Insecticidal Activity of *Bacillus thuringiensis* Strains on the Nettle Caterpillar, *Euprosterna elaeasa* (Lepidoptera: Limacodidae)

Angelica Plata-Rueda 1, Hughes Antonio Quintero 2, José Eduardo Serrão 3 and Luis Carlos Martínez 3,*

1 Department de Entomology, Federal University of Viçosa, Viçosa, Minas Gerais 36570–000, Brazil; angelicaplata@yahoo.com.mx
2 Department of Crop Protection, Monterrey Oil Palm Plantation, Puerto Wilches, Santander 687–061, Colombia; quinterohughes408@gmail.com
3 Department of General Biology, Federal University of Viçosa, Viçosa, Minas Gerais 36570–000, Brazil; jeserrao@ufv.br

* Correspondence: lc.martinez@outlook.com; Tel.: +55-31-3899-4012

Received: 10 April 2020; Accepted: 14 May 2020; Published: 15 May 2020

**Abstract:** In the present work, we evaluated the insecticidal activity of *Bacillus thuringiensis* (*Bt*) strains on *Euprosterna elaeasa* as an alternative for the organophosphate insecticide use in oil palm plantations in the Americas. The toxic effects of four *Bt*-strains (HD-1 var. *kurstaki*, SA-12 var. *kurstaki*, ABTS-1857 var. *aizawai*, and GC-91 var. *aizawai*) were evaluated against *E. elaeasa* caterpillars for toxicity, survival, anti-feeding, and mortality in field-controlled conditions. The *Bt*-strains, ABTS-1857 var. *aizawai* (LC₅₀ = 0.84 mg mL⁻¹), GC-91 var. *aizawai* (LC₅₀ = 1.13 mg mL⁻¹), and HD-1 var. *kurstaki* (LC₅₀ = 1.25 mg mL⁻¹), were the most toxic to *E. elaeasa*. The caterpillar survival was 99% without exposure to *Bt*-strains, and decreased to 52–23% in insects treated with the LC₅₀ and 10–1% in insects exposed to LC₉₀ after 48 h. Furthermore, *Bt*-strains decreased significantly the consumption of oil palm leaves of *E. elaeasa* 3 h after exposure. Mortality of *E. elaeasa* caterpillars caused by *Bt*-strains had similar lethal effects in the laboratory and in field conditions. Our data suggest that *Bt*-strains have insecticidal activity against *E. elaeasa* and, therefore, have potential applications in oil palm pest management schemes.

**Keywords:** anti-feeding effect; biopesticide; biological control; oil palm pest; survivorship; toxicity

1. Introduction

The nettle caterpillar, *Euprosterna elaeasa* Dyar (Lepidoptera: Limacodidae) is a significant pest of *Elaeis guineensis* Jacquin (Arecales: Arecaceae) from Brazil, Colombia, Ecuador, Guyana, Mexico, Panamá, Peru, Surinam, Trinidad and Tobago, and Venezuela [1,2]. This insect also damages other palm trees species, such as *Bactris gasipaes* Kunth, *Calappa botryophora* (Mart.) Kuntze, *Cocos nucifera* Linnaeus, and *Desmoncus polyacanthos* (Mart.) Kuntze [1,3]. The life cycle of *E. elaeasa* is 64 days (egg 5.1, larva 35.2, pupa 19.4, and adult 4.7) [4]. *Euprosterna elaeasa* damages oil palm leaves with a consumption rate of 66 cm²/caterpillar, and the damage causes an 80% loss of plant canopy with 1000 insects/leaf. It is also a reason behind Pestalotiopsis fungal disease in oil palm plantations [2,4].

In Colombia, chemical insecticides such as acephate, methamidophos, and monocrotophos are used on oil palm crops to control *E. elaeasa* [1,5]. Due to the high level of infestation and the rapid spread of *E. elaeasa* in oil palm trees, the use of insecticides is common practice [6,7]. However, recent studies have shown the presence of these insecticides in minimal quantities in palm oil [8,9]. Conventional insecticides are expensive and cause environmental pollution [10], atmosphere ozone-depletion [11],...
residual long [12], and insecticide resistance [13]. New alternatives that are more sustainable, different from organophosphates, are needed to replace the main insecticides historically used against *E. elaeasa* for the past 50 years [1,6]. The search for alternatives for *E. elaeasa* control is important, considering the impact generated by the use of insecticides in this agroecosystem [6]. Thus, the use of natural enemies, such as viruses, bacteria, and fungi, can be an alternative for oil palm pest control [14–16].

*Bacillus thuringiensis* (**Bt**) is a biocontrol agent for defoliating pests worldwide, and individual strains are specific to a small group of insect targets without effects on animals and environment [17]. *Bt* is a gram-positive spore-forming bacterium with entomopathogenic properties. In the sporulation, *Bt* produces crystalline or “Cry” inclusions, called δ-endotoxins, biosynthesized during the second phase of the growth cycle [18]. In this cycle, the Cry proteins are converted in active toxins upon insect ingestion [19]. Several Cry proteins displaying activity on insects have been identified: the Cry1 proteins are toxic to Lepidoptera [20], while the Cry3 proteins are toxic to Coleoptera [21,22]; also, a high number of different subgroups (Cry1Ac, Cry1Ba, Cry8Ca, Cry1Eb, Cry1J, etc.) are active against mosquitoes, Coleoptera, Diptera, Hemiptera, and Hymenoptera [21,23,24].

*Bt* was reported as a biological control agent for oil palm pests [6,25]. Different oil palm lepidopteran species may have different levels of susceptibility to a specific Cry protein that occurs in *Metisa plana* Walker (Psychidae) [16], *Opsiphanes cassina* Felder (Nymphalidae) [26], and *Tirathaba rufivena* Walker (Pyralidae) [27]. This microbiological agent provides biodiversity in agroecosystems and the delivery of ecosystem services to agricultural production, especially in pest population regulation [28]. Since the entomopathogenic bacterium infects their host through the midgut, they hold greater potential as biocontrol agents for *E. elaeasa*; however, the use of *Bt* on this insect has not been carried out.

This study evaluated the insecticidal activity of *Bt* strains as potential agents to control *E. elaeasa*, explained in different experiments: (i) toxicity test, (ii) survivorship, (iii) anti-feeding effect, and (iv) mortality in field conditions. The objective was to contribute to the development of strategies for controlling *E. elaeasa*, as a replacement for organophosphate insecticides.

### 2. Materials and Methods

#### 2.1. Insects

In the field, 2527 adults of *E. elaeasa* (males = 1284, females = 1243) were captured manually during the day, in 5-yr-old commercial plantations of oil palm in the county of Puerto Wilches, Santander, Colombia (N 07°20’, W 73°54’). The insects were transferred in plastic trays (30 × 50 × 50 cm) with perforated lids for ventilation to the Entomology Laboratory of the Oil Palm Monterrey Plantation (Puerto Wilches, Santander, Colombia) to establish a breeding colony in laboratory conditions. Adults were fed a honey solution daily (15 mL of honey and distilled water, in a 2:1 ratio) applied with a sponge. Males and females of *E. elaeasa* were isolated in glass containers (30 × 30 × 30 cm) covered with a nylon mesh and containing *E. guineensis* leaves. For egg development, 9800 eggs oviposited on the surface of the leaves were collected every 24 h and placed in Petri dishes (90 × 15 mm high) containing a paper towel saturated with water. After hatching, first-instar caterpillars (*n* = 7550) were placed individually in glass vials (5 × 25 cm) covered with cotton and fed every 24 h with *E. guineensis* leaves. Eggs and caterpillars were maintained in incubators at 27 ± 1 °C, with 75 ± 5% RH and 12:12 (L:D) photoperiod. Newly third instar *E. elaeasa* caterpillars were used in the laboratory and field condition bioassays.

#### 2.2. Concentration–Mortality Bioassay

Commercial *Bt* formulations commonly used to control Lepidoptera were used in all bioassays and selected for quality, high-efficiency, and non-toxicity (toxicity Class IV) [18]. The following *Bt* strains, HD-1 var. *kurstaki* (Dipel®, Abbott Laboratories, North Chicago, IL, USA), SA-12 var. *kurstaki* (Thuricide®, Certis USA LLC, Columbia, MD, USA), ABTS-1857 var. *aizawai* (XenTari®,
Valent Bioscience Corporation, Osage, Iowa, USA), and GC-91 var. aizawai (Agree®, Certis USA LLC, Columbia, MD, USA), were prepared in an aqueous solution with 0.1% Triton X-100 (strains and distilled water) to obtain a stock suspension (100 g L\(^{-1}\)) from which dilutions were prepared as needed. Six concentrations (0.156, 0.312, 0.625, 1.25, 2.5, and 5 mg mL\(^{-1}\)) were used to evaluate the toxicity of each Bt-strain to E. elaeasa caterpillars, construct concentration–mortality curves, and estimate the lethal concentrations (LC\(_{50}\) and LC\(_{90}\)). Distilled water with 0.1% Triton X-100 was used as control. The application of the concentrations was carried out by the feeding method using oil palm leaves. Pieces (10 × 10 mm) of oil palm leaves were cut, sterilized with 5% sodium hypochlorite with three successive series of distilled water, and dried at room temperature. Then, pieces of oil palm leaf were dipped in solutions of different concentrations of each Bt-strain for 10 s and allowed to air dry for a period of 1 h. Caterpillars were placed individually in Petri dishes, and a piece of oil palm leaf treated with Bt-strain was provided. Three replicates with 50 insects of each were used in concentration testing, and the experimental design was completely randomized. The dead insects were counted after 48 h Bt-strain exposure.

2.3. Time–Mortality Bioassay

Caterpillars of E. elaeasa were placed individually in Petri dishes and exposed to the lethal concentrations (LC\(_{50}\) and LC\(_{90}\)) of each of the Bt-strains determined in the dose–response relationship. A control was performed using distilled water with 0.1% Triton X-100. Exposure procedures and conditions were the same as described above for the concentration–mortality bioassay Section 2.2. The number of live insects was recorded every 6 h for 2 d. Three replicates of 50 insects were used by each Bt-strain and the experimental design was completely randomized.

2.4. Anti–Feeding Effect

Caterpillars of E. elaeasa were placed individually in Petri dishes with a piece of oil palm leaf (10 × 10 mm) treated with LC\(_{50}\) or LC\(_{90}\) of each Bt-strains and distilled water as control. Caterpillars were in contact with E. guineensis leaves for 3 h and, after this, the pieces were photographed with a digital photographic camera (D40, 18D55 mm, Nikon Corporation, Tokyo, Kantô, Japan) with a 15 cm macro focus in natural and flourishing light (SB-700 Nikon Corporation). The images were analyzed using the digital analysis software, UTHSCSA Image Tool v. 2.0 (University of Texas, Austin, TX, USA). The leaf area consumed by the caterpillar was measured in mm\(^{2}\), with pixels based on the RGB (red, 213 nm; green, 111 nm; blue, 56 bits) histogram. Twenty repetitions for each of the Bt-strain concentrations (LC\(_{50}\) and LC\(_{90}\)) and control were carried out in a completely randomized design.

2.5. Mortality in Semi–Controlled Test

The bioassay was conducted in 5-yr-old commercial oil palm plantations (cv ‘Tenera’ × ‘Deli Ghana’) in the county of Puerto Wilches (Santander, Colombia), with an average temperature of 27.98 °C, 81–93% relative humidity, 1455 to 2258 h of sunshine per year, and 2189 mm of annual rainfall. In these conditions, fifty palm trees were selected and E. elaeasa caterpillars were used for each Bt-strain in the controlled field test. For each palm tree, 50 caterpillars were placed on the leaf No. 17, according to the rules of phyllotaxy [29] and isolated with a nylon trap (0.5 × 0.5 × 1.20 m). Treatments consisted of adding each Bt-strain at the calculated LC\(_{90}\) concentration, and distilled water with 0.1% Triton X-100 as the control, with ten replications per treatment. Applications of 100 mL of Bt-strain per leaf were made by a manual pump (Royal Condor®, 5 L capacity, Soacha, Cundinamarca, Colombia), and the number of dead caterpillars was counted after 15 d Bt-strain exposure.

2.6. Statistical Analysis

The concentration–mortality data were submitted to Probit analysis to obtain a dose–response curve [30]. The time–mortality data were analyzed for survival analysis (Kaplan-Meier estimators, log-rank test) with the Origin Pro 9.1 software (OriginLab Corporation, Northampton, MA, USA).
Anti-feeding effect data were arcsine-transformed and submitted to one-way ANOVA, and a Tukey’s honestly significance difference (HSD) \((p < 0.05)\) test was also used for comparison of means. Mortality data in semi-controlled conditions were summarized in percentages and submitted to one-way ANOVA and a Tukey’s HSD \((p < 0.05)\); also, all values presented as mean ± SEM. Statistical procedures were analyzed by SAS 9.0 software (SAS Institute, Campus Drive Cary, NC, USA).

3. Results

3.1. Concentration–Mortality Bioassay

The dose–response model provided a good fit to the data \((p > 0.05)\), allowing the determination of toxicological endpoints, and confirms the toxicity of each Bt-strain to *E. elaeasa* Table 1. The bioassay showed that ABTS-1857 var. *aizawai* had LC\(_{50}\) = 0.84 mg mL\(^{-1}\) (range of 0.66–1.16 mg mL\(^{-1}\)), GC-91 var. *aizawai* had LC\(_{50}\) = 1.09 mg mL\(^{-1}\) (range of 0.74–1.72 mg mL\(^{-1}\)), HD-1 var. *kurstaki* had LC\(_{50}\) = 1.13 mg mL\(^{-1}\) (range of 0.84–1.56 mg mL\(^{-1}\)), and SA-12 var. *kurstaki* had LC\(_{50}\) = 1.25 mg mL\(^{-1}\) (range of 0.80–2.13 mg mL\(^{-1}\)). Mortality was <1% in the control group.

Table 1. Lethal concentration of *Bacillus thuringiensis* strains against *Euprosterna elaeasa* after 48 h exposure obtained from probit analysis (df = 5). The chi-square value refers to the goodness of fit test at \(p > 0.05\).

| Strain          | No. Insects | Lethal Concentration | Estimated Concentration (mg mL\(^{-1}\)) | 95% Confidence Interval (mg mL\(^{-1}\)) | Slope ± SE | \(\chi^2\) (p-Value) |
|-----------------|-------------|----------------------|------------------------------------------|------------------------------------------|------------|---------------------|
| HD-1 var. kurstaki | 150         | LC\(_{50}\)          | 1.133                                    | 0.845–1.561                              | 2.22 ± 0.25 | 1.23 (0.36)         |
|                 | 150         | LC\(_{90}\)          | 4.268                                    | 2.802–8.512                              |            |                     |
| SA-12 var. kurstaki | 150         | LC\(_{50}\)          | 1.258                                    | 0.805–2.136                              | 2.40 ± 0.41 | 1.89 (0.16)         |
|                 | 150         | LC\(_{90}\)          | 4.299                                    | 2.442–10.92                              |            |                     |
| ABTS-1857 var. aizawai | 150         | LC\(_{50}\)          | 0.840                                    | 0.664–1.075                              | 1.73 ± 0.35 | 1.34 (0.22)         |
|                 | 150         | LC\(_{90}\)          | 4.623                                    | 3.172–7.875                              |            |                     |
| GC-91 var. aizawai | 150         | LC\(_{50}\)          | 1.097                                    | 0.742–1.724                              | 2.40 ± 0.41 | 1.38 (0.22)         |
|                 | 150         | LC\(_{90}\)          | 4.579                                    | 2.647–9.894                              |            |                     |

3.2. Time–Mortality Bioassay

Survival rate was determined 48 h after *E. elaeasa* caterpillar exposure to Bt-strains at lethal concentrations, LC\(_{50}\) and LC\(_{90}\). Survival rates differed between treatments at LC\(_{50}\) (log-rank test, \(\chi^2 = 9.47, \text{df} = 3, \text{and} \ p < 0.001\)). *E. elaeasa* survival decreased from 99.9% in the control to 52.79% with SA-12 var. *kurstaki*, 51.37% with GC-91 var. *aizawai*, 35.62% with HD-1 var. *kurstaki*, and 23.12% with ABTS-1857 var. *aizawai* (Figure 1A).

At LC\(_{90}\), the survival rates of *E. elaeasa* were different according to the treatments (log-rank test, \(\chi^2 = 18.57, \text{df} = 4, \text{and} \ p < 0.001\)), decreasing from 99.9% (control) to 10.13% with SA-12 var. *kurstaki*, 9.87% with GC-91 var. *aizawai*, and 0% with both the HD-1 var. *kurstaki* and ABTS-1857 var. *aizawai* (Figure 1B).
Bt 6.83 mm aizawai consumed in comparison to control (Figure 2). The leaf area consumed by Insects 3.3.

1B). with mm2 mm2

\[ \chi^2 = 9.47, p < 0.001 \]

\[ \chi^2 = 18.57, p < 0.001 \]

3.3. Anti–Feeding Effect

The four Bt-strains caused an anti-feeding effect on E. elaeasa caterpillars, with lower leaf area consumed in comparison to control (Figure 2). The leaf area consumed by E. elaeasa differed between Bt-strains at LC50 (F4,19 = 9.51, p < 0.001), decreasing from 26.41 mm² (control) to 11.38 mm² with GC-91 var. aizawai, 8.89 mm² with ABTS-1857 var. aizawai, 8.44 mm² with SA-12 var. kurstaki, and 6.83 mm² with HD-1 var. kurstaki (Figure 2A). However, at LC90 all Bt-strains had similar anti-feeding effects among them (F4,19 = 27.36, p > 0.05; Figure 2B).

Figure 1. Survival curves of Euprosterna elaeasa caterpillars exposed to Bacillus thuringiensis strains, subjected to survival analyses using the Kaplan–Meier estimators’ log-rank test. Lethal dose of (A) LC50 (control) and (B) LC90 (control).

3.4. Mortality in Semi–Controlled Test

The mortality caused by the Bt-strains to E. elaeasa caterpillars was different in a semi–controlled test (F4,49 = 48.19; p < 0.05), as shown in Figure 3. Mortality caused by Bt-strains of LC90 to E. elaeasa
caterpillars was higher in HD-1 var. *kurstaki* and ABTS-1857 var. *aizawai* (92.1 ± 0.2% and 89.1 ± 2.1%, respectively) than with GC-91 var. *aizawai* and SA-12 var. *kurstaki* (86.8 ± 5.1% and 84.1 ± 4.9%, respectively), but they were all higher than in the control (2.67 ± 0.7%).

![Figure 3](image)

**Figure 3.** Mortality of *Euprosterna elaeasa* caterpillars by *Bacillus thuringiensis* strains to level LC₉₀ application on oil palm trees. Treatment means (percent mortality ± SEM) with different letters show significant differences by Tukey’s HSD test at the *p* < 0.05 level.

4. Discussion

The use of various *Bt*-strains was effective in causing mortality, compromising survivorship, and reducing the consumption rate of the nettle caterpillar, *E. elaeasa*. The *Bt*-strains HD-1 var. *kurstaki*, SA-12 var. *kurstaki*, ABTS-1857 var. *aizawai*, and GC-91 var. *aizawai* were toxic to *E. elaeasa* caterpillars and have a strong effect through oral exposure. *Bt*-strains caused mortality in *E. elaeasa* in a concentration-dependent manner, as demonstrated in other defoliating pests [19,31,32]. *Euprosterna elaeasa* caterpillars exposed to high concentrations of *Bt*-strains displayed muscle contractions, oral or anal secretions, and consequently, septicemia. In this context, symptoms in *E. elaeasa* caterpillars were consistent with the known effects of microbial disruption of insect midgut membranes. A set of results point to the effects on the digestive system of lepidopterous pests, such as *Diatraea saccharalis* Fabricius (Crambidae) [33], *Plutella xylostella* Linnaeus (Plutellidae) [34], and *Spodoptera frugiperda* JE Smith (Noctuidae) [35], after *Bt*-strains oral exposure. In general, few *Bt*-strains are effective against *E. elaeasa* at different concentrations and reinforce their use as an alternative to organophosphate insecticides on this species.

In this study, the survival time of *E. elaeasa* decreases mainly with HD-1 var. *kurstaki* and ABTS-1857 var. *aizawai*. However, periods of *Bt*-strains, from 24 to 48 h, were necessary to induce mortality in *E. elaeasa*. Survivorship of this insect is associated with the quick action in the midgut of several Cry proteins produced by *Bt*-strains and observed in other lepidopteran pests [36,37]. In this study, the compared effects of the *Bt*-strains on *E. elaeasa* occur at various periods. These time differences occur commonly between strains of the same subspecies (e.g., *Bt* var. *kurstaki* and *Bt* var. *aizawai*) [38], by specific variation of the δ-endotoxins originating from different *Bt*-strains [39], host immune
responses [40], and virulent factors of strain types [41]. In this sense, Bt toxins have been reported to reduce or inhibit larval growth, development, or weight, interrupting the insect’s lifecycle [42]. The rapid effect against E. elaesa suggests that the insecticidal activity of Bt-strains causes detrimental effects on neonates, with appreciable population reduction during the first days of infestation and can be essential for protecting oil palm trees.

The decrease in the consumption of oil palm leaves treated with LC_{50} and LC_{90} of Bt-strains suggests an anti-feeding effect on E. elaesa, represented by different rates of intoxication and, consequently, cessation of feeding. On the other hand, the concentration-dependent effect on total food consumption by both lethal concentrations indicates that intoxication of Bt-strains is cumulative. Effects of Bt-strains after 24 h exposure on Helicoverpa armigera Hübner (Noctuidae), Phyllocnistis citrella Stainton (Gracillariidae), and Tuta absoluta Meyrick (Gelechiidae) were observed [43–45], causing a dramatic reduction in initial leaf consumption. In E. elaesa, the reduced consumption (in both lethal concentrations) exposure regimes demonstrated that intoxication has serious deleterious effects that may translate into metabolic costs associated with the repair of midgut epithelium damage in caterpillar survivors. For instance, altered permeability and damage of the midgut interfere with food uptake, affect activity enzymes associated with digestion, and influence energy metabolism. Additionally, intoxication alters hemolymph pH and suppresses the immune response [19,39,40]. Our results demonstrate that the interplay of concentration and exposure regimen can produce anti-feeding effects, indicating that Bt-strains intoxication of E. elaesa caterpillars is cumulative.

The Bt-strains, HD-1 var. kurstaki, SA-12 var. kurstaki, ABTS-1857 var. aizawai, and GC-91 var. aizawai, showed lethal effects against E. elaesa in palm trees in the field, and results were consistent with those observed in the laboratory. However, mortality level at the larval stage was lower than those obtained under laboratory conditions. Efficacy of Bt-strains in field conditions may be due to environmental factors [46], toxins degradation [47], gut microbiota competition [48], and inactivation by the target organism [49]. The lethal effect of Bt and its effectiveness was also studied in other Limacodidae pests under field conditions as a potential biocontrol agent for Acharia apicalis Dyar [50], Acharia fusca Stoll [51], and Parasa lepida Cramer [52]. The results show that Bt-strains have a specific mode of action that affects a high number of E. elaesa caterpillars. In particular, HD-1 var. kurstaki and ABTS-1857 var. aizawai are the most effective in the field, and that maximum efficiency from strains should be used during this life stage.

5. Conclusions

The insecticidal effect of four Bt-strains for controlling E. elaesa were studied. The Bt-strains HD-1 var. kurstaki, SA-12 var. kurstaki, ABTS-1857 var. aizawai, and GC-91 var. aizawai cause mortality, reduces survivorship, and an anti-feeding effect on this insect, with the potential to control its field populations. The toxicity of the bacterium may efficiently manage E. elaesa caterpillars and reduce the insect’s damage to oil palm trees and transmission of the Pestalotiopsis fungal complex. Bt-strains have lethal and sublethal effects on E. elaesa, and are an alternative to organophosphate insecticides in oil palm plantations, aiding efforts to manage insecticide resistance.

Author Contributions: A.P.-R., H.A.Q., J.E.S., and L.C.M. conceived, designed, and conducted the experiments. All authors analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by Brazilian research agencies “Conselho Nacional de Desenvolvimento Científico e Tecnológico” CNPq (grant number 305165/2013-5), “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” CAPES (grant number 2815/11), and “Fundação de Amparo a Pesquisa do Estado de Minas Gerais” FAPEMIG (grant number APQ-01079-13).

Acknowledgments: We thank Adriana Casado for technical support.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
References

1. Genty, P.; Desmier de Chenon, R.; Morin, J.P. Les ravageurs du palmier à huile en Amerique latine. *Oléagineux* **1978**, *3*, 325–419.

2. Martínez, L.C.; Plata-Rueda, A. Lepidoptera vectors of Pestalotiopsis fungal disease: First records in oil palm plantations from Colombia. *Int. J. Trop. Insect Sci.* **2013**, *33*, 239–246. [CrossRef]

3. Howard, F.W.; Giblin-Davis, R.; Moore, D.; Abad, *Insects on Palms*; Cabi: London, UK, 2001; p. 400.

4. Alvarado, H.; de la Torre, R.A.; Barbera, E.; Martínez, L.; Bustillo, A. Ciclo de vida y tasa de consumo de *Euprosterna elaeasa* Dyar (Lepidoptera: Limacodidae) defoliador de la palma de aceite. *Rev. Palmas* **2014**, *35*, 41–51.

5. Reyes, A.R.; Cruz, M.A.; Genty, P. The root absorption technique for controlling oil-palm pests. *Oléagineux*** **1988**, *43*, 363–370.

6. Martínez, O.L.; Plata-Rueda, A.; Martínez, L.C. Oil palm plantations as an agroecosystem: Impact on integrated pest management and pesticide use. *Outlooks Pest Manag.* **2013**, *24*, 225–229. [CrossRef]

7. Hernández-Lambrario, R.; Caballero-Gallardo, K.; Olivero-Verbel, J. Toxicity and antifeedant activity of essential oils from three aromatic plants grown in Colombia against *Euprosterna elaeasa* and *Acharia fusca* (Lepidoptera: Limacodidae). *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 695–700. [CrossRef]

8. Yeoh, C.B.; Kuntom, A.; Dorasamy, S.; Omar, M.R.; Nor, M.Y.M.; Noh, M.R.M. 2006. Determination of acephate, methamidophos and monocrotophos in crude palm oil. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 960–964. [CrossRef]

9. Yeoh, C.B.; Chong, C.L. Acephate, methamidophos and monocrotophos residues in a laboratory-scale oil refining process. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 593–598. [CrossRef]

10. Goulson, D. An overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* **2013**, *50*, 977–987. [CrossRef]

11. Last, J.M. Global change: Ozone depletion, greenhouse warming, and public health. *Annu. Rev. Public Health* **1993**, *14*, 115–136. [CrossRef]

12. Shipp, J.L.; Wang, K.; Ferguson, G. Residual toxicity of avermectin b1 and pyridaben to eight commercially produced beneficial arthropod species used for control of greenhouse pests. *Biol. Control** **2000**, *17*, 125–131. [CrossRef]

13. Samantsidis, G.R.; O’Reilly, A.O.; Douris, V.; Vontas, J. Functional validation of target-site resistance mutations against sodium channel blocker insecticides (SCBIs) via molecular modeling and genome engineering in *Drosophila*. *Insect Biochem. Mol. Biol.* **2019**, *104*, 73–81. [CrossRef] [PubMed]

14. Zeddám, J.L.; Cruzado, J.A.; Rodríguez, J.L.; Ravallec, M. A new nucelopolyhedrovirus from the oil-palm leaf-eater *Euprosterna elaeasa* (Lepidoptera: Limacodidae): Preliminary characterization and field assessment in Peruvian plantation. *Agric. Ecosyst. Environ.* **2003**, *96*, 69–75. [CrossRef]

15. Bakeri, S.A.; Ali, S.R.A.; Tajuddin, N.S.; Kamaruzaman, N.E. Efficacy of entomopathogenic fungi, *Paecilomyces* spp., in controlling the oil palm bagworm, *Pteroma pendula* (Joannis). *J. Oil Palm Res.* **2009**, *21*, 693–699.

16. Kamarudin, N.; Alii, S.R.A.; Masri, M.M.M.; Ahmad, M.N.; Manan, C.A.H.C.; Kamarudin, N. Controlling *Metisa plana* Walker (Lepidoptera: Psychidae) outbreak using *Bacillus thuringiensis* at an oil palm plantation in Slim River, Perak, Malaysia. *J. Oil Palm Res.* **2017**, *29*, 47–54. [CrossRef]

17. Schneef, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D.R.; Dean, D.H. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 775–806. [CrossRef]

18. Bravo, A.; Likitvivatanavong, S.; Gill, S.S.; Soberón, M. *Bacillus thuringiensis*: A story of a successful bioinsecticide. *Insect Biochem. Mol. Biol.* **2011**, *41*, 423–431. [CrossRef]

19. Castro, B.M.D.C.; Martínez, L.C.; Barbosa, S.G.; Serrão, J.E.; Wilcken, C.F.; Soares, M.A.; Silva, A.A.D.; Carvalho, A.G.D.; Zanuncio, J.C. Toxicity and cytopathology mediated by *Bacillus thuringiensis* in the midgut of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). *Sci. Rep.* **2019**, *9*, 6667. [CrossRef]

20. Armengol, G.; Escobar, M.C.; Maldonado, M.E.; Orduz, S. Diversity of Colombian strains of *Bacillus thuringiensis* with insecticidal activity against dipteran and lepidopteran insects. *J. Appl. Microbiol.* **2007**, *102*, 77–88. [CrossRef]

21. Gill, S.S.; Cowles, E.A.; Pietrantonio, P.V. The mode of action of *Bacillus thuringiensis* endotoxins. *Annu. Rev. Entomol.* **1992**, *37*, 615–634. [CrossRef]
22. Naimov, S.; Weemen-Hendriks, M.; Dukiandjiev, S.; de Maagd, R.A. *Bacillus thuringiensis* delta-endotoxin Cry1 hybrid proteins with increased activity against the Colorado potato beetle. *Appl. Environ. Microbiol.* 2001, 67, 5328–5330. [CrossRef] [PubMed]

23. Zhong, C.; Ellar, D.J.; Bishop, A.; Johnson, C.; Lin, S.; Hart, E.R. Characterization of a *Bacillus thuringiensis* δ-endotoxin which is toxic to insects in three orders. *J. Invertebr. Pathol.* 2000, 76, 131–139. [CrossRef] [PubMed]

24. da Silva Rolim, G.; Plata-Rueda, A.; Martínez, L.C.; Ribeiro, G.T.; Serrão, J.E.; Zanuncio, J.C. Side effects of *Bacillus thuringiensis* on the parasitoid *Palmistichus elaeis* (Hymenoptera: Eulophidae). *Ecotoxicol. Environ. Safe.* 2020, 189, 109978. [CrossRef] [PubMed]

25. Basri, W.M.; Ramlah, A.S.; Norman, K. Status report on the use of *Bacillus thuringiensis* in the control of some oil palm pests. *Elaeis* 1994, 6, 82–101.

26. González, G.R.; Acuña, R.S.; Moizant, R.C.; Maestre, R.B.; Quintana, A.D.; Marcano, J.F. Agricultural technology of oil palm (*Elaeis guineensis* Jacq.) and integrated management of its defoliator * Opsiphanes cassina* Felder (Lepidoptera: Brassolidae) in commercial plantations at Monagas State, Venezuela. *Rev. Cient. UDO Agr.* 2012, 12, 584–598.

27. Prasetyo, A.E.; Lopez, J.A.; Eldridge, J.R.; Zommick, D.H.; Susanto, A. Long-term study of *Bacillus thuringiensis* application to control *Tirathaba rufiova*, along with the impact to *Elacidobius kamericanus*, insect biodiversity and oil palm productivity. *J. Oil Palm Res.* 2018, 30, 71–82.

28. Xiao, Y.; Wu, K. Recent progress on the interaction between insects and *Bacillus thuringiensis* crops. *Philos. T. R. Soc. B* 2019, 374, 20180316. [CrossRef]

29. Martínez, L.C.; Plata-Rueda, A.; Zanuncio, J.C.; Serrão, J.E. *Leucothryeus femoratus* (Coleoptera: Scarabaeidae): Feeding and behavioral activities as an oil palm defoliator. *Fla. Entomol.* 2013, 96, 55–63. [CrossRef]

30. Finney, D.J. *Probit Analysis*; Cambridge University Press: Cambridge, UK, 1964; p. 333.

31. Berretta, M.F.; Pedarros, A.S.; Sauka, D.H.; Perez, M.P.; Onco, M.I.; Benintende, G.B. Susceptibility of agricultural pests of regional importance in South America to a *Bacillus thuringiensis* Cry1Aa protein. *J. Invertebr. Pathol.* 2020, 107,354. [CrossRef]

32. Chakrabarty, S.; Jin, M.; Wu, C.; Chakraborthy, P.; Xiao, Y. *Bacillus thuringiensis* vegetative insecticidal protein family Vip3A and mode of action against pest lepidopteran. *Pest Manage. Sci.* 2020, 76, 1612–1617. [CrossRef]

33. Daquila, B.V.; Scudeler, E.L.; Dossi, F.C.A.; Moreira, D.R.; Famphile, J.A.; Conte, H. Action of *Bacillus thuringiensis* (Bacillales: Bacillaceae) in the midgut of the sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae). *Ecotoxicol. Environ. Safe.* 2019, 184, 109642. [CrossRef] [PubMed]

34. Santos, M.S.; Dias, N.P.; Costa, L.L.; De Bortoli, C.P.; Souza, E.H.; Santos, A.C.F.; De Bortoli, S.A.; Polanczyk, R.A. Interactions of *Bacillus thuringiensis* strains for *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) susceptibility. *J. Invertebr. Pathol.* 2019, 168, 107255. [CrossRef] [PubMed]

35. Del Valle Loto, F.; Carrizo, A.E.; Romero, C.M.; Baigori, M.D.; Pera, L.M. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from northern Argentina: Esterases, profiles, and susceptibility to *Bacillus thuringiensis* (Bacillales: Bacillaceae) in the midgut of the sugarcane borer *Diatraea saccharalis*. *Fla. Entomol.* 2019, 102, 347–352. [CrossRef]

36. Venette, R.C.; Luhman, J.C.; Hutchison, W.D. Survivorship of field-collected European corn borer (Lepidoptera: Crambidae) larvae and its impact on estimates of resistance to *Bacillus thuringiensis* Berliner. *J. Entomol. Sci.* 2000, 35, 208–212. [CrossRef]

37. Bommireddy, P.L.; Leonard, B.R. Survivorship of *Helicoverpa zea* and *Heliotris virescens* on cotton plant structures expressing a *Bacillus thuringiensis* vegetative insecticidal protein. *J. Econ. Entomol.* 2008, 101, 1244–1252. [CrossRef] [PubMed]

38. Mashitoly, T.A.; Abolmaaty, A.; El-Said El-Zemaitry, M.; Hussien, M.I.; Alm, S.R. Enhanced toxicity of *Bacillus thuringiensis* subspecies kurstaki and aizawai to black cutworm larvae (Lepidoptera: Noctuidae) with *Bacillus* sp. NF2 and *Pseudomonas* sp. FNFD1. *J. Econ. Entomol.* 2011, 104, 41–46. [CrossRef]

39. Liao, C.; Brooks, L.; Trouwlie, K.C.; Akhurst, R.J. Binding of Cry δ-endotoxins to brush border membrane vesicles of *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). *Insect Sci.* 2005, 12, 231–240. [CrossRef]

40. Rahman, M.M.; Roberts, H.L.; Schmidt, O. Tolerance to *Bacillus thuringiensis* endotoxin in immune-suppressed larvae of the flour moth *Ephestia kuehniella*. *J. Invertebr. Pathol.* 2007, 96, 125–132. [CrossRef]
41. Kim, M.J.; Han, J.K.; Park, J.S.; Lee, J.S.; Cho, J.I.; Kim, K.S. Various enterotoxin and other virulence factor genes widespread among Bacillus cereus and Bacillus thuringiensis strains. *J. Microbiol. Biotechnol.* **2015**, *25*, 872–879. [CrossRef]

42. Prodhan, M.; Haider, Z.; Shirale, D.K.; Islam, M.; Hossain, M.; Paranjepe, V.; Shelton, A.M. Susceptibility of field populations of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenée) to Cry1Ac, the protein expressed in Bt eggplant (*Solanum melongena* L.) in Bangladesh. *Insects* **2019**, *10*, 198. [CrossRef]

43. Singh, G.; Rup, P.J.; Koul, O. Acute, sublethal and combination effects of azadirachtin and *Bacillus thuringiensis* toxins on *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae. *Bull. Entomol. Res.* **2007**, *97*, 351–357. [CrossRef] [PubMed]

44. Amiri-BeSheli, B. Efficacy of *Bacillus thuringiensis*, mineral oil, insecticidal emulsion and insecticidal gel against *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *Plant Prot. Sci.* **2008**, *44*, 68–73. [CrossRef] [PubMed]

45. Amizadeh, M.; Hejazi, M.J.; Niknam, G.; Arzanlou, M. Compatibility and interaction between *Bacillus thuringiensis* and certain insecticides: Perspective in management of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biocontrol Sci. Technol.* **2015**, *25*, 671–684. [CrossRef]

46. Hilbeck, A.; Otto, M. Specificity and combinatorial effects of *Bacillus thuringiensis* Cry toxins in the context of GMO environmental risk assessment. *Frontiers Environ. Sci.* **2015**, *3*, 71. [CrossRef]

47. Pang, A.S.; Gringorten, J.L. Degradation of *Bacillus thuringiensis* δ-endotoxin in host insect gut juice. *FEMS Microbiol. Lett.* **1998**, *167*, 281–285. [CrossRef]

48. Broderick, N.A.; Robinson, C.J.; McMahon, M.D.; Holt, J.; Handelsman, J.; Raffa, K.F. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biol.* **2009**, *7*, 11. [CrossRef]

49. Miranda, R.; Zamudio, F.Z.; Bravo, A. Processing of Cry1Ab δ-endotoxin from *Bacillus thuringiensis* by *Manduca sexta* and *Spodoptera frugiperda* midgut proteases: Role in protoxin activation and toxin inactivation. *Insect Biochem. Mol. Biol.* **2001**, *31*, 1155–1163. [CrossRef]

50. Jaramillo-Celis, R.; Jiménez-Lacharme, F.; Hidalgo-Salvatierra, O. Susceptibility of the larvae of *Sibine apicalis* (Dyar) to *Bacillus thuringiensis* var. *kurstaki*. *Turrialba* **1974**, *24*, 106–107.

51. Martínez, L.C.; Plata-Rueda, A.; Serrao, J.E.; Zanuncio, J.C. Life history traits and damage potential of an invasive pest *Acharia fusca* (Lepidoptera: Limacodidae) on oil palm. *Ann. Entomol. Soc. Am.* **2014**, *107*, 1086–1093. [CrossRef]

52. Arumugam, G.; Karuppiah, H.; Seeramulu, B.; Pauchamy, R.; Sundaram, J. Occurrence of natural lectin with bacterial agglutination property in the serum of lepidopteran pest, *Parasa lepida*. *Entomol. Sci.* **2019**, *22*, 239–249. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).