

# Susceptibility of *Xylotrechus arvicola* (Coleoptera: Cerambycidae) to five Cry toxins

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## Introduction

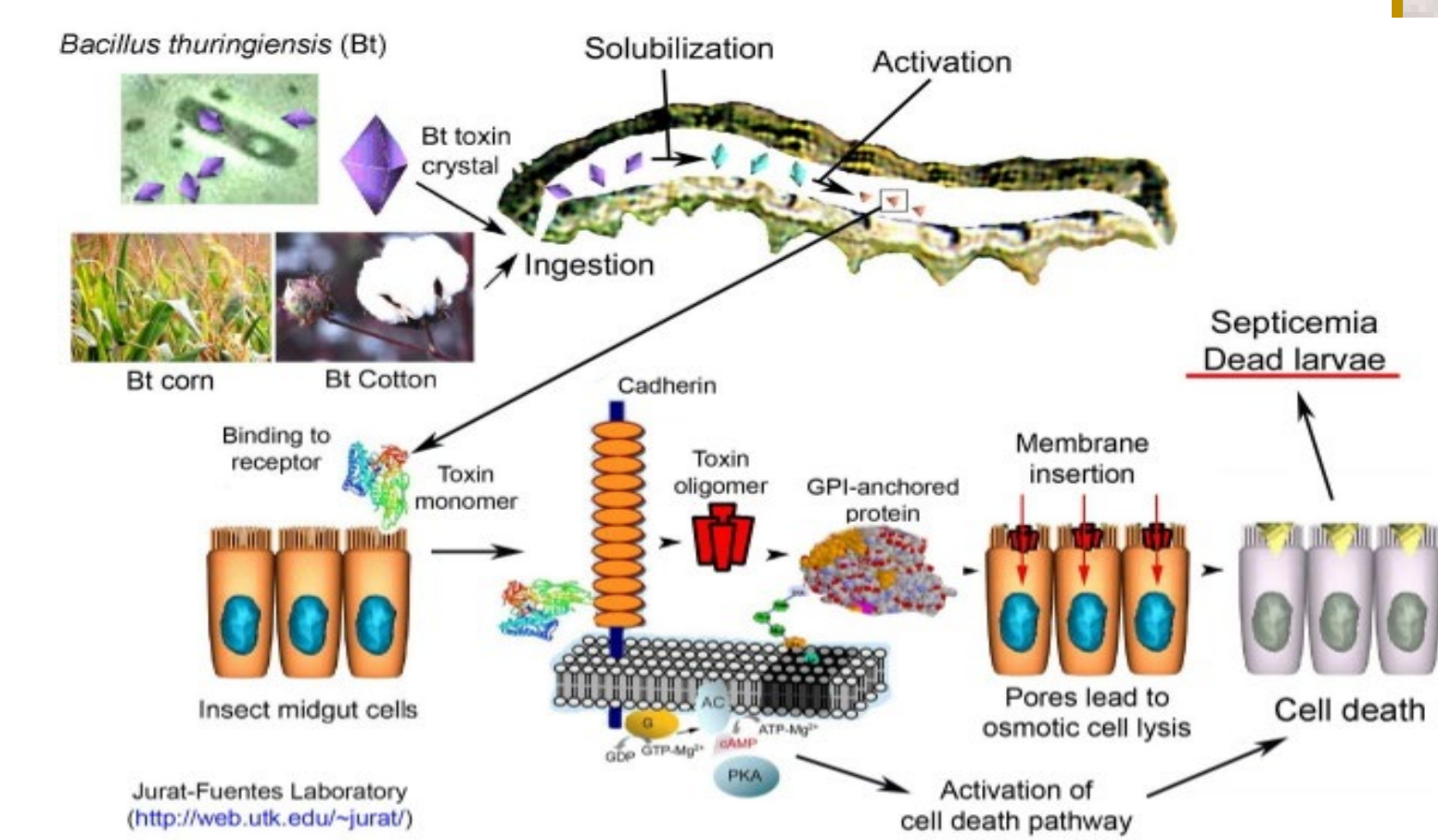
*Xylotrechus arvicola* is a significant pest in vineyards (*Vitis vinifera*) in the main wine-producing regions of Iberian Peninsula.



*X. arvicola* larvae bore the grapevine wood and make galleries, which cause structural damages to the plant and a decrease in the quality and quantity of its production. Cry toxins that exhibited toxicity against coleopteran larvae may be an alternative for its control.



## Cry proteins mode of action



## Materials and Methods

### Insect pest collections and *X. arvicola* rearing

**Vineyards:** 1. Insects captured with CROSSTRAP®

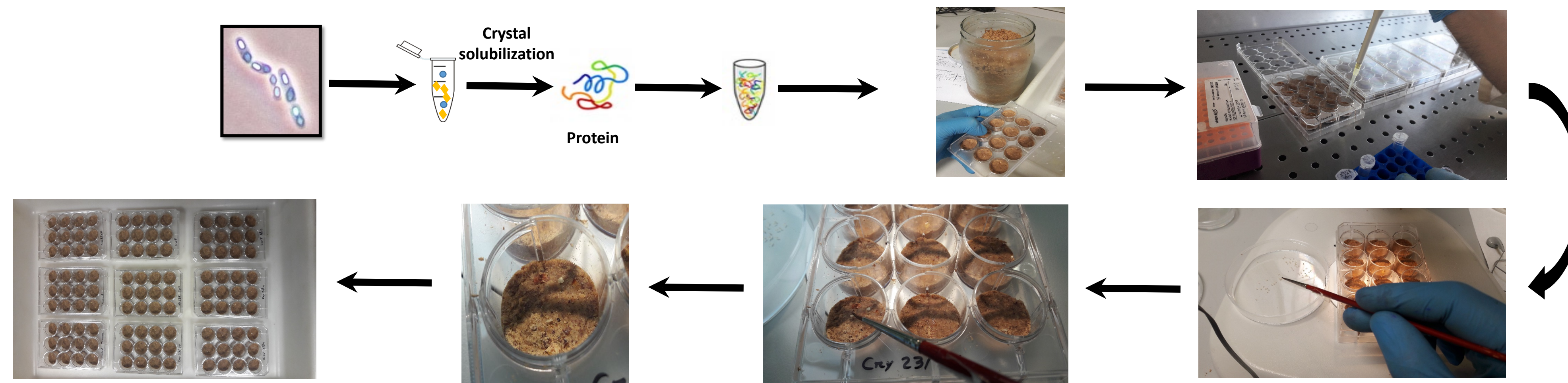


**Laboratory:** 2. Insect mate, lay eggs on substrates for oviposition, and neonate larvae are collected in a Petri dish



### Test Toxic Activity

**Single dose bioassays:** 1. Surface contamination method over semi synthetic diet and introduction of *X. arvicola* neonate larvae

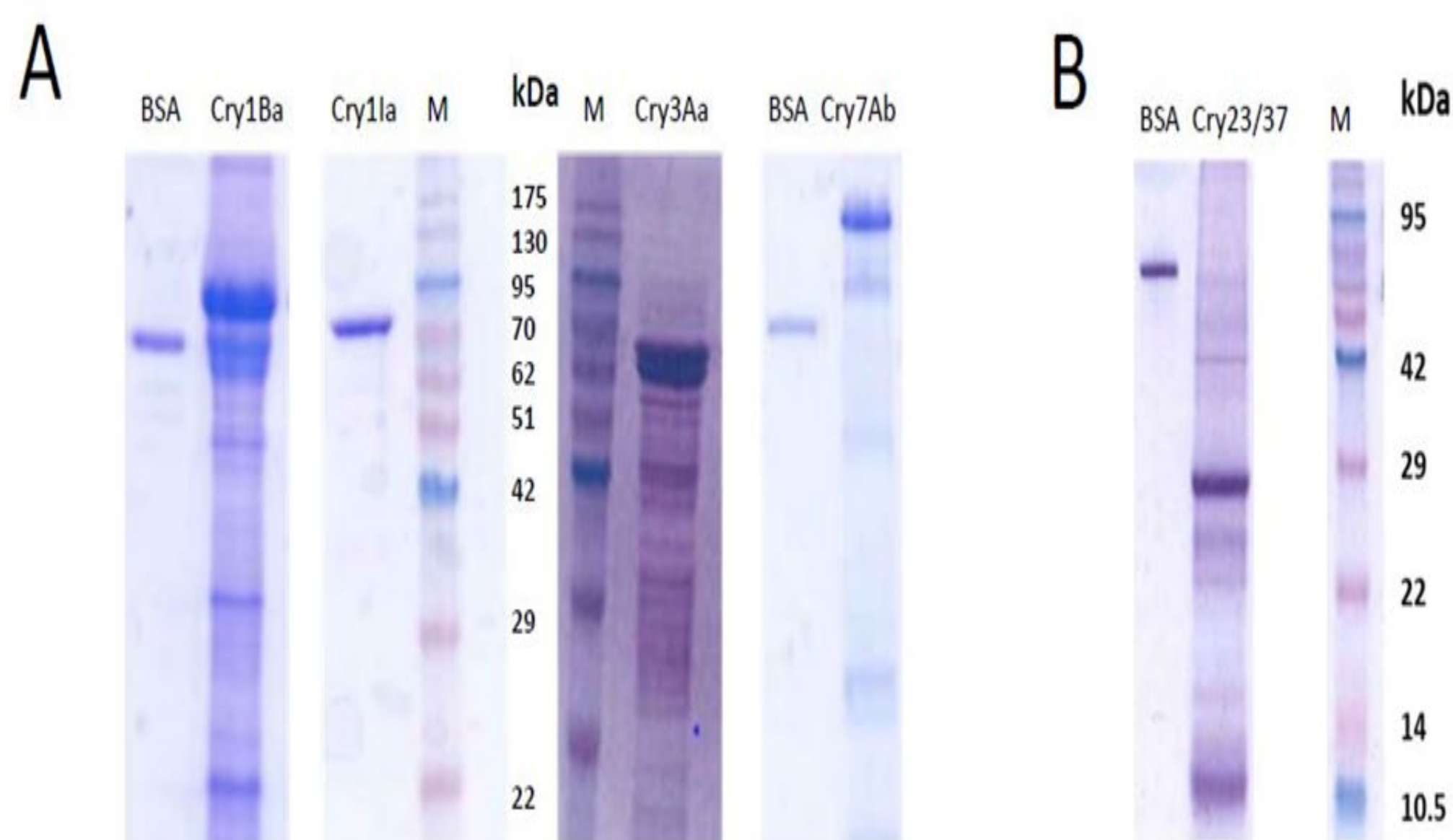


### Cry proteins evaluated

Protein	Abbreviation	Strain	Source	Solubilization buffer
Cry1Ba1	Cry1B	Bt (EG11916)	Eogen Inc., Langhorne, USA	Na <sub>2</sub> CO <sub>3</sub> : 50 mM, pH 10.5
Cry1Ia7	Cry1Ia	<i>E. coli</i>	Universidad Pública de Navarra, Spain	Na <sub>2</sub> CO <sub>3</sub> : 50 mM, pH 12
Cry3Aa1	Cry3Aa	<i>E. coli</i> (4AA1)	Bacillus Genetic Stock Collection, Columbus, USA	Na <sub>2</sub> CO <sub>3</sub> : 50 mM, pH 12
Cry7Ab2	Cry7Ab	Bt (HD867)	Bacillus Genetic Stock Collection, Columbus, USA	Na <sub>2</sub> CO <sub>3</sub> : 50 mM, pH 10.5
Cry23Aa/Cry37Aa	Cry23/37	Bt (EG10327)	Agricultural Research Culture Collection, NRRL, USA	Na <sub>2</sub> CO <sub>3</sub> : 50 mM, NaCl 100 mM, pH 11.3

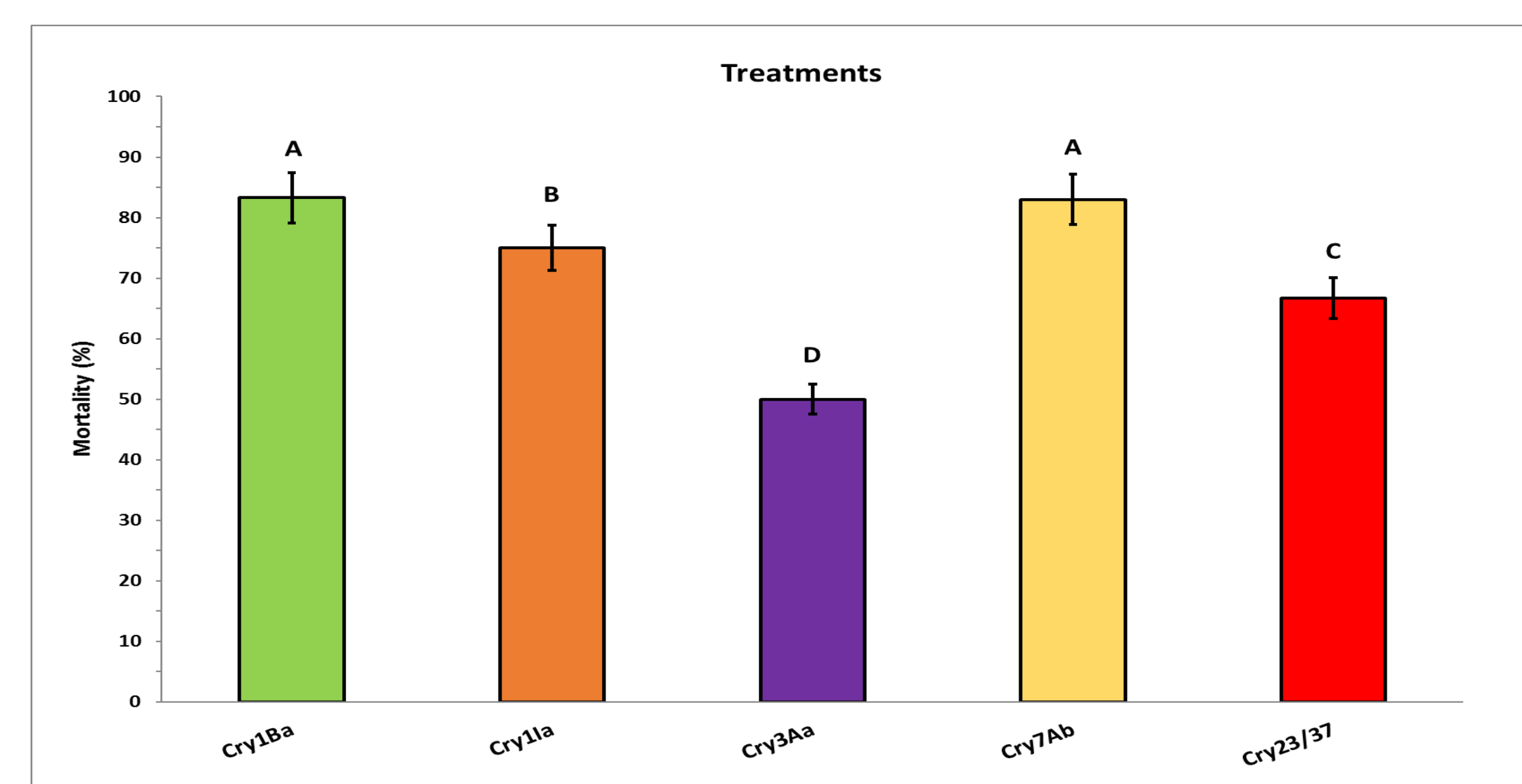
## Results and discussion

### Protein Profile



Analysis of expression of strains that produced a single Cry protein (A), and the Bt EG10327 strain which produced Cry23Aa (about 30 kDa) and Cry37Aa (about 15 kDa) (B). BSA: 750 ng bovine serum albumin (BSA). M: molecular mass marker (Pink pre-stained protein ladder Pink B4MWP02, Nippon genetics).

### Toxic Activity



Corrected mortality (% ± SE) of *X. arvicola* neonate larvae exposure to 1 mg/cm<sup>2</sup> of Cry proteins applied over an artificial diet. Abbott's formula was used for correction. Different letters on the bars indicate statistically significant differences (LDS test) among the mortality rates.

➤ The evaluation of insecticide active substances against coleopteran pests with a long and cryptic biological cycle is a challenge.

➤ We have successfully applied the bioassay protocol used on *X. arvicola* larvae with other pesticides.

➤ However, the accuracy of the results could be discussed, since some toxicological effects do not depend on the Cry proteins which can be degraded in 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 have, at least, preliminary toxic information.

## Acknowledgments

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## Conclusions

The Cry proteins evaluated have demonstrated different toxicity activity against the insect pests assayed, with over 50% mortality rates in all cases. Cry1Ba and Cry7Ab showed the most aggressive responses.

The larval stage tested is previous to drilling in the plant, which makes spray treatments feasible.

The results can help in designing combinations of Cry proteins as biopesticides to apply them by the time these larvae hatch to increase vine wood protection.