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Proceeding Paper

Susceptibility of *Xylotrechus arvicola* (Coleoptera: Cerambycidae) to Five Cry Toxins †

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Abstract: The beetle *Xylotrechus arvicola* is a significant pest in vineyards (*Vitis vinifera*) in the wine-producing regions of the Iberian Peninsula. *X. arvicola* larvae bore the grapevine wood and make galleries, which cause structural damage to the plant and a decrease in the quality and quantity of its production. The susceptibility of *X. arvicola* larvae to five coleopteran toxic Cry proteins (Cry1B, Cry1I, Cry3A, Cry7A, and Cry23/37) was evaluated under laboratory conditions. After 30 days, Cry proteins showed larvicidal activity against *X. arvicola*, with mortality rates over 50%, with the proteins Cry1Ba and Cry7Ab being the most aggressive, with mortality rates over 80%. The evaluated Cry proteins can be applied in the environmentally friendly control of *X. arvicola* larvae since they are able to kill them. The larval stage tested is prior to drilling into the plant, which makes spray treatments feasible. The results can help in the design of combinations of Cry proteins as biopesticides to be applied by the time these larvae hatch to increase vine wood protection.

Keywords: vineyards; insect pest; Xylophagous polyphagous; *Bacillus thuringiensis*; crystal proteins

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1. Introduction

Xylotrechus arvicola Olivier (Coleoptera: Cerambycidae) is a significant grape pest (*Vitis vinifera*) in the Iberian wine-producing regions [1]. *X. arvico*la females lay the eggs on crevices or under the rhytidome of the vine [2]. The hatched larvae enter the stems, producing galleries due to their feeding. In about 2 years, they pupate, resulting in the emergence of adults in approximately one month. The larvae cause structural damage to the stems and lead to the spread of fungi within the wood [3]. They can only be controlled with chemically synthesized systemic pesticides [4], which are legally complicated to use in vineyards.

The controlled evaluation of pesticides against this insect species is challenging, as the conditions for a laboratory rearing that fulfills the biological cycle have not yet been established. However, adult insects can be captured in the fields, and larvae can be maintained some time on a semisynthetic diet. In this way, insecticides with different modes of action have been evaluated on both stages [4], but active substances with a low environmental impact are still needed.

The most successful pesticide products in organic farming are based on *Bacillus thuringiensis* (Bt), a bacterium that produces pesticidal crystal proteins (Cry proteins). Each Cry protein has a narrow insect toxicity spectrum, and the most studied are proteins with lepidopteran species as a target. However, several Cry proteins were reported to be toxic

to a few coleopteran species or to have activity against both orders [5]. Still, most of the genera within the Coleoptera order have not yet been evaluated with pest species [6].

The aim of this research was to evaluate, under laboratory conditions and for the first time, the toxicological potential of different Cry proteins with known coleopteran activity against a larval stage of the coleopteran cerambycid *X. arvicola*.

2. Experiments

2.1. X. arvicola Collection and Rearing

The protocol followed was adapted from a previous study evaluating insecticide activity against *X. arvicola* adults: insect adults were captured using the Crosstrap® interception traps (Econex, Siscar, Murcia, Spain) [7] in vineyards located in Gordoncillo (León, Spain). The captured insects were paired and put into glass jars, then allowed to mate. The base was covered with filter paper. The insects had access to substrates for oviposition and bowls for drinking. Laid eggs were taken out and put into Petri dishes. *X. arvicola* neonate larvae used in the tests were obtained from these eggs. Adults and larvae, before and after the application of treatments, were kept in a chamber with controlled temperature (24 ± 1 °C), humidity (60 ± 5%), and a photoperiod of 16:8 (light:darkness).

2.2. Cry Proteins

The Cry1Ba, Cry1Ia, Cr3Aa, Cry7Ab, and Cry23/37 proteins were prepared as reported by Rodríguez-González et al. [8]. In sum, they were obtained from recombinant strains of *B. thuringiensis* and *Escherichia coli* that produced a single protein. The bacteria were washed and lysed in a Carbonate buffer. The quantity and quality of the Cry protein in preparations was evaluated by 12% SDS-PAGE analysis. Each solution was lyophilized to powder for storage.

2.3. Bioassays of Cry Proteins on Artificial Diet to X. Arvicola Larvae

Bioassays were carried out using newly *X. arvicola* neonate larvae (≤24 h) by the surface contamination method [9]. An artificial diet was used to fill 12-well bioassay trays (2 cm^2/well) (Greiner CELLSTAR® 12 well plates, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The diet was surface-sterilized for 10 min under UV light. Each well was inoculated with 100 µL of the protein solution, obtained after suspending the lyophilized powder distilled water at a concentration of 100 µg/mL and allowing it to dry under a laminar flow hood. Once dried, each well contained approximately 1 µg/cm^2 of Cry protein, and one larva was transferred to each well and confined with a lid. Three replicates of 12-well plates (36 larvae in total) were used per treatment. Larval mortality was rated within 30 days. The larvae were considered dead if they did not react when prodded. A diet inoculated with 100 µL of Na_2CO_3 50 mM, pH 10.5 buffer was used as the control treatment.

2.4. Statistical Analysis

Recorded mortalities were corrected with Abbott's formula [10] for each treatment. These data were used to calculate the means and standard error of the means (SEM) for the mortality values observed for each Bt Cry protein treatment.

Mortality rates from the five Cry treatments were put through one-way ANOVA. Differences among the treatments were examined by mean comparisons using the post-hoc Least Significant Difference (LSD) comparison test, considering a p value ≤ 0.05 as statistically significant. Statistical analyses were performed using the SPSS version 26 software (IBM, 1968, Armonk, NY, USA) (SPSS).

3. Results

A conventional bioassay was set up to evaluate *X. arvicola* larval susceptibility to Cry proteins. It provided positive results, since treatment mortality was higher than the control (one-way ANOVA test, F = 2.097; df = 5, 66; $p = 0.043$) and provided small errors

(Figure 1). All protein treatments showed statistically significant differences in mortality rates, except Cry1Ba and Cry7Ab treatments, which showed similar mortality rates with the best larvicidal efficacy (killed 83% of treated larvae). Cry1Ia and Cry23/37 rendered intermediate larval mortality rates, with Cry1Ia showing higher rates. Cry3Aa (killed 50% of larvae) was the protein with the lowest mortality rate.

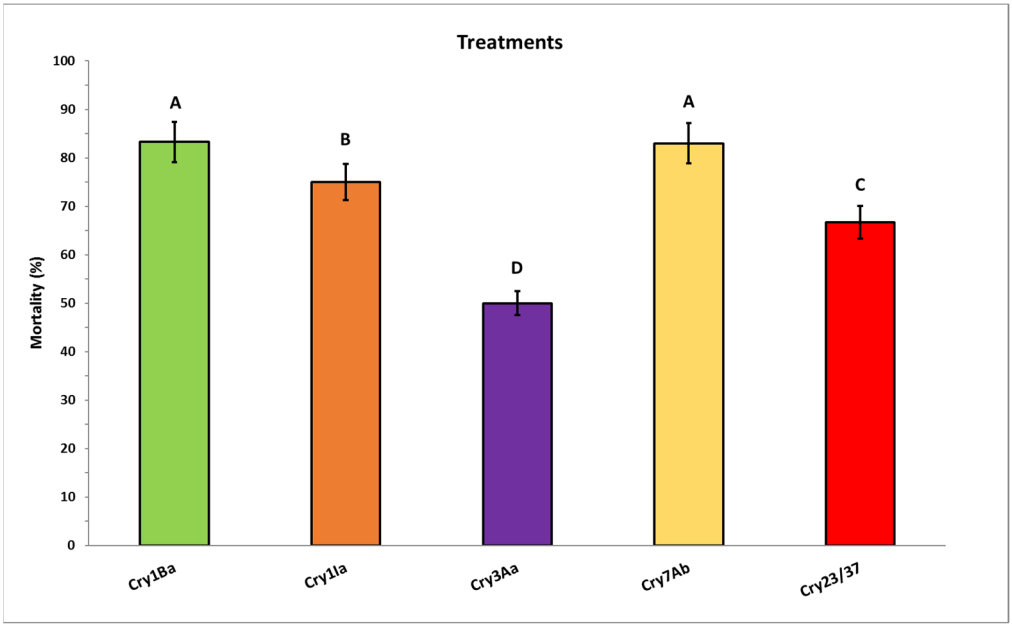

Figure 1. Corrected mortality (% ± SE) of *X. arvicola* neonate larvae exposure to 1 μg/cm^2 of Cry proteins applied over artificial diet. The Abbott's formula was used for correction. Different capital letters on the bars indicate statistically significant differences (LDS post-test) among the mortality rates.

4. Discussion

The evaluation of insecticide active substances against coleopteran pests with a long and cryptic biological cycle is a challenge. The laboratory tests are the initial steps used in evaluation, but are arduous to precisely set up. We successfully applied the bioassay protocol used on *X. arvicola* larvae with other pesticides [11]. However, the accuracy of the results could be debated, since some toxicological effects do not depend on the Cry proteins, which can degrade over a long treatment period. Nevertheless, we can assess the results, since we assume the generally accepted fact that the main insect toxicity effect of Cry preparation relies on the Cry proteins in the sample, and, with the reported data, we at least have preliminary toxic information.

The evaluated cry proteins belong to very different classes and show high activity for *X. arvicola* larvae, ranging from 50% to 83% mortality after 30 days of evaluation. Chen et al. [12] reported similar results, showing the toxic effect of the Bt strain Bt866 (with a *cry3Aa* gene) against two cerambycid species, *Apriona germari* and *Anoplophora glabripennis*.

Our current studies suggested that Cry proteins may minimize the damage caused by the *X. arvicola* larvae. It may be beneficial to develop these proteins as a bio-insecticide and apply them to vineyards during the emergence of *X. arvicola* adults between June and July in the wine-producing regions with PDO [13].

5. Conclusions

The Cry proteins evaluated have demonstrated different rates of toxicity activity against the assayed insect pests, with mortality rates over 50% in all cases. Cry1Ba and Cry7Ab showed the most aggressive responses. The tested larval stage is prior to drilling in the plant, which makes spray treatments feasible. The results can help in the design

of combinations of Cry proteins for use as biopesticides, to be applied by the time these larvae hatch, to increase vine wood protection.

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECPS2020-08821/s1.

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