF1 treatment plots in Tenerife, Gran Canaria and La Palma. Visual scale consisted in five categories: Category 0: no damage, Category 1: 5-20 % leaf damage (percentage of leaf area showing damage), Category 2: 21-40% leaf damage, Category 3: 41-60% leaf damage and Category 4: > 60% leaf damage (Fuentes et al., 2017a). Systematic biweekly samplings were performed until fruit emergence, which varied depending the trial; in 2014 trials in Tenerife nine samplings were carried out from 16 April to 19 August, in Gran Canaria eight samplings between 19 May and 1 September and in La Palma ten samplings between 9 May and 11 September. In 2015 in Tenerife trial seventeen samplings were carried out from 9 October to 10 June.

Thereafter, the average percentage of foliar damage was calculated using the formula of Townsend-Heuberger (1943):

$$\left(\frac{\sum(\mathrm{nxv})}{\mathrm{V\,x\,N}}\ \mathrm{x}\ 100\right)$$

Where *n* is the number of sample units in each category, *v* the value of each category (0, 1, 2, 3, 4 or 5), *V* the value of the highest category and *N* the total number of sample units.

2.3.2. Fruit damage

Fruit damage was evaluated once bunches were emitted in the same 28-32 selected plants for foliar damage evaluation. Several prospections were performed to evaluate the bunch emergency as it varied considerably between plants; being this period in 2014 trials in Tenerife from 17 September to 16 October, in Gran Canaria from 16 September to 4 November and in La Palma from 24 August to 6 October, and finally in 2015 trial in Tenerife from 7 August to 18 September. For determining the percentage of fruit damage, only the data obtained the last day of prospections was used, before fruit pocketing, moment in which fruit is wrapped with a plastic bag to avoid fruit damage.

Fruit damage was estimated by determining the percentage of damaged banana handles respect to the total present on each bunch. A small fruit perforation characteristic of *C. chalcites* feeding in any finger (fruit) of the handle was considered as the entire handle affected.

2.3.3. Larval mortality

The effectiveness of ChchNPV-TF1 respect to conventional insecticides was also evaluated by comparing the mean percentage of larval mortality obtained in conventional and ChchNPV-TF1 plots, on the basis of mortality data from each collection time. In all the cases, larvae were collected 4-5 days after each treatment. Larvae were also collected prior to insecticides application to verify the absence of virus and to estimate larval densities. In both treatment plots larval density at the beginning of the assay was similar (data not shown). We attempt to collect ≈25 larvae at each collection time, however the infrastructures avoid us to collect in each collection time and in some collection times there were not enough larvae. Collected larvae were individualized in sterile plastic pots of 25 ml containing artificial diet (Cabello and Hernández, 1988). Larvae were kept under laboratory conditions in an insect rearing chamber at a temperature of 25±2°C, relative humidity of 70±15% and photoperiod of 16:8 hours (L:D), until death or pupation.

In 2014 trials, in Tenerife although 7 treatments were performed larvae were collected just in three of them. In Gran Canaria larvae were collected only after two treatment applications, while in La Palma although larvae were attempted to collect in the three treatments we were unable to collect them due to the low *C. chalcites* incidence. Finally, in 2015 trial in Tenerife larvae were collected in seven of the nine treatments. In Tenerife trials conventional treatments consisted mostly in alternation of Bt and indoxacarb, while in Gran Canaria and La Palma Bt was only applied.

2.4. Statistical analyses

The mean percentages of larval mortality of both control strategies within each island were normalized by arcsine transformation and subjected to t-tests, using Statistix v.10 program, Analytical Software, (Tallahassee, FL, USA). Similarly, mean percentages of fruit damage obtained in both control strategies within each island data were subjected to Mann-Whitney U test, using the same program, as data were not normally distributed after data transformation.

3. RESULTS

3.1. ChchNPV-TF1 efficacy reducing foliar damage

In 2014 trials, in Tenerife conventional treatment plot initially presented 1.3 to 2.6-fold less foliar damage than ChchNPV-TF1 treatment plot, but by the middle of the sampling period the damage of the two plots was equalled, and both showed a final foliar damage of 31% (Fig. 1A). In Gran Canaria trial, foliar damage in ChchNPV-TF1 was practically identical to that of conventional plot throughout all the sampling period, ranging from 4-5% to 33% (Fig. 1B). In contrast, in La Palma a low foliar feeding damage was registered, especially in ChchNPV-TF1 treatment plot, probably due to the low prevalence of *C. chalcites* in La Palma during the assay. Foliar damage was initially higher in conventional plot (18%) than in ChchNPV-TF1 treatment plot (4%), but by the middle foliar damage in both plots matched and by the end conventional treatment plot showed higher foliar damage (18%) than ChchNPV-TF1 (4%) (Fig. 1C).

Finally in 2015 trial in Tenerife, conventional plot showed 1.25 to 2.5-fold less foliar damage than ChchNPV-TF1 treatment plot, but towards the last months the damage was equalized in both plots, and by the end of the assays the ChchNPV-TF1 treatment plot even showed 1.4 to 1.9-fold lower damage than conventional plot (Fig. 1D).

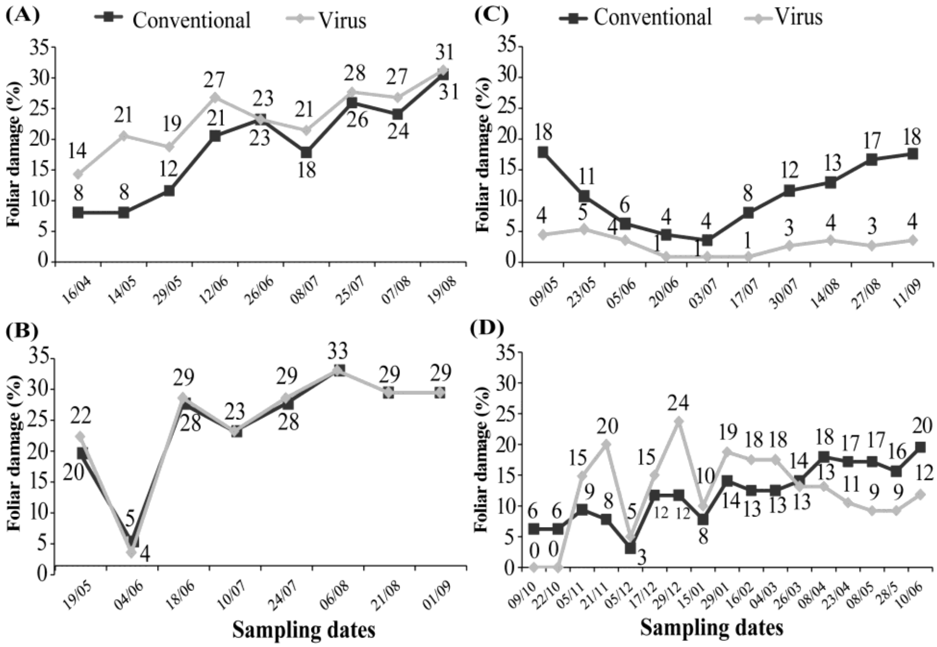

Figure 1. Foliar damage evolution produced by *C. chalcites* during all the sampling period in conventional and ChchNPV-TF1 treatment plots in 2014 trials in (A) Tenerife, (B) Gran Canaria and (C) La Palma, and 2015 trial in (D) Tenerife.

3.2. ChchNPV-TF1 efficacy reducing fruit damage

In 2014 trials (Fig. 2A), in Tenerife the average percentage of damaged banana handles was surprisingly higher in ChchNPV-TF1 treatment plot (4.8±1.9%) than in conventional treatment plot (0.2±0.2%) (U Mann-Whitney, P=0.04). In contrast, in Gran Canaria the average percentage of damaged banana handles was similar between ChchNPV-TF1 treatment plot (1.1±0.7%) and conventional treatment plot (2.9±1.2%) (U Mann-Whitney, P=0.15). Finally, in La Palma trial few damage was observed, being similar between ChchNPV-TF1 treatment plot (0±0%) and conventional plot (0.3±0.3%) (U Mann-Whitney, P=0.33).

In 2015 trial in Tenerife (Fig. 2B), in contrast to that of 2014 trial, the average percentage of damaged banana handles was similar in ChchNPV-TF1 treatment plot (4.0±1.7%) and in conventional treatment plot (1.9±1.1%) (U Mann-Whitney, P=0.44) (Fig. 2A). When comparing both years, the average percentage of damaged banana handles was similar in ChchNPV-TF1 treatment plot (U Mann-Whitney, P=0.99) and in conventional treatments (U Mann-Whitney, P=0.15).

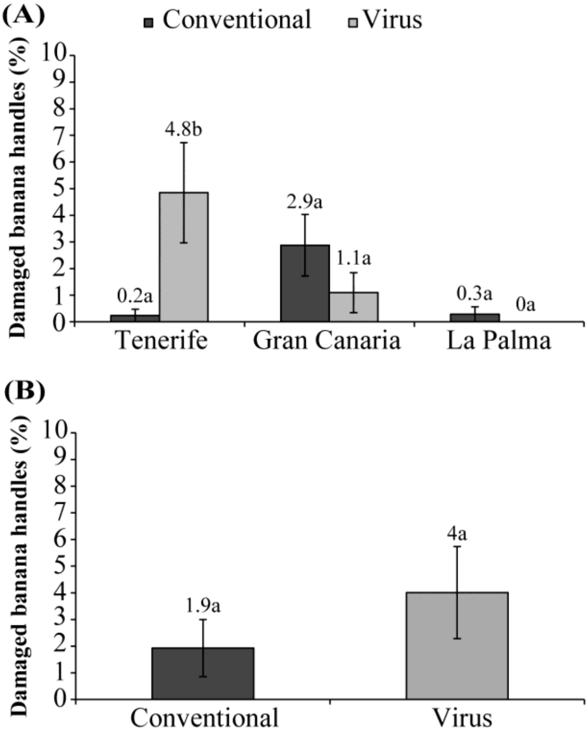

Figure 2. Mean percentage of banana handles damaged by *C. chalcites* in conventional and ChchNPV-TF1 treatment plots in 2014 trials in (A) Tenerife, Gran Canaria and La Palma, and 2015 trial in (B) Tenerife. Values followed by identical letters did not differ significantly between treatments within each island (Mann-Whitney U, P>0.05). Vertical lines indicate the conventional error.

3.3 ChchNPV-TF1 efficacy reducing larval density

In general, the number of larvae collected in conventional and ChchNPV-TF1 treatment plots were similar in each trial, being 54 versus 64, 41 versus 30, and 165 versus 187 in 2014 trials in Tenerife and Gran Canaria, and in 2015 trial in Tenerife, respectively.

In 2014 trials (Fig. 3A), in Tenerife the percentage of larval mortality (mean larval mortality±standard Error) was significantly higher in ChchNPV-TF1 treatment plot (68±10%) than in conventional treatment plot (17±2.9%) (t=3.96; d.f.=4; P=0.02). Similarly, in Gran Canaria a higher mortality was recorded in ChchNPV-TF1 treatment plot (28±13%) than in conventional plot (5.0±3.5%) (t=3.13; d.f.=2; P=0.04). Regrettably, in La Palma trial a very low incidence of *C. chalcites* was found in both plots, especially in ChchNPV-TF1 treatment plot, hindering the collection of larvae. Therefore, we could not obtain sufficient larvae to estimate the percentage of larval mortality.

Finally, in 2015 trial in Tenerife (Fig. 1B) the percentage of larval mortality was also higher in ChchNPV-TF1 treatment plot (68%±4.1%) than in conventional treatments (25%±11.3%) (U Mann-Whitney, P=0.03). When comparing both years, no significant differences were found between ChchNPV-TF1 treatment plots (t=0.01; d.f.=8; P=0.99) and conventional treatment plots (t=0.29; d.f.=6; P=0.78).

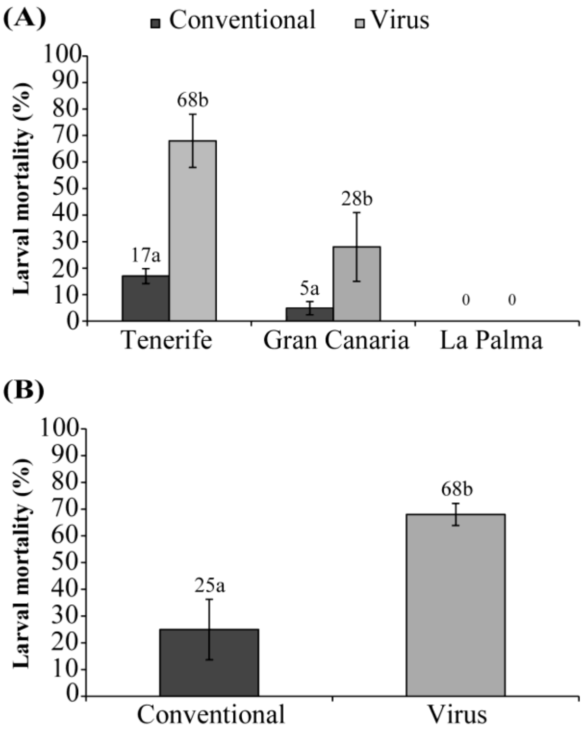

Figure 3. Mean percentage of larval mortality in conventional and ChchNPV-TF1 treatment plots in 2014 trials in (A) Tenerife, Gran Canaria and La Palma, and 2015 trial in (B) Tenerife. Values followed by identical letters did not differ significantly between treatments within each island (Two-Sample T Tests at P>0.05 and Mann-Whitney U). Vertical lines indicate the conventional error.

4. DISCUSSION

The present study aims to evaluate the efficacy of ChchNPV-TF1 in protecting bananas from *C. chalcites* feeding damage in commercial banana crops on the Canary Islands. The similar number of larvae collected in conventional and ChchNPV-TF1 treatment plots suggested a similar *C. chalcites* incidence and distribution within each plot, being possible to compare the effectiveness of the baculovirus treatment versus conventional treatment in each trial. Otherwise, the low number of applications performed in Gran Canaria and La Palma in relation with those performed in Tenerife, might be related with a lower prevalence of *C. chalcites* infestation in comparison with that found in Tenerife. Previous studies also reported that Tenerife Island presented a higher prevalence of *C. chalcites* infestations than other islands such as Gran Canaria or La Palma (Fuentes et al., 2017a). Therefore, control programs in Tenerife might be somewhat more complicated.

In general, ChchNPV-TF1 was as effective as conventional insecticides reducing foliar damage in Tenerife and Gran Canaria, while in La Palma mean foliar damage was even lower in ChchNPV-TF1 treatment plot. Previous studies had also indicated that ChchNPV-TF1 was as effective or even more than conventional treatments reducing foliar damage (Fuentes et al., 2017b; Simón et al., 2015). However, foliar feeding damage is unlikely to have had a substantial effect on final crop production as *C. chalcites* feed preferentially on young leaves, and particularly the young leaves of young plants, and therefore, major defoliation would be needed to cause crop losses (Fuentes et al., 2017b). Similarly, feeding damage to leave of mature plants is negligible and has not detectable effect on fruit production. In contrast, direct feeding damage to fruit had a clear and important influence on the commercial value of fruit, and particularly that of spring period as it coincides with the development of the fruit after flowing which is the most susceptible period for determining the quantity and quality of the harvested fruit (Fuentes et al., 2017b). Therefore, more efforts were done in determining fruit feeding damage. In general, fruit damage in ChchNPV-TF1 treatment plot was similar to that obtained in conventional treatment plots, except in 2014 trial in Tenerife, in which the number of fruit handles affected by *C. chalcites* damage was slightly higher than in conventional treatment plot.

Finally regarding larval mortality, a higher mortality was recorded in ChchNPV-TF1 treatment plot than in conventional treatment plot, which was specially pronounced in Tenerife due to the higher *C. chalcites* incidence

(Fuentes et al., 2017a). This higher mortality indicates that the virus has a prolonged effect over time in comparison with conventional treatments, and so, the OBs had a greater persistence on banana plant with respect to conventional treatments. Similarly, recent studies with ChchNPV-TF1 in young plants grown in pots under greenhouses and open-field conditions resulted in an extended period of larval mortality than conventional treatments (Fuentes et al., 2017b; Simón et al. 2015). Therefore, ChchNPV applications might favour to maintain host populations below damage levels by performing fewer applications, as shown in other host-pathogen systems (Arthurs and Lacey, 2004; Cunningham, 1995; Fuxa, 1991; Moscardi and Sosa-Gómez, 1992).

The reasons for the lower efficacy of the baculovirus treatment in 2014 trial in Tenerife in comparison with that of Gran Canaria or La Palma are unclear. Previous studies have demonstrated that the efficacy of different insecticides might vary from year to year under field-conditions, due to variation in environmental conditions or to the variable susceptibility of the insect colony (Arrizubieta et al., 2016; Gupta et al., 2007; Ignoffo and Garcia, 1992; Robertson et al., 1995). Additionally, Tenerife trials were performed in commercial plantations with high *C. chalcites* incidence compared with plantations in Gran Canaria or La Palma, and this clearly resulted in a greater number of treatments, being pest control somewhat more complicated. Moreover, in 2014 trial in Tenerife treatment aplications started more or less with the population peak of *C. chalcites* during the first flight in spring, during this period damage to fruit is specially important as it coincides with the development of the fruit after flowing, which is the most susceptible period for determining the quantity and quality of the harvested fruit (Fuentes et al., 2017a). So, if the virus takes longer to kill the larvae in comparison with conventional insecticides (Cherry et al., 2000; Grzywacz el al., 2008; Fuentes et al., 2017b), larvae that ingested a lethal dose continue feeding (Cory and

Hoover, 2006; Cory and Myers, 2004; Vasconcelos et al., 2005), leading to produce more damage to banana handles. Therefore, viral applications should be performed long before the fruit development. Furthermore, as fruit damage in banana crops is usually produced by late *C. chalcites* instars (Del Pino et al., 2011, Domínguez et al., 2012), and that with the age larvae become less susceptible to the virus (Cory and Myers, 2004; Bernal et al., 2014), ChchNPV-TF1 treatments must be applied against early larval instars to avoid economic damage to hosts plants.

Taking into account this information, in 2015 a new trial was designed in Tenerife applying the virus during the entire crop cycle taking more or less the same number of treatments as in 2014, but more extended in time. By this way, the long-term effect of the virus would be more appreciable. And so, in 2015 trial treatments started in autumn to coincide with the second peak of *C. chalcites* population and finished in September, covering all banana growth period. Following this timing, the effectiveness of the baculovirus reducing fruit feeding damage was similar to that of the conventional treatments.

Therefore, we demonstrated that once timing applications are adjusted at appropriate structure of plant age and density of the host, a successful protection with ChchNPV-TF1 is obtained. Previous studies also reported that NPV treatments could be as effective as chemical insecticides or even sometimes better (Cherry et al., 2000; Gómez-Bonilla et al., 2013; Gupta et al., 2007; Moscardi, 1999; Lasa et al., 2007; Moore et al., 2004). Therefore, such king of crop protection strategies, based on preventing quantitative crops losses rather than pest outbreaks (Bancal et al., 2007; Cerda et al., 2017), represented a promising way to reduce pesticide use.

These all information is really valuable for establishing integrated pest management (IPM) programs against *C. chalcites* in banana crops, which are mandatory since 2014 in any crops grown in Spain (Royal decree 1311/2012, which incorporates Directive 2009/128/EEC). Additionally, integrated banana crop management might favour to get a higher quality organic production that might reach a higher price in market. IPM is an ecologically based pest control strategy that favors natural mechanisms of pest control with minimal disruption from broad-spectrum pesticides (Flint and van Bosch, 1981; Pedigo, 1996). In this sense, ChchNPV-TF1 provides an attractive alternative to the commonly used Bt, to avoid the appearance of resistance due to the repeated used of Bt. Additionally, the absence of cross-resistance between Bt and NPV and the fact that populations resistant to Bt used to be more susceptible to NPV (Raymond et al., 2006; Sarfraz et al., 2010), might favoured the integration of ChchNPV-TF1 in IPM.

5. ACKNOWLEDGMENTS

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6. REFERENCES

Arthurs, S.P., Lacey, L.A., 2004. Field evaluation of commercial formulations of the codling moth granulovirus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards. Biological Control 31, 388-397.

ASPROCAN, 2016. 2015 annual statistics, Asociación de Organizaciones de Productores de Plátanos de Canarias. http://platanodecanarias.net/wp-content/uploads/2016/10/ESTADISTICAS_2015.pdf (accessed 12.4.17)

Arrizubieta, M., Simón, O., Torres-Vila, L.M., Figueiredo, E., Mendiola, J., Mexia, A., Caballero, P., Williams, T., 2016. Insecticidal efficacy and persistence of a co-occluded binary mixture of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) variants in protected and field-grown tomato crops on the Iberian Peninsula. Pest Management Science 72, 660-670.

Bancal, M.O., Robert, C., Ney, B., 2007. Modelling wheat growth and yield losses from late epidemics of foliar diseases using loss of green leaf area per layer and pre-anthensis reserves. Annals of Botany 100, 777-789.

Bernal, A., Williams, T., Hernández-Suárez, E., Carnero, A., Caballero, P., Simón, O., 2013. A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: The basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banana crops. Biological Control 67, 101-110.

Bernal, A., Simón, O., Williams, T., Caballero, P., 2014. Stage-specific insecticidal characteristics of a nucleopolyhedorvirus isolate from *Chrysodeixis chalcites* enhanced by optical brighteners. Pest Management Science 70, 798-804.

Cabello, T., Hernández, M.D., 1988. Actividad de alimentación de las larvas de *Agrotis segetum* (Denis y Schiffermüller) y *A. ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) y niveles de daños en maíz. Boletín de Sanidad Vegetal Plagas 14, 295-305.

Cerda, R., Avelino, J., Gary, C., Tixier, P., Lechevallier, E., Allinne, C., 2017. Primary and secondary yield losses caused by pests and diseases: assessment and modelling in coffee. PloS ONE DOI: 10.1371/journal.pone.0169133.

Cherry, A.J., Rabindra, R.J., Parnell, M.A., Geetha, N., Kennedy, J.S., Grzywacz, D., 2000. Field evaluation of *Helicoverpa armigera* nucleopolyhedrovirus formulations for control of the chickpea pod-borer, *H. armigera* (Hübn), on chickpea (*Cicer arietinum* var. Shoba) in southern India. Crop Protection 19, 51-60.

Cory, J.S., Hoover, K., 2006. Plant-mediated effects in insect-pathogen interactions. Trends in Ecology and Evolution 21, 278-286.

Cory, J.S., Myers, J.H., 2004. Adaptation in an insect host-plant pathogen interaction. Ecology Letters 7, 632-639.

Cunningham, J.C., 1995. Baculoviruses as microbial insecticides, in: Reuveni, R. (Ed.), Novel approaches to integrated pest management. Lewis Publishers, Boca Raton, FL., pp. 261-92.

Del Pino, M., Carnero, A., Hernández-Suárez, E., Cabello, T., 2011. La lagarta o bicho camello, *Chrysodeixis chalcites* (Esper, 1789), una plaga emergente en los cultivos de platanera de Canarias. Phytoma 225, 21-26.

Domínguez, E., López-Cepero, J., Nogueroles, C., 2012. Identificación y control de plagas y enfermedades, in: Nogueroles, C. (Ed.), Calidad y sostenibilidad en el cultivo de la platanera en Canarias. ASPROCAN, Tenerife, Spain, pp.145-170.

Flint, M.L., van den Bosch, R., 1981. Introduction to integrated pest management, Plenum press, New York.

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017a. *Chrysodeixis chalcites*, a pest of banana crops on the Canary Islands: incidence, economic losses and current control measures. Crop Protection Submitted.

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017b. *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV): natural occurrence and efficacy as a biological insecticide on young banana plants in greenhouse and open-field conditions on the Canary Islands. PlosOne Submitted.

Fuxa, J.R., 1991. Insect control with baculoviruses. Biotechnology Advances 9, 425-42.

Galán-Saúco, V., 1992. Los frutales tropicales en los subtropicos, II. Plátano (Banano), Mundi-Prensa, Madrid.

Gómez-Bonilla, Y., López-Ferber, M., Caballero, P., Murillo, P., Muñoz, D., 2013. Granulovirus formulations efficiently protect stored and field potatoes from *Phthorimaea operculella* and *Tecia solanivora* in Costa Rica. Biological Control 58, 215-224.

González-Concepción, C., Gil-Fariña, M.C., Pestano-Gabino, C., 2008. Multivariate modelling of the Canary Islands banana output. The role of farmer income expectation. Journal of Social Sciences 4: 88-97.

Grzywacz, D., Mushobozi, W.L., Parnell, M., Jolliffe, F., Wilson, K., 2008. Evaluation of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) for the field control of African armyworm (*Spodoptera exempta*) in Tanzania. Crop Protection 27, 17-24.

Gupta, R.K., Raina, J.C., Arora, R.K., Bali, K., 2007. Selection and field effectiveness of nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hübner). International Journal of Virology.3, 45-59.

Horowitz, A.R., Weintraub, P.G., Ishaaya, I., 1998. Status of pesticide resistance in arthropod pests in Israel. Phytoparasitica 26, 231–240.

Hughes, P.R., Wood, H.A., 1981. A synchronous per oral technique for the bioassay of insect viruses. Journal of Invertebrate Pathology 37, 154-159.

Ignoffo, C.M., Garcia, C., 1992. Combinations of environmental factors and simulated sunlight affecting activity of inclusion bodies of the *Heliothis* (Lepidoptera: Noctuidae) nucleopolyhedrosis virus. Environmental Entomology 21, 210-213.

Knox, C., Moore, S.D., Luke, G.A., Hill, M.P., 2015. Baculovirus-based strategies for the management of insect pests: a focus on development and application in South Africa. Biocontrol Science and Technology, 25, 1-20.

Lasa, R., Pagola, I., Ibáñez, I., Belda, J.E., Williams, T., Caballero, P., 2007. Efficacy of a *Spodoptera exigua* multiple nucleopolyhedrovirus as a biological insecticide for beet armyworm control in greenhouses of southern Spain. Biocontrol Science and Technology 17, 221-232.

MAPAMA, 2016. Superficies y producciones anuales de cultivo de acuerdo con el Reglamento (C) 543/2009. http://www.mapama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/ (accessed 12.4.17).

Méndez-Hernández, C., Perera-González, S., Rodríguez-Serrano, M., 2017. Guía de lucha contra las plagas de la platanera. Tabla de productos fitosanitarios autorizados contra plagas y enfermedades en platanera. http://www.agrocabildo.org/publica/Publicaciones/subt_48_Anexo%20I.pdf.

Moore, S.D., Pittaway, T., Bouwer, G., Fourie, J.G., 2004. Evaluation of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) for control of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on citrus in South Africa. Biocontrol Science and Technology 14, 239-250.

Moscardi, F., Sosa-Gómez, D.R., 1992. Use of viruses against soybean caterpillars in Brazil, in: Copping, L.G., Green, M.B., Rees, R.T. (Eds.), Pest management in soybean, Elsevier, London, pp. 98-109.

Moscardi, F., 1999. Assessment of the application of baculoviruses for the control of Lepidoptera. Annual Review of Entomology 44, 257-289.

Pedigo, L.P., 1996. Entomology and Pest Management, Prentice-Hall Pub., Englewood Cliffs, NJ.

Raymond, B., Sayyed, A.H., Wright, D.J., 2006. The compatibility of a nucleopolyhedrosis virus control with resistance management for *Bacillus thuringiensis*: Co-infection and cross-resistance studies with the diamondback moth, *Plutella xylostella*. Journal of Invertebrate Pathology 93, 114-120.

Robertson, J.L., Preisler, H.K., Ng, S.S., Hickle, L.A., Gelernter, W.D., 1995. Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. Journal of Economy Entomology 88, 1-10.

Robinson, J.C., Galán Saúco, V., 2010. Bananas and plantains, CABI, UK.

Sarfraz, R.M., Cervantes, V., Myers, J.H., 2010. Resistance to *Bacillus thuringiensis* in the cabbage looper (*Trichoplusia ni*) increases susceptibility to a nucleopolyhedrovirus. Journal of Invertebrate Pathology 105, 204-206.

Simón, O., Bernal, A., Williams, T., Carnero, A., Hernández-Suárez, A., Muñoz, D., Caballero, P., 2015. Efficacy of an alphabaculovirus-based biological insecticide for control of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) on tomato and banana crops. Pest Management Science 71, 1623-1630.

Townsend, G.R., Heuberger, J.W., 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter 27, 340-343.

Vasconcelos, S.D., Hails, R.S., Speight, M.R., Cory, J.S., 2005. Differential crop damage by healthy and nucleopolyhedrovirus-infected *Mamestra brassicae* L. (Lepidoptera: Noctuidae) larvae: a field examination. Journal of Invertebrate Pathologhy 88, 177-179.

CHAPTER V

General discussion

This study arises from the need of defining *Chrysodeixis chalcites* damage thresholds in banana crops and the potential use of a novel biological insecticide that is currently under development (Caballero et al., 2014), with the aim of developing an adequate integrated pest management (IPM) program in banana crops on the Canary Islands. *C. chalcites* is an important pest that produces significant economic losses on harvested bananas, but control of this pest is performed mainly by the application of chemical insecticides. However, a low number of active compounds are authorized for this crop in the European Union (Del Pino et al., 2011; Fuentes et al., 2017a), leading to the repeatedly use of the same pesticides and favouring the development of resistance (Horowitz et al., 1998) and the accumulation of hazardous residues on the fruits that hinder banana commercialization. Such kinds of problems have raised public concern and since 2014 IPM programs are mandatory in any crop system in Spain (Royal decree 1311/2012, which incorporates the European Directive 2009/128/EEC).

Pest population assessment is one of the fundamental aspects used to determine the frequency of pest control interventions in IPM programs. Moreover, effective decision-making in IPM also relies on understanding the relationships between pest numbers, plant responses to injury and the resultant economic losses (Higley and Pedigo, 1993; Pedigo, 1996). Therefore, **Chapter II** aimed to estimate the *C. chalcites* incidence, damage levels, production losses due to direct damage and treatment costs.

The prevalence of *C. chalcites* infestations was quite high, ranging from 42-50% on Gran Canaria to 100% on El Hierro. Feeding damage by *C. chalcites* was most severe on the islands of El Hierro, La Palma and Tenerife, whereas on Gran Canaria was of minor importance. Curiously, these infestation and feeding damage levels keep relation with the time of appearance of fruit damage in the archipelago, which began in 2002 in banana crops in El Hierro, followed by damages in southern plantations in La Palma and Tenerife. The last island to be affected was Gran Canaria in 2012, in southern banana crops (Del Pino et al., 2011; Perera and Molina, 2007). Previous observations reported major prevalence of *C. chalcites* infestations on southern-facing slopes, although in the present study this assumption was only confirmed in Gran Canaria Island. This might be due to the fact that control programs were carried out more severely in those plantations with high incidence or damage, equilibrating those differences. The major *C. chalcites* infestations observed in southern slopes are likely influenced by the cropping system established on each slope, as in the warmer and dryer southern-facing slopes mesh-built greenhouses are more common, favouring the development of *C. chaclites* by shortening the duration of the biological cycle of the pest, and leading to more generations throughout the year (Barrionuevo et al., 2012; Danks, 2000; Mironidis and Savopoulou-Soultani, 2008).

In general, damages were similar in both plantation locations except in Gran Canaria and Tenerife, where more foliar and fruit damage were registered in southern-facing slopes, respectively. No major differences were either observed in feeding damage according to the seasons. This might be due to the fact that in banana crops grown under mesh, *C. chalcites* generations follow one another throughout the year with no diapause (Del Pino et al., 2011; Domínguez et al., 2012; García et al., 1992; Perera and Molina, 2007). However, in the present study we showed that damage to fruit in the spring is particularly important, as it coincides with development of the fruit after flowering, being this period especially important for determining the quality and quantity of harvested bananas (Del Pino et al., 2011; Galán-Saúco, 1992). Moreover, *C. chalcites* has two predominant flights during the year, one in spring (May-June) and the other in autumn (September-October) (Del Pino et al., 2011). Therefore, the first flight covers the period of fruit development and monitoring and controlling *C. chalcites* during the spring would be of particular value for the effective protection of developing fruits. Crop protection strategies, based on preventing quantitative crop losses rather than pest outbreaks represent a promising approach to rationalize pesticide use.

According to the results obtained in this study, larvae of *C. chalcites* feed preferably on young leaves, especially those of young plants. In young plants, larvae perforate leaves (Del Pino et al., 2011; Domínguez et al., 2012; Perera and Molina, 2007; Simón et al., 2015; Vilardebo and Guérout, 1964). However, as shown in the present thesis, foliar damage to young plants is unlikely to have substantial effects on final crop production, as only major defoliation would significantly reduce crop yields (Turner and Hunt, 1987). Likewise, feeding damage to mature plants is also negligible and has not detectable effect on final crop production. However, direct feeding damage to fruit is the main factor that injures bananas and produces important economic losses. Therefore, fruit damages were also estimated in packaging plants and they agreed with the results obtained through field surveys on fruit damage. Taking into account the percentage of the discarded bananas and the annual banana production in each island, we estimated annual losses of approximately 2.68 million euros. It has to be emphasized that losses were quite high despite the adoption of control measures.

In **Chapter II** it is shown that in addition to direct losses, control measures targeted at *C. chalcites* unequivocally contribute to final banana commercial value. According to the interviews performed to different banana growers in different areas of the islands, indoxacarb, chlorpyrifos and *Bacillus* *thuringiensis* (Bt) were the three main insecticides commonly used in banana crops, as occurs in other lepidopteran-crop systems (Broza and Sneh, 1994; Moore et al., 2004). Taking into account that around three treatments are performed throughout the complete life cycle and that insecticides are used alone, the cost derived from the purchase and application of these insecticides might exceed 240 €/Ha, 345 €/Ha and 258 €/Ha for indoxacarb, chlorpyrifos and Bt, respectively. Therefore, considering direct feeding damage and control costs on some islands such as El Hierro and La Palma, fruit feeding losses exceed the cost of insecticide purchase and application. Thus, it is necessary to design effective IPM programs that improve the ratio of production costs: crop yields. Actually, banana growers use direct observations on feeding damage or evidence of larval infestations as the main criteria for implementing control measures. Therefore, estimating treatment thresholds might facilitate correct decisions in IPM.

In addition to design appropriate IPM programs, novel insecticides are needed for insecticide rotations with the few that remain authorized, as continuous use of a single dominant insecticide leads to the rapid development of resistance (Horowitz et al., 1998). Among the possible alternative control agents, the nucleopolyhedrovirus of *C. chalcites* (ChchNPV) was proven highly effective under controlled conditions, in laboratory and small-scale greenhouse trials on young banana plants (Bernal et al., 2013a; Simón et al., 2015).

It is known that Lepidopteran nucleopolyhedroviruses (genus *Alphabaculovirus, Baculoviridae*) present a high genotypic diversity that has a clear effect over the phenotypic traits of the viral population (Arrizubieta et al., 2014; Bernal et al., 2013b; Cory et al., 2005; Erlandson, 2009; Hodgson et al., 2002). Those phenotypic trails such as pathogenicity, speed of kill or OB production are of major importance when selecting the active material of a biological insecticide. For this reason the genotypic diversity should be studied in detail. The next step in this thesis was to determine the genotypic diversity of ChchNPV variants present in *C. chalcites* populations from the Canary Islands, with the aim of selecting the most appropriate one and evaluate its efficacy in young banana plants grown at small-scale under greenhouses and open field conditions (**Chapter III**), and secondly in mature plants with fruits in commercial plantations (**Chapter IV**).

The low ChchNPV infection prevalence (2.3%) was similar to the prevalence of other host-pathogen systems (Gómez-Valderrama et al., 2010; Ordoñez-García et al., 2015; Valicente and Barreto, 1999). Curiously, ChchNPV prevalence on *C. chalcites* populations on Canary Islands was similar to that obtained in a smaller-scale study performed in 2006 (Bernal et al., 2013a). Unlike a previous study that was carried out for a short period of time and in a low number of plantations with high infestations, the present study covered two years and included all types of plantations, adjusting prevalence data better to the reality. Similar to previous results (Bernal et al., 2013a), the majority of virus-infected larvae were collected in Tenerife and El Hierro. Although the highest numbers of larvae were collected in Tenerife, suggesting a major prevalence of infestations, there was not a clear relationship between prevalence of infestation and prevalence of infection. In contrast, the prevalence of ChchNPV infection in greenhouse structures was two fold higher than that of open-fields, probably due to the highest prevalence of infestations in southern slopes, or more likely to the reduced ultraviolet radiation under mesh-built structures, protecting the OBs against a rapid UV degradation (Ignoffo and García, 1992; Lasa et al., 2007a).

In the line with the results obtained in 2006, ChchNPV-TF1 was the most prevalent isolate in the archipelago. This data suggest that ChchNPV-TF1 presented a long-term prevalence over *C. chalcites* populations found in banana crops. However, in the present study, the remaining isolates were also found distributed across the different islands, while in 2006 they were only found in the south of Tenerife (Bernal et al., 2013a). The highest incidence of ChchNPV-TF1 in the archipelago led us to use this isolate rather than a specific mixture with increased pathogenicity and virulence (Bernal et al., 2013b; Caballero et al., 2014). Firstly, the stability of the mixture was not corroborated in field conditions, which may favour transmission of different proportions of certain genotypes resulting in different phenotypes (Cory et al., 2005; Cory and Myers, 2009; Graham et al., 2004; Hodgson et al., 2002). Secondly, the lower productivity of the mixture might influence clearly the production of progeny virus (Bernal et al., 2013b; Caballero et al., 2014), by reducing the prevalence of new NPV infections. And finally, as field assays were performed on commercial banana crops in several islands, ChchNPV-TF1, which is the isolate most adapted to local *C. chalcites* populations, was selected.

The next step was to evaluate the efficacy of ChchNPV-TF1 in protecting banana young plants against *C. chalcites* feeding damage in greenhouse and open-field conditions (**Chapter III**), as ~65% of the banana production is produced in open field conditions in the Canary Islands (Fuentes et al., 2017b; MAPAMA, 2016) and there were no previous data under such is conditions. Firstly, the optimal viral concentration to be applied in both crop conditions was evaluated. Greenhouse conditions are more stable than open-field crops (Galán-Saúco et al., 1998; Robinson and Galán-Saúco, 2010), and the harsher open-field conditions might affect viral persistence, and hence, viral effective doses (Gzywacz et al., 2008; Lasa et al., 2007a). Surprisingly, 10^9 OBs/l was the optimal concentration under both conditions. Previous studies also showed that this concentration protected young banana plants efficiently from *C. chalcites* feeding damage under greenhouse conditions (Simón et al., 2015). Moreover, similar viral concentrations have been reported in other baculovirus-based biological control agents (Barrera et al., 2017; Grzywacz et al., 2008; Lasa et al., 2007b).

Overall, ChchNPV-TF1 at 10^9 OBs/l was as effective as conventional treatments, indoxacarb and Bt, in controlling pest infestations and foliar damage under greenhouse and open-field conditions. However, previous studies reported a lower viral concentration (10^8 OBs/l) as effective as conventional treatments. This difference might be related with environmental conditions, as previous studies were carried out in autumn, whereas in the present study, assays were performed during summer periods of high temperatures and strong sunlight that could reduce OB efficacy. It could also be explained by a lower susceptibility of *C. chalcites* populations to NPV infection, due to variation of population susceptibility to pathogens (Robertson et al., 1995) or to the development of insect resistance to NPVs (Jehle et al., 2017; Reeson et al., 1998).

Contrary to conventional treatments, ChchNPV-TF1 produced an extended period of larval mortality as, in larvae collected up to 7 days post-treatment, a high level of NPV mortality was recorded, suggesting that larvae acquired a lethal dose several days after application. Indoxacarb and Bt treatments, meanwhile, produced a rapid peak in mortality that declined over time. This is in agreement with other studies that showed that conventional treatments are not as prevalent as NPVs (Belda et al., 2000; Cherry et al., 2000; Gupta et al., 2007; Lasa et al., 2007b; Kolodny-Hirsch et al., 1997). These results may indicate a greater persistence of ChchNPV-TF1 OBs on the banana plants with respect to conventional treatments, providing an extended period of pest control. Therefore, treatment timing acquires more importance when integrating NPV into IPM programs, as the NPV protection seems to be slower but prolonged over time.

The final chapter (**Chapter IV**) aims to evaluate the efficacy of ChchNPV-TF1 in protecting banana fruits from *C. chalcites* damage in commercial banana crops, under the real banana crops conditions, in comparison with the conventional treatment carried out by banana growers.

The similar number of larvae captured in plots treated with both control strategies suggested a similar prevalence of *C. chalcites* infestations. Moreover, the lower number of control treatments performed in Gran Canaria and La Palma in comparison with those performed in Tenerife might indicate that the prevalence of *C. chalcites* infestations is lower, as banana growers implemented control measures with the mere presence of feeding damage or larval infestations. In line with previous results (Simón et al., 2015), ChchNPV-TF1 was as effective or even more than convectional insecticides in reducing foliar damage. The major contribution of the present study is that, in general, ChchNPV-TF1 was as effective as the conventional treatment in reducing fruit damage. Similarly to small-scale assays, the higher mortality recorded in the ChchNPV-TF1 treated plot might also indicate an extended period of larval mortality. ChchNPV applications might favour the maintenance of host populations below damage levels by performing fewer applications, as shown in other host-pathogen systems (Arthurs and Lacey, 2004; Cunningham, 1995; Fuxa, 1991; Moscardi and Sosa-Gómez, 1992).

However, in the 2014 trial in Tenerife, the number of bunches affected by *C. chalcites* damage was slightly higher than in conventional treated plots. The reasons behind this are unclear. The efficacy of different insecticides could vary from year to year under field-conditions, due to variation in environmental conditions or in the susceptibility of the insect colony (Arrizubieta et al., 2016; Gupta et al., 2007; Ignoffo and Garcia, 1992; Robertson et al., 1995). In addition, the high prevalence of *C. chalcites* infestations in Tenerife makes control more complicated. More likely, the fact that NPVs take longer to kill the larvae (Cherry et al., 2000; Fuentes et al., 2017b ; Grzywacz el al., 2008;), and persist longer in the environment (Fuentes et al., 2017b; Gupta et al., 2007; Kolodny-Hirsch et al., 1997; Lasa et al., 2007a), make NPV treatment-timing adjustment more complex. In 2014, trial treatments started in spring, at the same time as fruit development and together with the first harmful pest outbreak (Fuentes et al., 2017a, 2017b; Del Pino et al., 2011), which might have hindered the extended period of virus performance. This could be the reason for the reduced effectiveness of ChchNPV-TF1 in decreasing fruit damage, in comparison with conventional treatments. spring is the most sensitive period for determining the quantity and quality of harvested bananas (Fuentes et al., 2017b), as it coincides with fruit development and *C. chalcites* peak population. Therefore, should the virus need more time to act, viral applications should be performed long before fruit development.

In 2015, a new trial was designed in Tenerife starting treatment applications in autumn, coinciding with the second *C. chalcites* outbreak peak, and prolonged during the entire banana growth cycle. Contrary to 2014 trial, banana fruit damage was similar between both control strategies. Therefore, it seems that once timing applications are adjusted to the appropriate structure of plant age and host density, a successful protection with ChchNPV-TF1 is obtained. Previous studies also reported that NPV treatments could be as effective as chemical insecticides or even sometimes better (Cherry et al., 2000; Gómez-Bonilla et al., 2013; Gupta et al., 2007; Lasa et al., 2007; Moore et al., 2004; Moscardi, 1999). Therefore, such kind of crop protection strategies, based on preventing quantitative crop losses rather than pest outbreaks (Bancal et al., 2007; Cerda et al., 2017), represents a promising way to reduce pesticide use. Fruit development does not occur at the same time in all the plants, therefore, preventive and periodic treatments should be performed from the initial fruit development.

All the information obtained in the present thesis is highly valuable for establishing adequate IPM programs against *C. chalcites* in banana crops. Taking into account that ChchNPV-TF1 is as effective as conventional treatments, ChchSNPV-TF1 based products could be incorporated into IPM programs, given also their compatibility with biological and chemical control agents (Espinel et al., 2009; Gupta et al., 2007;). Moreover, the low number of active substances authorized in this crop and their repeated used might facilitate the inclusion of ChchNPV-TF1 in IPM programs against *C. chalcites* in banana crops, with the aim of rotating it with the currently used compounds to prevent the use of a single compound and thus delay the development of resistance. Finally, the fact that a low number of active compounds are permitted in this crop and that these are used repeatedly, defines precisely the market for a ChchNPV-TF1 based product.

References

Arrizubieta, M., Williams, T., Caballero, P., Simón, O., 2014, Selection of a nucleopolyhedrovirus isolate from *Helicoverpa armigera* as the basis for a biological insecticide. Pest Management Science70, 967-976.

Arrizubieta, M., Simón, O., Torres-Vila, L.M., Figueiredo, E., Mendiola, J., Mexia, A., Caballero, P., Williams, T., 2016. Insecticidal efficacy and persistence of a co-occluded binary mixture of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) variants in protected and field-grown tomato crops on the Iberian Peninsula. Pest Management Science 72, 660-670.

Arthurs, S.P., Lacey, L.A., 2004. Field evaluation of commercial formulations of the codling moth granulovirus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards. Biological Control 31, 388-397.

Bancal, M.O., Robert, C., Ney, B., 2007. Modelling wheat growth and yield losses from late epidemics of foliar diseases using loss of green leaf area per layer and pre-anthensis reserves. Annals of Botany. 100, 777-789.

Barrera, G.P., Gómez-Valderrama, J.A., Villamizar-Rivero, L.F., 2017. Efficacy of microencapsulated nucleopolyhedroviruses from Colombia as biological insecticides against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Acta Agronómica 66, 267-274.

Barrionuevo, M.J., Murúa, M.G., Goane, L., Meagher, R., Navarro, F., 2012. Life table studies of *Rachiplusia nu* (Guenée) and *Chrysodeixis* (=*Pseudoplusia*) *includens* (Walker) (Lepidoptera: Noctuidae) on artificial diet. Florida Entomology 95, 944-951.

Belda, J.E., Mirasol, E., Escribano, A. Rapallo, S., Caballero, P., 2000. Eficacia de nucleopoliedrovirus (VPNse) en el control de *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) en pimiento en invernadero. Boletín de Sanidad Vegetal Plagas 26, 619-628.

Bernal, A., Williams, T., Hernández-Suárez, E., Carnero, A., Caballero, P., Simón, O., 2013a. A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: The basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banana crops. Biological Control 67, 101-110.

Bernal, A., Simón, O., Williams, T., Muñoz, D., Caballero, P., 2013b. A *Chrysodeixis chacites* single-nucleopolyhedrovirus population from the Canary Islands is genotypically structured to maximize survival. Applied and Environmental Microbiology 79, 7709-7718.

Broza, M., Sneh, B., 1994. *Bacillus thuringiensis* spp. kurstaki as an effective control agent of lepidopteran pests in tomato fields in Israel. Journal of Economic Entomology 87, 923-928.

Caballero, P., Bernal, A., Simón, O., Carnero, A., Hernández-Suárez, E., Williams, T., 2014. Novel genotypes of *Chrysodeixis chalcites* single nucleopolyhedrovirus (ChchSNPV), a procedure for its production and use as a biological control agent. European Patent PCT-760.

Cerda, R., Avelino, J., Gary, C., Tixier, P., Lechevallier, E., Allinne, C., 2017. Primary and secondary yield losses caused by pests and diseases: assessment and modelling in coffee. PloS ONE 12, e0169133.

Cherry, A.J., Rabindra, R.J., Parnell, M.A., Geetha, N., Kennedy, J.S., Grzywacz, D., 2000. Field evaluation of *Helicoverpa armigera* nucleopolyhedrovirus formulations for control of the chickpea pod-borer, *H.armigera* (Hübn), on chickpea (*Cicer arietinum* var. Shoba) in southern India. Crop Protection 19, 51-60.

Cory, J.S., Green, B.M., Paul, R.K., Hunter-Fujita, F., 2005. Genotypic and phenotypic diversity of a baculovirus population within an individual insect host. Journal of Invertebrate. Pathology. 89, 101-111.

Cory, J.S. and Myers, J.H., 2009. Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis. Journal of Animal Ecology 78, 646-655.

Cunningham, J.C., 1995. Baculoviruses as microbial insecticides, in: Reuveni, R. (Ed.), Novel approaches to integrated pest management, Boca Raton, FL., pp. 261-92.

Danks, H.V., 2000. Measuring and reporting life-cycle duration in insect and arachnids. European Journal of Entomology 97, 285-303.

Del Pino, M., Carnero, A., Hernández-Suárez, E, Cabello, T., 2011. La lagarta o bicho camello, *Chrysodeixis chalcites* (Esper, 1789), una plaga emergente en los cultivos de platanera de Canarias. Phytoma 225, 21-16.

Domínguez, E., López-Cepero, J., Nogueroles, C., 2012. Identificación y control de plagas y enfermedades, in: Nogueroles, C. (Ed.), Calidad y sostenibilidad en el cultivo de la platanera en Canarias. ASPROCAN, Tenerife, Spain, pp.145-170.

Eberle, K.E., Jehle, J.A., Huber, J., 2012. Microbial control of crop pest using insect viruses, in: Abrol, D.P., Shankar, U. (Eds.), Integrated pest management: principles and practice. CAB International, Oxfordshire, UK, pp. 281-298.

Erlandson, M.A., 2009. Genetic variation in field populations of baculoviruses: mechanisms for generating variation and its potential role in baculovirus epizootiology. Virologica Sinica 24, 458-469.

Espinel, C., Gómez; J., Villamizar; L., Cotes; A.M., Léry, X., López-Ferber, M., 2009. Biological activity and compatibility with chemical pesticides of a Colombian granulovirus isolated from *Tecia solanivora.* IOBC/wprs Bulletin Vol. 45, pp. 145-148.

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017a. *Chrysodeixis chalcites,* a pest of banana crops on the Canary Islands: incidence, economic losses and current control measures. Crop Protection Submitted

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017b. Insecticidal efficacy of *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchSNPV) in banana plants under semi-field conditions in greenhouses and open-field. Plos ONE Submitted.

Fuxa, J.R., 1991. Insect control with baculoviruses. Biotechnology Advances 9, 425-42.

Galán-Saúco, V., 1992. Los frutales tropicales en los subtrópicos. II. Plátano (Banano), Mundi-Prensa, Madrid, Spain.

Galán-Saúco, V., Cabrera-Cabrera, J., Hernández-Delgado, P.M., Rodríguez-Pastor M.C., 1998. Comparison of protected and open-air cultivation of Grand Naine and Dwarf Cavendish banana. Acta Horticulturae 490, 247-259.

García, R., Ortega, G., Pérez, J.M., 1992. Insectos de Canarias. Cabildo Insular de Gran Canaria, Las Palmas de Gran Canaria.

Gómez-Bonilla, Y., López-Ferber, M., Caballero, P., Murillo, P., Muñoz, D., 2013. Granulovirus formulations efficiently protect stored and field potatoes from *Phthorimaea operculella* and *Tecia solanivora* in Costa Rica. Biological Control 58, 215-224.

Gómez-Valderrama, J.A., Guevara-Agudelo, E.J., Barrera-Cubillos, G.P., Cotes-Prado, A.M., Villamizar-Rivero, L.F., 2010. Aislamiento, identificación y caracterización de nucleopoliedrovirus nativos de *Spodoptera frugiperda* en Colombia. Revista Facultad Nacional de Agronomia 63, 5511-5520.

Graham, R.I., Tyne, W.I., Possee, R.D., Sait, S.M., Hails, R., 2004. Genetically variable nucleopolyhedroviruses isolated from spatially separate populations of the winter moth *Operophtera brumata* (Lepidoptera: Geometridae) in Orkney. Journal of Invertebrate Pathology 87, 29-38.

Grzywacz, D., Mushobozi, W.L., Parnell, M., Jolliffe, F., Wilson, K., 2008. Evaluation of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) for the field control of African armyworm (*Spodoptera exempta*) in Tanzania. Crop Protection 27, 17-24.

Gupta, R.K., Raina, J.C., Arora, R.K., Bali, K., 2007. Selection and field effectiveness of nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hübner). International Journal of Virology 3, 45-59.

Higley, L.G., Pedigo, L.P., 1993. Economic injury level concepts and their use in sustaining environmental quality. Agriculture, Ecosystems and Environment 46, 1-4.

Hodgson, D.J., Vanbergen, A.J., Hartley, S.E., Hails, R.S., Cory, J.S., 2002. Differential selection of baculovirus genotypes mediated by different species of host food plant. Ecology Letters 5, 512-518.

Horowitz, A.R., Weintraub, P.G., Ishaaya, I., 1998. Status of pesticide resistance in arthropod pests in Israel. Phytoparasitica 26, 231-240.

Ignoffo, C.M. and Garcia, C., 1992. Combinations of environmental factors and simulated sunlight affecting activity of inclusion bodies of the Heliothis (Lepidoptera: Noctuidae) nucleopolyhedrosis virus. Environmental Entomology 21, 210-213.

Jehle, J.A., Schule-Bopp, S., Undorf-Spahn, Fritsch, Schottel, J.L., 2017. Evidence of a second type of resistance against *Cydia pomonella* granulovirus I field populations of codling moths. Applied and Environmental Microbiology 83, e02330-16.

Knox, C., Moore, S.D., Luke, G.A., Hill, M.P., 2015. Baculovirus-based strategies for the management of insect pests: a focus on development and application in South Africa. Biocontrol Science and Technology 25, 1-20.

Kolodny-Hirsch, D.M., Sitchawat, T., Jansiri, T., Chenrchaivachirakul, A., Ketunuti, U., 1997. Field Evaluation of a Commercial Formulation of the *Spodoptera exigua* (Lepidoptera: Noctuidae) Nuclear Polyhedrosis Virus for Control of Beet Armyworm on Vegetable Crops in Thailand. Biocontrol Science and Technology 7, 475-488.

Lasa, R., Ruiz-Portero, C., Alcázar, M.D., Belda, J.E., Caballero, P., Williams, T., 2007a. Efficacy of optical brightener formulations of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as a biological insecticide in greenhouses in southern Spain. Biological Control 40, 89-96.

Lasa, R., Pagola, I., Ibáñez, I., Belda, J.E., Williams, T., Caballero, P., 2007b. Efficacy of *Spodoptera exigua* multiple nucleopolyhedrovirus as a biological insecticide for beet armyworm control in greenhouses of southern Spain. Biocontrol Science and Technology 17, 221-232.

MAPAMA, 2016. Superficies y producciones anuales de cultivo de acuerdo con el Reglamento (C) 543/2009. http://www.magrama.gob.es/estadistica/pags/anuario/2015-Avance/AE_2015_Avance.pdf).

Mironidis, G.K., Savopoulou-Soultani, M., 2008. Development, survivorship, and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) under constant and alternating temperatures. Environmental Entomology 37, 16-28.

Moore, S.D., Pittaway, T., Bouwer, G., Fourie, J.G., 2004. Evaluation of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) for control of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on citrus in South Africa. Biocontrol Science and Technology 14, 239-250.

Moscardi, F., Sosa-Gómez, D.R., 1992. Use of viruses against soybean caterpillars in Brazil, in: Copping, L.G., Green, M.B., Rees, R.T. (Eds.), Pest management in soybean, Elsevier, London, pp. 98-109.

Moscardi, F., 1999. Assessment of the application of baculoviruses for the control of Lepidoptera. Annual Review of Entomology 44, 257-289.

Ordoñez-García, M., Rios-Velasco, C., Berlanga-Reyes, D.I., Acosta Muñiz, C.H., Salas-Marina, M.A., Cambero-Campos, O.J., 2015. Occurrence of natural enemies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Chihuahua, Mexico. Florida Entomologist 98, 843-847.

Pedigo, L.P., 1996. Entomology and Pest Management, Prentice-Hall Pub, Englewood Cliffs, NJ.

Perera, S., Molina, M.J., 2007. Plagas y enfermedades en el cultivo ecológico de laplatanera, in: Nogueroles, C., Líbano, J. (Eds.), El cultivo ecológico de la platanera en Canarias. Gabinete de Proyectos Agroecológicos S.L., Tenerife, Spain, pp. 77-118.

Reeson, A.F., Wilson, K., Gunn, A., Hails, R.S., Goulson, D., 1998. Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. Proceedings of the Royal Society B 265, 1787-1791.

Robertson, J.L., Preisler, H.K., Ng, S.S., Hickle, L.A., Gelernter, W.D., 1995. Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. Journal of Economy Entomology 88, 1-10.

Robinson, J.C., Galán Saúco, V., 2010. Bananas and plantains. CABI, Wallingford, UK.

Simón, O., Bernal, A., Williams, T., Carnero, A., Hernández-Suárez, A., Muñoz, D., Caballero, P., 2015. Efficacy of an alphabaculovirus-based biological insecticide for control of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) on tomato and banana crops. Pest Management Science 71, 1623-1630.

Turner, D.W., Hunt, N., 1987. Planting date and defoliation influence the time of harvest of bananas. Sci. Hortic. 32, 233-248.

Valicente F.H., Barreto, M., 1999. Levantamento dos inimigos naturais da lagarta do cartucho do milho, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), na região de Cascavel, PR. Anais da Sociedade Entomológica do Brasil 28, 333-337.

Vilardebo, A., Guérout, R., 1964. *Plusia chalcytes* Esp. (Lépidoptère Noctuidae) nuisible au bananier dans les régions cotières de L´ouest Africain. Fruits 19, 195-208.

CONCLUSIONES

1. La prevalencia de las infestaciones de *C. chalcites* fue alta y similar durante los dos años de prospecciones. En las vertientes orientadas al norte y las orientadas al sur se encontró una densidad de población similar, excepto en Gran Canaria donde hubo una mayor incidencia de *C. chalcites* en las vertientes orientadas al sur. La densidad de población de *C. chalcites* no varió a través de las estaciones y las generaciones siguieron una a otra durante todo el año en los cultivos de platanera.

2. Los daños de alimentación foliar y en fruta variaron significativamente a través de las islas, localización de la plantación y estación. En general, los mayores daños se registraron en El Hierro, La Palma y Tenerife, registrándose menores daños en Gran Canaria. Los daños de alimentación fueron similares en las dos localizaciones de las plantaciones, excepto en Gran Canaria y Tenerife donde se registraron más daños en las vertientes sur. Los daños por alimentación en la fruta fueron similares en las estaciones, aunque en primavera el daño en fruta fue más relevante.

3. La cantidad de plátanos dañados cambió significativamente según las islas, produciéndose las mayores pérdidas en El Hierro. Las pérdidas fueron más frecuentes en primavera, coincidiendo con el desarrollo del fruto tras la floración. En general, a pesar de las medidas de control, las pérdidas por la alimentación de *C. chalcites* en el fruto pueden alcanzar el 1,5% de la producción anual (≈2,68 millones de euros/ año).

4. Indoxacarb resultó ser el principal producto insecticida utilizado para el control de *C. chalcites*, utilizándose monosistematicamente con una

frecuencia media de 3 tratamientos por ciclo de cultivo. El coste del control usando este insecticida podría alcanzar los 240€/ha, representado el 0.64% del valor de producción de una hectárea.

5. La prevalencia de las infecciones por ChchNPV en poblaciones naturales de *C. chalcites* fue del 2,3%, pero osciló entre 0-4,8% en Tenerife y fue normalmente baja (0-2%) en el resto de islas. La prevalencia del virus en plantaciones de invernaderos de malla fue el doble que en plantaciones al aire libre. El aislado nativo ChchNPV-TF1 fue la variante más abundante y extendida, y por ello, fue seleccionado para los ensayos de campo.

6. La aplicación de ChchNPV-TF1 a 10^9 OBs/l redujo significativamente la prevalencia de *C. chalcites* y el daño foliar en plantas jóvenes de platanera en condiciones de invernadero y aire libre, de la misma forma que los productos normalmente usados, indoxacarb y bioinsecticida basado en Bt. Sin embargo, ChchNPV-TF1 produjo una mayor mortalidad en las larvas recogidas a lo largo del tiempo, lo que sugiere una mayor persistencia de OBs de ChchNPV-TF1 en plantas de platanera, proporcionando un mayor periodo de control de la plaga comparado con los tratamientos convencionales.

7. Finalmente, ChchNPV-TF1 fue tan efectivo con los tratamientos convencionales reduciendo el daño foliar y en fruta en invernaderos de malla comerciales, excepto en el ensayo de 2014 en Tenerife, en el cual, el daño en fruta fue mayor en la parcela tratada con el virus. Sin embargo, la aplicación de ChchNPV-TF1 el año siguiente en invernadero de malla de Tenerife a lo largo de todo el ciclo de cultivo proporcionó un control del daño de *C. chalcites* satisfactorio y

comparable al proporcionado por insecticidas químicos y basados en Bt.

8. En general, los resultados de esta tesis proporcionan una información útil sobre la importancia del ChchNPV-TF1 como un componente fundamental para implementar un programa de manejo integrado de plagas en los cultivos de platanera de Canarias.

CONCLUSIONS

1. The prevalence of *C. chalcites* infestations was high and similar during the two years of prospections. A similar population density was detected between northern-facing and southern-facing slopes, except in Gran Canaria where southern-facing slopes showed higher incidence. *C. chalcites* population density did not varied across seasons, suggesting that generations follow one another throughout the year on banana crops.

2. Foliar and fruit feeding damage varied significantly across the islands, plantation location and season. In general, more damages were registered in El Hierro, La Palma and Tenerife, being damages lower in Gran Canaria. Feeding damages were similar in both plantation locations, except in Gran Canaria and Tenerife where more damages were registered in southern-facing slopes. Fruit feeding damages were similar across the seasons, although damage to fruit in spring was more relevant.

3. The weight of damaged bananas varied significantly across the islands, showing El Hierro the highest losses. Losses were more evident in spring, which coincide with fruit development after flowering. Overall, besides control measures, *C. chalcites* fruit feeding losses can reach to 1.5% of annual production (≈2.68 million euros/year).

4. The main insecticidal product used to control *C. chalcites* was indoxacarb, which is used monosystematically with an average frequency of 3 treatments per crop cycle. Control cost using this insecticide would reach the 240 €/ha, representing the 0.64% of the production value for one hectare.

5. The prevalence of ChchNPV infections in *C. chalcites* natural populations was 2.3%, but varied from 0-4.8% on Tenerife and was usually low (0-2%) on the other islands. The virus prevalence in mesh-built greenhouse plantations was twice that in open field plantations. The native isolate ChchNPV-TF1 was the most abundant and widespread variant, and so, it was selected for field trails.

6. Application of 10^9 OBs/l of ChchNPV-TF1 significantly reduced *C. chalcites* prevalence and foliar damage in young banana plants under greenhouse and open-field conditions, as did the commonly used indoxacarb and Bt-based bioinsecticide. However, ChchNPV-TF1 produced an increased mortality in larvae collected over time, suggesting a greater persistence of ChchNPV-TF1 OBs on banana plant, providing an extended period of pest control compared to conventional treatments.

7. Finally, ChchNPV-TF1 was as effective as conventional treatments reducing foliar and fruit damage in commercial mesh-built greenhouses, except in 2014 trial in Tenerife in which fruit damage was slightly higher in virus treatment plot. However, the application of ChchNPV-TF1 the following year in Tenerife mesh-built greenhouse throughout the crop cycle provided a control of *C. chalcites* damage satisfactory and comparable to that provided by chemical and Bt-based insecticides.

8. In general, the results of this thesis provide useful information on the importance of the ChchNPV-TF1 as a fundamental component to implement an integrated pest management program on the Canary Islands banana crops.

LIST OF PUBLICATIONS

Indexed publications:

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017. *Chrysodeixis chalcites*, a pest of banana crops on the Canary Islands: incidence, economic losses and current control measures. Crop Protection under review.

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017. *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV): natural occurrence and efficacy as a biological insecticide on young banana plants in greenhouse and open-field conditions on the Canary Islands. PlosOne under review.

Fuentes, E.G., Hernández-Suárez, E., Simón, O., Williams, T., Caballero, P., 2017. Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV): an effective biological control agent against *C. chalcites* damage in commercial banana crops on the Canary Islands. Pest Management Science, to be submitted.

Divulgation publications:

Fuentes Barrera, E.G., 2014. Una nueva alternativa en el control de la lagarta de la platanera. Agropalca, 23.

Hernández Suárez, E., **Fuentes Barrera, E. G**., Piedra-Buena Díaz, A., 2016. Incidencia en campo del virus de la lagarta de la platanera (*Chrysodeixis chalcites*). Agropalca, 32.

Fuentes Barrera, E. G., Cartaya Delgado, N., García Luque, M., Piedra-Buena Díaz, A., 2016. Estimación de las pérdidas económicas producidas por la lagarta de la platanera (*Chrysodeixis chalcites*). Agropalca, 32.

Fuentes Barrera, E. G., Piedra-Buena Díaz, A., Hernández Suárez, E., 2016. Incidencia y daños de la lagarta de la platanera (*Chrysodeixis chalcites*). Agropalca, 35.

Book published:

Fuentes Barrera, E. G., Piedra-Buena Díaz, A., Hernández Suárez, E., 2017. Gestión Integrada de *Chrysodeixis chalcites* en platanera. In print.

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Chrysodeixis chalcites (Lepidoptera: Noctuidae) is an important pest of banana crops on the Canary Islands, which is usually controlled using chemical insecticides. However, a low number of active compounds are authorized, and the repeated use favours the development of resistance and the accumulation of hazardous residues on the fruits. In addition, since 2014 Integrated Pest Management (IPM) programs are mandatory in any crop system in Spain.

The results of the present study are part of a wider approach to define *C. chalcites* damage thresholds in banana crops and the potential use of a novel biological control agent from the Canary Islands, the C. chalcites nucleopolyhedrovirus (ChchNPV-TF1). Firstly, the incidence and feeding damage inflicted by *C. chalcites* as well as production losses were estimated. Secondly, the effectiveness of ChchNPV-TF1 controlling *C. chalcites* foliar and fruit damage was measured under greenhouse and open-field conditions.

Tesis Doctoral - Ernesto G. Fuentes Barrera

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