Research Article

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Efficacy of an alphabaculovirus-based biological insecticide for control of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) on tomato and banana crops

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Abstract

BACKGROUND: *Chrysodeixis chalcites* (Esper) is a major pest of tomato in Mediterranean countries and attacks banana in the Canary Islands (Spain). The efficacy of *Chrysodeixis chalcites* single nucleopolyhedrovirus (ChchSNPV-TF1) was evaluated in plant growth chambers and greenhouse trials performed on tomato and banana plants respectively. Treatments were applied using a compressed air sprayer.

RESULTS: Mean (± SE) lethal infection varied from 77 ± 10% to 94 ± 3% in second-instar larvae fed for 2 days on tomato plants treated with 2×10^6 to 5×10^7 virus occlusion bodies (OBs) L^{-1}, increasing to ∼100% infection after 7 days. Mortality of larvae collected from banana at different intervals post-application varied from 54 ± 10% to 96 ± 4% in treatments involving $1 \times 10^8 - 1 \times 10^9$ OBs L^{-1}, whereas indoxacarb (Steward 30% WG) and *Bacillus thuringiensis* var. *kurstaki* (Biobit 16% WP) treatments produced between 22 ± 6% and 32 ± 5% pest mortality. All treatments significantly reduced plant defoliation compared with untreated controls. Application of 1×10^9 OBs L^{-1} was 3–4-fold more effective than chemical or *B. thuringiensis* treatments. Larvae acquired lethal infection more rapidly when feeding on tomato than banana plants, but this difference disappeared following >60 min of feeding.

CONCLUSION: This information should prove useful in the registration of ChchSNPV-TF1 as a bioinsecticide in the Canary Islands and Europe.
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Keywords: tomato looper; ChchSNPV; greenhouse trials; application rates

1 INTRODUCTION

The golden twin-spot moth or tomato looper, *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae), is regarded as a major pest of greenhouse-grown crops, particularly in countries surrounding the Mediterranean, the Middle-East and sub-Saharan Africa.[1] This highly polyphagous pest feeds on tomatoes, peppers, beans, crucifers and ornamental plants, among others.[2] In southern Europe it also feeds on soya,[3,4] artichokes[5] and fodder crops such as lucerne and clover.[6] It is one of the four most important pests of European greenhouse crops,[7−10] including greenhouses in northern Europe.[11,12] Finally, this insect has recently been reported in Canada and sporadically in parts of the United States.[13]

In Spain, major damage has been reported in horticultural crops in Almería, southern Spain,[3] and in banana crops in the Canary Islands, where *C. chalcites* is considered to be an emerging pest that occurs throughout the production cycle.[14] For banana plants, injury mainly occurs on the young unfolding leaves, which are cut or perforated by *C. chalcites* larvae of all instars.[14,15] More importantly, late-instar larvae often feed on the epidermis of developing banana fruits, producing serious skin injuries that result in >30% losses in bananas grown under greenhouse conditions in the Canary Islands.[14,15]

Attempts at biological control of *C. chalcites* on the basis of predator or parasitoid releases have met with partial success,[16] or are presently in the process of evaluation.[14] Biological insecticides based on *Bacillus thuringiensis* var. *kurstaki* have proved to be effective in greenhouse and field crops[17,18] and are used to control this pest on bananas. Similarly, the use of pheromone-based

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control measures can be effective for *C. chalcites* monitoring[2,13] or mating disruption.[19]

Chemical-based control measures usually involve applications of chlorpyrifos, fenamiphos or indoxacarb, among others.[2,20] Synthetic insecticide treatments require multiple applications which increase production costs, hamper the commercialisation of products that can occasionally contain pesticide residues, and have led to the development of resistance in certain populations of this pest.[14,16,17,21] However, the low number of active substances authorised by the European Union for use in banana (http://www.agrocabildo.org/publica/Publicaciones/subt_48_Anexo%20II.pdf) limits the possible use of alternative active substances, thus increasing the risks associated with the development of pesticide resistance to chemical control measures by this pest.

Insect baculoviruses have an established record as the basis for highly effective biological insecticides against certain lepidopteran pests.[22] Previous studies indicated that an alphabaculovirus, named *Chrysodeixis chalcites* single nucleopolyhedrovirus (ChchSNPV), from Tenerife, Canary Islands (Spain), was highly pathogenic to *C. chalcites* larvae under laboratory conditions.[23,24] This isolate was named Tenerife isolate 1 (ChchSNPV-TF1). The pathogenicity and speed of kill of ChchSNPV-TF1 was comparable with that of other baculovirus-based insecticides.[22,25]

The performance of a virus-based insecticide as a pest control agent depends largely on the quantity of virus occlusion bodies (OBs) applied to the crop[26] and the feeding behaviour of the pest.[27] Feeding in polyphagous lepidopteran larvae is often influenced by the species and phenology of the crop plant.[28,29] For example, in tomato, *C. chalcites* feeds on the parenchyma on the underside of leaves and then rolls the edges of the leaves together and begins to eat through the leaves, making them appear skeletonised.[30] As the efficacy of a virus-based insecticide depends on the ability to deliver a lethal quantity of OBs to feeding larval stages of the pest,[31] we determined the influence of OB dose on virus-induced pest mortality in growth chamber experiments on tomato plants. Using these results, we then compared the performance of applications of ChchSNPV-TF1 OBs with those of two other insecticides frequently used for control of this pest: one based on *Bacillus thuringiensis* var. *kurstaki* and the other, a modern synthetic insecticide, indoxacarb. Given the relationship between acquisition of infection and efficacy of crop protection, in a final study we compared the rate of acquisition of infection of *C. chalcites* larvae feeding on virus-treated tomato and banana leaves to determine whether leaf structural or chemical differences in these crops significantly affect the probability of disease acquisition following application of ChchSNPV OBs.

2 EXPERIMENTAL METHODS

2.1 Insects and viruses

A laboratory colony of *C. chalcites* was started using pupae received from the Instituto Canario de Investigaciones Agrarias (ICIA), Tenerife (Spain), in 2007. This colony was refreshed periodically with pupae from the Canary Islands (Spain). Insects were reared at 25 °C, 70 ± 5% humidity and a photoperiod of 16:8 h (light:dark) on a semi-synthetic diet.[32] Adults were fed 30% w/v diluted honey. The ChchSNPV-TF1 isolate originated from a single infected larva collected during a natural epizootic in a banana crop in Tenerife, as described previously.[23]

ChchSNPV-TF1 OBs were produced by inoculating sixth-instar laboratory-reared larvae with 9.02×10^8 OBs mL^{-1} using the droplet feeding method.[33] For this, OBs were suspended in 10% sucrose solution and 0.001% Fluorella blue food dye. Larvae that drank inoculum within 10 min were placed in groups of 150 in 1.5 L plastic containers with ∼100 mL of semi-synthetic diet.[32] Larvae were checked daily for signs of polyhedrosis disease. Dead and moribund insects that had stopped moving were collected and stored at −20 °C. OBs were extracted by thawing and homogenising the cadavers in water, and purified by filtration through muslin and centrifugation at 2000 × *g* for 5 min.[23,34] Purified OBs were resuspended in sterile water, and their concentration was determined by counting triplicate samples using an improved Neubauer haemocytometer (Hawksley, Lancing, UK) under phase contrast microscopy at × 400. Purified OBs were stored at 4 °C for up to 1 month prior to use in laboratory and greenhouse assays. The identity of these OBs was confirmed by restriction endonuclease analysis using *Bgl*II, which had proved to be valuable for discrimination between ChchSNPV genotypes in previous studies.[23,24,34] The biological activity of laboratory-produced OBs was estimated in second-instar *C. chalcites* using the droplet feeding bioassay technique and compared with the LC_{50} values of the original stock inoculum and published OB activity values for this virus. For this, groups of 25 newly moulted second-instar *C. chalcites* from the laboratory colony were starved for 8–12 h at 26 °C and then allowed to drink from an aqueous suspension containing 10% (w/v) sucrose, 0.001% (w/v) Fluorella blue and OBs following a fivefold dilution series: 1×10^5, 2×10^4, 4×10^3, 8×10^2 and 1.6×10^2 OBs mL^{-1}, previously calculated to result in between 95 and 5% mortality.[23,24,34] An OB-free solution was also fed to a group of insects as a control. Mortality was recorded daily until death or pupation. As the volume of inoculum suspension ingested by each insect was not quantified, results were treated as lethal concentrations, rather than lethal doses. Results were subjected to probit analysis using the POLO-PC program.[35]

2.2 Efficacy of ChchSNPV-TF1 on tomato plants in growth chambers

A trial was performed using flowering tomato plants (*Solanum lycopersicum* L., Solanaceae, var. Raf) maintained in a walk-in plant growth room (3.38 m width × 4.95 m length × 2.40 m height) illuminated with eight fluorescent tubes (60 W). OB suspensions (2×10^6, 1×10^7 and 5×10^7 OBs L^{-1}) or water, as a control, were applied until run-off using a compressed-air hand sprayer (Matabi 5; Antzuola, Guipúzcoa, Spain). All treatments included 0.1% (v/v) Agral 90 (Syngenta Agro S.A., Madrid, Spain), nonylphenoxy polyethoxy ethanol, a non-ionic liquid wetting and spreading agent.

At 1 h post-application, when plants were completely dry, tomato branches with seven leaves were removed. The base of each branch was placed in a 25 mL glass jar containing a hydroponic nutrient solution or Hoaglands solution,[36] and these branches were then placed individually in 2 L glass containers. Finally, each container was artificially infested with 200 second-instar larvae from the laboratory colony. At the same time, 25 larvae from the laboratory colony were individualised as controls to determine whether the laboratory colony harboured an inapparent alphabaculovirus infection. The conditions of the growth chamber were controlled as follows: 25 ± 1 °C, 70 ± 5% humidity and a 16:8 h (light:dark) photoperiod.

Groups of 25 larvae were collected from each branch at four different intervals post-application: 2 h and 2, 5 and 7 days. Collected larvae were individually placed in 25 mL plastic cups with artificial diet, reared at 25 °C and 70 ± 5% humidity, in constant darkness,

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and checked daily until death or pupation. Bioassays were performed on four occasions. As groups of 25 larvae were sampled from containers on four occasions, samples were not independent in time, so that percentage mortality results were subjected to repeated-measures analysis of variance (ANOVA) in SPSS v.12 (SPSS Inc., Chicago, IL). The characteristics of the variance-covariance matrix were examined by applying Maunchly's sphericity test.[37] The significance of treatment effects at each sample time were determined by within-subject comparisons among the estimated means using Tukey's test ($P \leq 0.05$).

2.3 Efficacy of ChchSNPV-TF1 in banana plants grown in greenhouses

Experiments on the efficacy of ChchSNPV-TF1 were performed using young banana plants (*Musa acuminata*, var. Dwarf Cavendish) grown in mesh-structure greenhouses in Güimar, Tenerife, Canary Islands (Spain). The experiments involved five treatments: (i) 0.004% (w/v) indoxacarb (Steward 30% WG; DuPont, Wilmington, DE); (ii) 0.05% *B. thuringiensis* var. *kurstaki* (Biobit 16% WP; Aragro, Madrid, Spain) as a bioinsecticide; (iii) ChchSNPV-TF1 1×10^8 OBs L^{-1}; (iv) ChchSNPV-TF1 1×10^9 OBs L^{-1}; (v) water control. Indoxacarb and *Bt* treatments were applied to banana crops at the product label recommended rates (http://www.agrocabildo.org/publica/Publicaciones/subt_48_Anexo%20II.pdf).

All treatments included 0.1% (v/v) Agral 90 wetter-sticker and were applied using a compressed-air handheld sprayer (Solo® 402; Solo NZ Ltd, Hamilton, New Zealand). Experimental plots were arranged in a fully randomised design. Plots comprised four rows with seven plants per row (4 × 7 m), totalling 28 young banana plants, of which 18 were border plants and ten were central plants. Plants were present at 1 m intervals with a 1 m space between rows, giving each plot an area of 28 m^2. The first trial was performed using eight replicate plots per treatment in October–November 2011 (2011 trial). The second trial was performed using a fully randomised plot design in September 2012 (2012 trial), with three replicate plots per treatment. Plants were artificially infested with ∼80–120 *C. chalcites* eggs placed in groups of ∼20–30 on each of the four youngest leaves of each plant. This variation in egg number reflected the natural variation in the size of *C. chalcites* egg masses, but the total number of eggs placed on plants in each plot was similar among all treatments (∼2800 eggs $plot^{-1}$). Five days after the infestation, when larvae had reached the second instar, plots were sprayed with a 1 L volume of each treatment, equivalent to 357 L ha^{-1}. All applications were made between 07:00 and 10:00 a.m.

In the first trial, sampling from border plants was used to estimate the prevalence of infection, whereas central plants were used to determine larval survival and defoliation. For this, the percentage of larval mortality was determined by collecting 25 *C. chalcites* larvae 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 artificial diet until death or pupation. Numbers of surviving larvae and foliar feeding injury were estimated for the ten central plants of each plot. The densities of larvae on central plants were not affected by the periodic collection of larvae performed for border plants. Survival of larvae and plant damage were estimated by direct counting of the number of larvae and the foliar feeding damage immediately before the application (time 0) and at 7 days post-application. Percentages of surviving larvae were calculated on the basis of the initial number of larvae in each treatment (taken as a nominal value of 100%), whereas recent foliar feeding damage was estimated by counting the final number of foliar perforations characteristic of *C. chalcites* feeding damage on each leaf in each treatment and subtracting the initial number of perforations present prior to the insecticidal treatments. In the second trial, only the percentage of larval mortality was evaluated, using the same methods as described for the first trial. The percentages of virus-induced mortality for each treatment were subjected to repeated-measures ANOVA using SPSS v.12. Within-subject comparisons among the estimated means were determined by Tukey's test ($P \leq 0.05$).

2.4 Rate of acquisition of viral infection

The rate of acquisition of a lethal infection on each species of host plant was evaluated using tomato and banana leaves. Concentrations of 2×10^6 OBs L^{-1}, or water as a control, were applied using a compressed-air handheld sprayer (Solo 402). All applications were performed until run-off to provide a standardised criterion for application, reflecting the high-volume applications performed on these crops. All treatments included 0.1% (v/v) Agral 90 as wetter-sticker. When plants were completely dry, the stem of each branch (tomato) or each leaf (banana) was placed in a 25 mL glass jar containing Hoagland's solution, and these branches were then individually placed in 2 L glass containers. Finally, each container was artificially infested with 500 second-instar larvae from the laboratory colony. The containers were maintained in a walk-in plant growth room (3.38 m width × 4.95 m length × 2.40 m height) illuminated by eight fluorescent tubes (60 W). At the same time, 25 larvae from the laboratory colony were individualised and reared on semi-synthetic diet as controls to determine whether the laboratory colony harboured an inapparent alphabaculovirus infection. Groups of 25–30 larvae were randomly collected from treated plants at 5, 10, 15, 20, 40, 60, 80, 100 and 120 min and at 4, 6, 9, 12 and 24 h following the infestation process. Collected larvae were individually placed in 25 mL plastic cups with semi-synthetic diet and checked daily until death or pupation. The experiment was performed on five occasions, each representing a replicate performed at a different moment in time. Percentage mortality values in each treatment were normally distributed and were subjected to repeated-measures ANOVA using SPSS v.12 (SPSS Inc.). The significance of treatment effects at each sample time was determined by within-subject comparisons among means using Tukey's test ($P \leq 0.05$).

3 RESULTS

3.1 Identity and biological activity of ChchSNPV OBs for use in efficacy trials

Following the production of ChchSNPV-TF1 OBs in larvae reared in plastic containers, the identity and biological activity of semi-purified OBs were confirmed by *Bgl*II digestion of viral DNA and insect bioassay in second-instar *C. chalcites*. The *Bgl*II profile of the virus produced in laboratory-infected larvae was identical to that described previously[23,34] (Fig. 1A), which confirmed the identity and the absence of cross-contamination during laboratory production of OBs. Additionally, the LC_{50} of laboratory-produced ChchSNPV-TF1 OBs for second instars was 1.23×10^3 OBs mL^{-1}, very similar to the LC_{50} value of the original inoculum (1.45×10^3 OBs mL^{-1}) and previously described LC_{50} values.[23,24,34] Concentration–mortality regression slopes were fitted in parallel (test for non-parallelism: $\chi^2 = 1.56$; df = 1; $P > 0.05$),

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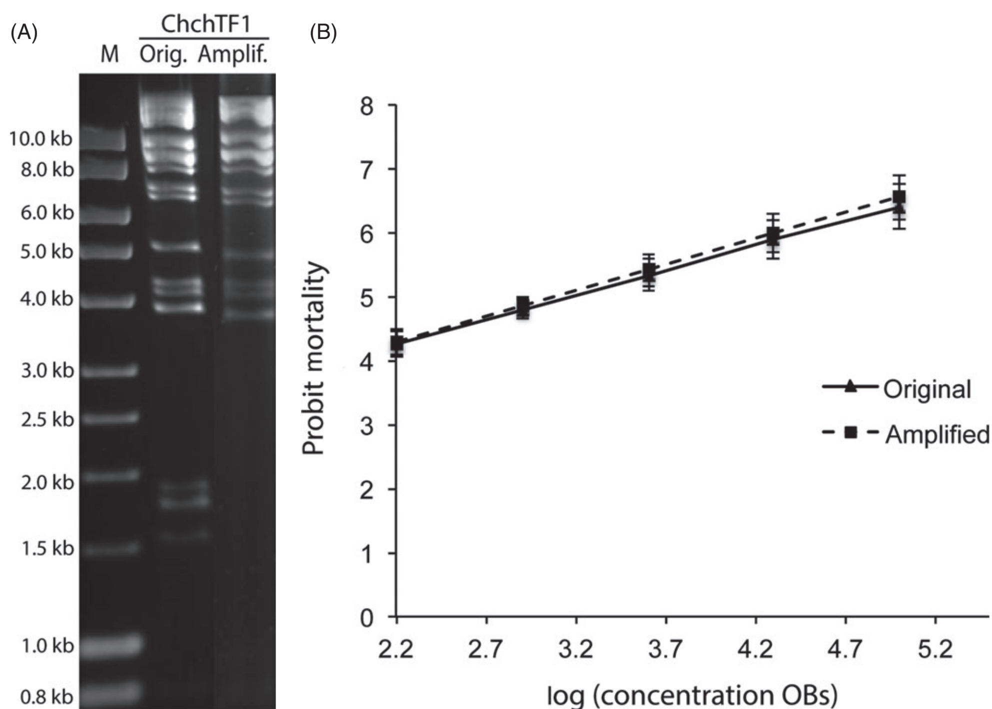

Figure 1. (A) Restriction endonuclease analysis (*Bgl*II) following digestion of genomic DNA of the original inoculum (Orig.) and the laboratory-amplified (Amplif.) OBs of ChchSNPV-TF1. The DNA 1 kb Marker Ladder (Nippon, Tokyo, Japan) was used as a molecular size marker (M). (B) Probit regression lines of the original inoculum (Original, triangular points) and that obtained after laboratory production (Amplified, square points) were determined by bioassay in second-instar *Chrysodeixis chalcites* larvae.

and a test for equality ($\chi^2 = 3.23$; df = 2; $P > 0.05$) indicated that the potency of the laboratory-produced inoculum did not differ significantly from that of the source inoculum (Fig. 1B).

3.2 Efficacy of ChchSNPV-TF1 on tomato plants in growth chambers

No virus mortality was registered in control larvae reared in the laboratory to determine the prevalence of infection, indicating that the *C. chalcites* population used to infest plants was healthy. The number of *C. chalcites* larvae that acquired a lethal virus infection increased significantly over time ($F_{3,9} = 54.203$; $P < 0.001$). The 2×10^6 OBs L^{-1} treatment resulted in 55 ± 9% (mean ± SE) lethal infection in larvae collected at 2 h post-application and reared in the laboratory until death or pupation, indicating rapid acquisition of infection. The percentage of lethal infection increased to 97 ± 3% in larvae collected at 7 days post-application (Fig. 2). Similar results were obtained with OB concentrations of 1×10^7 and 5×10^7 OBs L^{-1}, indicating no significant effect of OB concentration over the time course of the experiment (concentration*time interaction: $F_{6,18} = 1.581$; $P = 0.210$).

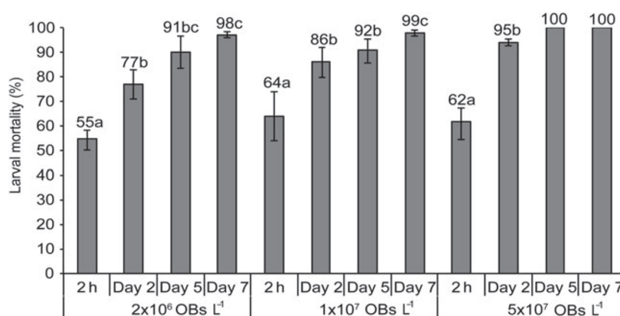

Figure 2. Mean (± SE) percentage mortality of larvae collected from tomato plants at different times post-application and reared in the laboratory until death or pupation. Experimental treatments involved one of three different OB concentrations. Values above the bars indicate means, and those followed by identical letters did not differ significantly for comparisons of time points within each virus concentration treatment (repeated-measures ANOVA; Tukey's test, $P > 0.05$).

3.3 Efficacy of ChchSNPV-TF1 in banana plants grown in greenhouses

Lethal polyhedrosis disease was not observed in larvae collected at time point zero (pretreatment sample) in the first or second trial. However, following OB treatment, virus mortality varied from 1 to 2% in control larvae in both trials, depending on the sample time, possibly owing to low levels of cross-contamination from adjacent virus treatments.

In the first trial, treatment effects on larval mortality differed significantly during the experiment (treatment*time interaction: $F_{12,72} = 2.306$; $P = 0.015$). Application of 1×10^8 OBs mL^{-1} resulted in 54 ± 10% (mean ± SE) lethal infection in larvae sampled at 1 day post-application, which increased to 81 ± 11% lethal infection in larvae sampled at 5 days post-application (Fig. 3A). In contrast, application of 1×10^9 OBs mL^{-1} resulted in 86 ± 7% lethal infection in larvae collected at 1 day post-application to 92 ± 5% lethal infection in larvae sampled at 3 days post-application. Indoxacarb and *Bt* treatments resulted in 22–33% mortality and 13–32% mortality respectively, depending on sample time.

Significant reductions in larval densities on experimental plants were observed in all treatments relative to initial larval densities, compared with the control ($F_{4,35} = 41.323$; $P < 0.001$). Prior to the application of treatments, the mean number of larvae registered in the control, indoxacarb, *Bt* and ChchSNPV-TF1 1×10^8 OBs L^{-1}

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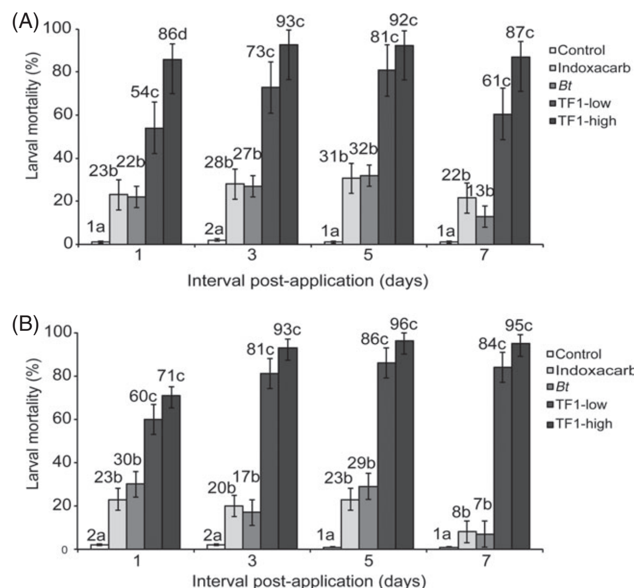

Figure 3. Mean (± SE) percentage mortality of larvae collected from banana plants at four different intervals after treatment and reared in the laboratory until death or pupation. Experimental treatments involved application of water (control), indoxacarb, *Bacillus thuringiensis* var. *kurstaki* (*Bt*) and two concentrations of ChchSNPV, 1×10^8 OBs L^{-1} (TF1-low) and 1×10^9 OBs L^{-1} (TF1-high), in 2011 (A) and in 2012 (B) in greenhouse trials. Values above the bars indicate means, and those followed by identical letters did not differ significantly for comparisons of treatments within each time point (repeated-measures ANOVA; Tukey's test, $P > 0.05$).

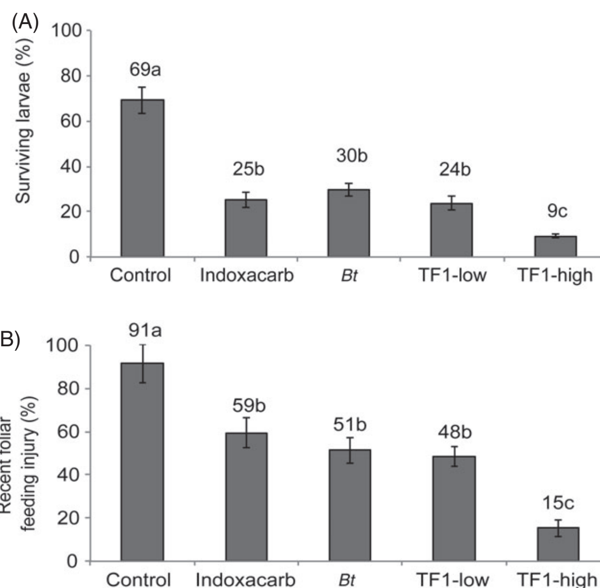

Figure 4. Mean (± SE) percentage of surviving larvae (A) and recent foliar feeding damage (B) in banana plants treated with water (control), chemical (indoxacarb), *Bt* (and two ChchSNPV-TF1 concentrations, TF1-low (1×10^8 OBs L^{-1}) and TF1-high (1×10^9 OBs L^{-1}), sampled at 7 days post-application. Values above the bars indicate means, and those followed by identical letters did not differ significantly (ANOVA; Tukey's test, $P > 0.05$).

and 1×10^9 OBs L^{-1} plots was 30.2, 18.7, 26.4, 28.6 and 33.5 larvae plant^{-1} respectively, whereas at 7 days post-application these densities had fallen to 20.8, 4.7, 7.9, 7.0 and 3.1 larvae plant^{-1}, which represents 69, 25, 30, 24 and 9% survival of experimental larvae respectively (Fig. 4A). Survival values for larvae of central plot plants were independent of sampling performed at earlier times post-application on plants at the edge of experimental plots. The greatest reduction in numbers of larvae was observed in the treatment involving 1×10^9 OBs mL^{-1} (shown as TF1-high in Fig. 4A), whereas the treatments involving indoxacarb, *Bt* and ChchSNPV-TF1 at 1×10^8 OBs mL^{-1} had similar densities of larvae (Fig. 4A).

Foliar feeding damage values were significantly affected by treatment ($F_{4,35} = 11.118$; $P < 0.001$), likely reflecting the differences in pest densities in each treatment (Fig. 4B). The highest levels of defoliation were registered in control plants, whereas the lowest levels of defoliation were observed in plants treated with ChchSNPV-TF1 at 1×10^9 OBs mL^{-1}. Levels of defoliation in treatments involving indoxacarb, *Bt* and ChchSNPV-TF1 at 1×10^8 OBs mL^{-1} were intermediate and were similar among these treatments (Tukey's test, $P > 0.05$) (Fig. 4B).

In the second trial, all treatments resulted in a significant increase in mortality compared with the control treatment during the experiment (treatment*time interaction: $F_{12,24} = 9.254$; $P < 0.001$). Application of ChchSNPV-TF1 at 1×10^8 OBs mL^{-1} resulted in 60 ± 6% (mean ± SE) lethal infection in larvae sampled at 1 day post-application, which increased to 86 ± 7% lethal infection in larvae sampled at 5 days post-application (Fig. 3B). In contrast, application of 1×10^9 OBs mL^{-1} resulted in 71 ± 5% lethal infection in larvae sampled at 1 day post-application, which increased to 96 ± 4% lethal infection in larvae sampled at 5 days post-application. In the indoxacarb treatment, mortality fell from 23 ± 5% to 8 ± 4% during the trial, whereas mortality in the *Bt* treatment fell from 30 ± 7% to 7 ± 6% during the same period.

3.4 Rate of acquisition of viral infection

No lethal virus disease was observed in control larvae reared in the laboratory to determine the prevalence of infection, indicating that the *C. chalcites* population was healthy. The percentage of *C. chalcites* larvae that acquired a lethal infection increased significantly over time ($F_{1,13} = 17.654$; $P < 0.001$). During the first hour of exposure, a significantly higher proportion of larvae acquired lethal infection when feeding on tomato compared with those that fed on banana plants (Fig. 5). The greatest difference was observed in the sample taken at 40 min, in which 23 ± 3% (mean ± SE) and 13 ± 2% of larvae had already acquired a lethal infection on tomato and banana plants respectively. However, larvae from subsequent sample times showed no significant host-plant-related differences in virus mortality ($P > 0.05$). By 12 h exposure, 62 ± 3% and 62 ± 5% of larvae from either tomato or banana had acquired a lethal infection, and this increased to 68 ± 2% and 70 ± 3% in larvae sampled following a 24 h period of feeding on treated banana or tomato plants respectively (Fig. 5).

4 DISCUSSION

The present study aimed to determine the efficacy of different concentrations of ChchSNPV-TF1 OBs for the control of *C. chalcites* larvae on tomato plants in growth chambers and on banana plants cultivated in greenhouses. Application of 2×10^6 OBs L^{-1} to tomato plants in growth chambers was as effective in producing lethal infection in *C. chalcites* larvae as OB applications that were fivefold or 25-fold higher. As pesticide applications to tomato plants usually involve volumes of 600–1000 L ha^{-1}, depending on plant phenology, the growth chamber treatments

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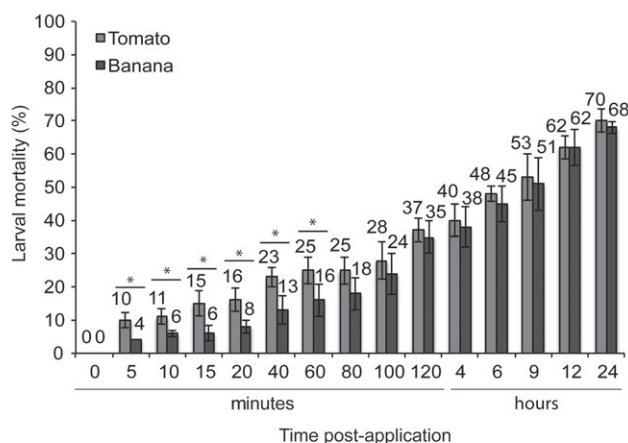

Figure 5. Mean (± SE) percentage mortality of larvae collected at different time points and reared in the laboratory following application of 2×10^6 OBs mL^{-1} of ChchSNPV-TF1 to banana and tomato leaves. Values above the bars indicate means. Horizontal lines with asterisks indicate significant differences between host plants (Tukey's test, $P \leq 0.05$).

that we used would be equivalent to $1.2 \times 10^8 - 2 \times 10^9$ OBs ha^{-1} of ChchSNPV-TF1. This appeared to be a useful starting point to define application rates for *C. chalcites* control in field- or greenhouse-grown tomatoes. Crucially, this rate is approximately 1000-fold lower than OB application rates determined for other commercial-virus-based insecticides such as *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) or *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV).[38,39]

In greenhouse trials on young banana plants, the low-rate virus treatment (1×10^8 OBs mL^{-1}) resulted in larval mortality that was significantly higher than those of chemical and *Bt* treatments, whereas larval survival on treated plants and defoliation levels were similar to those of chemical and *Bt* treatments. Application of the higher rate of 1×10^9 OBs mL^{-1} resulted in exceptionally high levels of pest mortality (~90%) in larvae collected at various intervals post-application, and significantly reduced levels of feeding damage compared with the other treatments.

Surprisingly, larvae treated with indoxacarb or *Bt* survived in high numbers during the first trial, and the efficacy of these treatments appeared to decrease over time. Although larvae become increasingly resistant to baculovirus infection as they age,[40] the lower susceptibility of later larval stages can be compensated for through increased consumption of OBs produced by other NPV-infected larvae dying earlier or OB-contaminated leaf surfaces leading to a higher ingested dose of OBs.[41] The apparently low efficacy of indoxacarb or the *Bt* insecticide currently used in banana crops in the Canary Islands (Spain) also suggests the possible existence of resistance to these products in the pest population,[17,21] although this was not determined in the present study.

The potential of ChchSNPV-TF1 as a bioinsecticide is likely to be affected by numerous factors, including the timing of applications in combination with the age structure of the pest population, as susceptibility to infection decreases with larval stage.[24] In this study, OBs were applied to banana plants when the majority of the pest population was in the second instar. At this stage, larvae are highly susceptible to infection and cause little damage to plants compared with that of later instars. Indeed, foliar feeding by *C. chalcites* early instars on banana plants does not usually translate into yield losses or cosmetic damage to banana fruits. In contrast, late instars often feed on fruits, resulting in economically significant losses when infestation levels are high.[14,15]

Effective spray targeting of the feeding sites of the pest is also likely greatly to influence the performance of baculovirus insecticides, the effectiveness of which depends on larval feeding behaviour and the probability of consuming a lethal dose of OBs.[42] Early instars of *C. chalcites* feed on the undersides and internal parts of young leaves. Given this behaviour, OBs applied to the underside of banana leaves would be expected to provide the principal source of inoculum, especially under greenhouse conditions, where UV protection conferred by the leaves, in addition to the UV filtering activity of the greenhouse structure, is likely markedly to improve the persistence of OBs on plant surfaces.[43,44] Nonetheless, the persistence of ChchSNPV OBs on greenhouse-grown banana crops in the Canary Islands needs to be confirmed experimentally.

The highest concentration of ChchSNPV-TF1 OBs was more effective in reducing plant defoliation than the chemical and *Bt* insecticides in banana crops. In many instances, alphabaculovirus treatments can be as effective as chemical insecticides.[38,45] In contrast, when pest populations have developed resistance to synthetic insecticides, virus-based insecticides represent a unique opportunity for pest control, as cross-resistance between synthetic insecticides and virus insecticides has never been reported owing to the markedly different mode of action of these agents.[44,46]

In the present study, an application volume of 1 L containing 1×10^9 OBs was necessary efficiently to protect banana young plants present in a 28 m^2 plot. This is equivalent to 3.57×10^{11} OBs for the treatment of 1 ha of plants at the same phenological stage. However, given that pesticides are usually applied to banana crops at volumes of 1600–2000 L ha^{-1}, depending on plant phenology, the rate of 1×10^9 OBs L^{-1} would be equivalent to approximately $1.6 \times 10^{12} - 2.0 \times 10^{12}$ OBs ha^{-1}. Higher OB application rates are likely to be necessary to protect fully grown banana plants efficiently, given the high volume applications needed effectively to cover the foliage of large-sized plants.

ChchSNPV-TF1 appeared to be slightly more effective in controlling *C. chalcites* larvae on tomato plants than on banana plants, as a lower concentration of OBs was required to produce a very high prevalence of pest mortality. In banana field trials, not only may environmental factors such as temperature, leaf surface exudates and sunlight have reduced treatment efficacy,[42] but also the phenological stage of banana crops grown in the open field may explain the higher concentration of OB applications required for efficient pest control compared with those required for effective protection of young banana plants grown in greenhouses.

Infection acquisition experiments revealed that larvae that fed on tomato plants acquired a lethal infection approximately twice as rapidly as those that fed on banana. However, this effect was only observed to be significant during the first hour of exposure to virus-treated plants, whereas no significant plant-related differences were observed at subsequent sample times. It is possible that the hardness of banana leaves compared with those of tomato may have reduced larval feeding rates during the first hour of exposure. The first 48 h of feeding are considered crucial in the acquisition of a viral infection.[44,47] Previous studies with SeMNPV recorded 15–31% lethal infection, depending on OB concentration, in *S. exigua* larvae collected at 6 h post-application.[39,44] However, larvae that feed on plants contaminated with ChchSNPV-TF1 acquired lethal infection far more rapidly than *S. exigua* larvae. After 20 min, between 8 and 16% of *C. chalcites* larvae from banana and tomato plants, respectively, had acquired a lethal infection and died during subsequent rearing. Similarly, by 6 h, the prevalence of lethal infection reached 45–48%. These findings underline the importance of pest feeding behaviour during the

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hours immediately following OB applications to the rate at which larvae become lethally infected. When both crop plants were grown under the same greenhouse conditions, similar results were obtained, suggesting that ChchSNPV-TF1 treatments in tomato crops are likely to be as effective as in banana crops.

The specificity of the virus-based insecticide is another issue that merits mention, as other noctuid pests and numerous species of natural enemies are usually present in greenhouse and field crops grown under integrated pest management programmes. Previous studies have indicated that ChchSNPV is pathogenic to two plusiids, *Autographa* (*Plusia*) *gamma* and *Trichoplusia ni*, but not to other pest noctuids, including *Helicoverpa armigera*, *Spodoptera exigua* and *S. littoralis*, even at very high inoculum concentrations of up to 3×10^9 OBs mL^{-1}.[48]

A low number of active substances are authorised in banana crops: indoxacarb 30% WG (Steward, DuPont) and 0.05% of *B. thuringiensis* var. *kurstaki* 16% WP (Novo-Biobit) are the most commonly used products (http://www.agrocabildo.org/publica/Publicaciones/subt_48_Anexo%20II.pdf). However, to reduce the probability of resistance in the pest population, the use of novel active substances, particularly biorational products, is recommended.[49,50] Alphabaculovirus-based products are readily incorporated into integrated pest management programmes, as they can be applied like chemical insecticides using conventional equipment and are fully compatible with natural enemy populations, thus reducing grower dependence on synthetic chemical products.

In conclusion, ChchSNPV-TF1 appears to have outstanding potential as a biological control agent against *C. chalcites* larvae on tomato and banana plants. Based on these and related findings, a unique combination of virus genotypes has recently been the subject of a patent application[51] and is planned to be registered as a crop protection product for use in banana and tomato crops in the Canary Islands and elsewhere in Europe in the near future.

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