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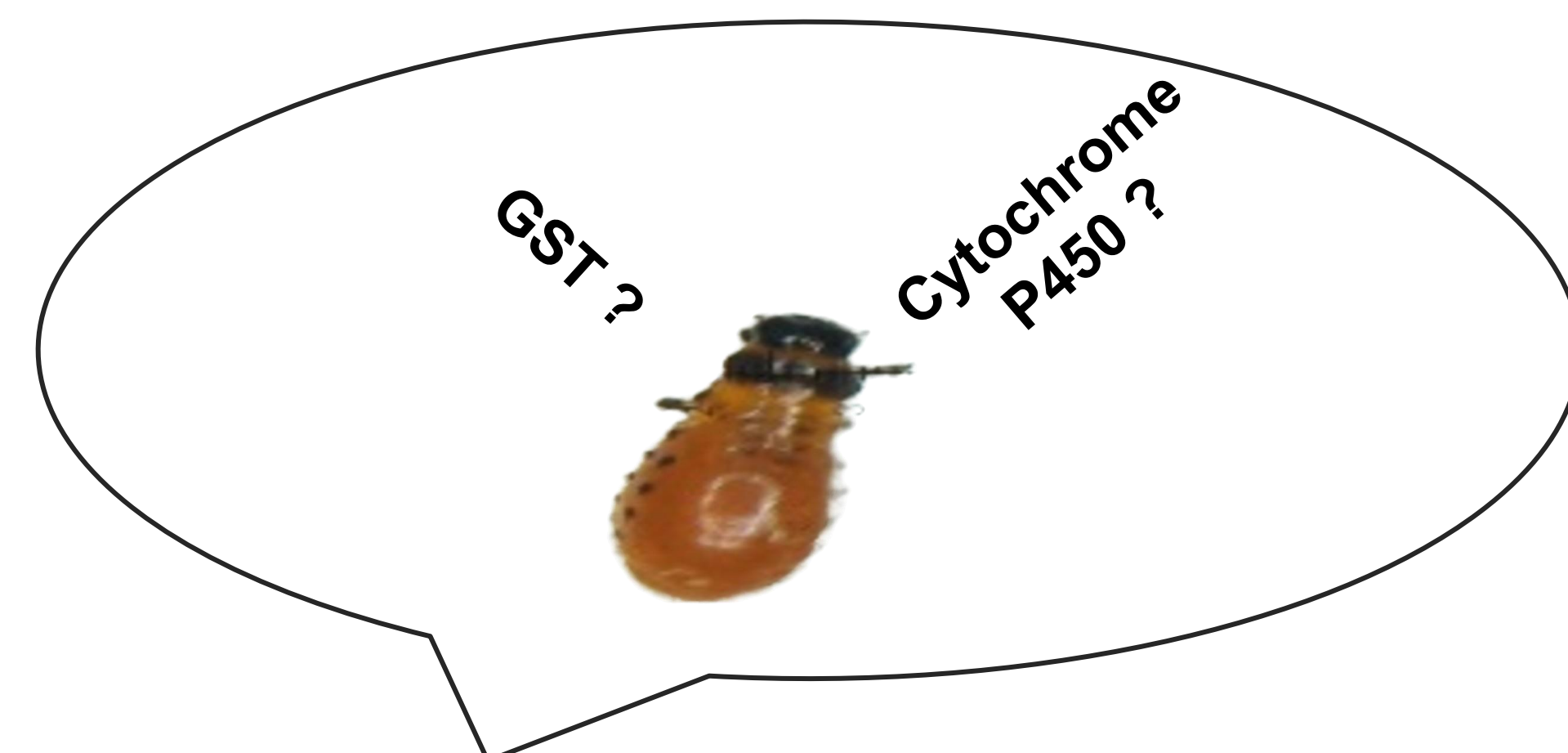
Abstract

Cultivation of potatoes has come under considerable threat from insects such as the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*. Multiple approaches have been implemented to control this pest, but it has developed resistance to most insecticides available on the market. The aim of this study was to deepen our understanding of the molecular mechanisms underlying this resistance towards two insecticides, cyantraniliprole and thiamethoxam, in *L. decemlineata* larvae. To achieve this objective, molecular targets linked to detoxification processes, such as seven cytochromes P450 (CYP) and three glutathione S-transferases (GST), were measured using qRT-PCR in *L. decemlineata* larvae. Multiple changes in transcript levels were noted following the different conditions assessed. Overexpression of CYP6a13 (5.8-fold) transcript levels was observed following thiamethoxam exposure in a larvae population that exhibited resistance to the compounds investigated. A 31.1-fold increase was in addition observed in transcript levels for the same target in thiamethoxam-resistant *L. decemlineata* larvae when compared to larvae susceptible to the same compound. It is also interesting that several transcripts coding for GSTs were elevated following treatments to both compounds in a larvae population displaying susceptibility to both pesticides: GST (15.6-fold for thiamethoxam and 10.7-fold for cyantraniliprole), GST1 (36.6-fold for thiamethoxam and 34.1-fold for cyantraniliprole) and GST1-Like (8.3-fold for thiamethoxam and 9.7-fold for cyantraniliprole). These results set the stage for subsequent RNA interference approaches directed against the overexpressed targets to evaluate their impact against insecticide resistance in this potato pest.

Objective

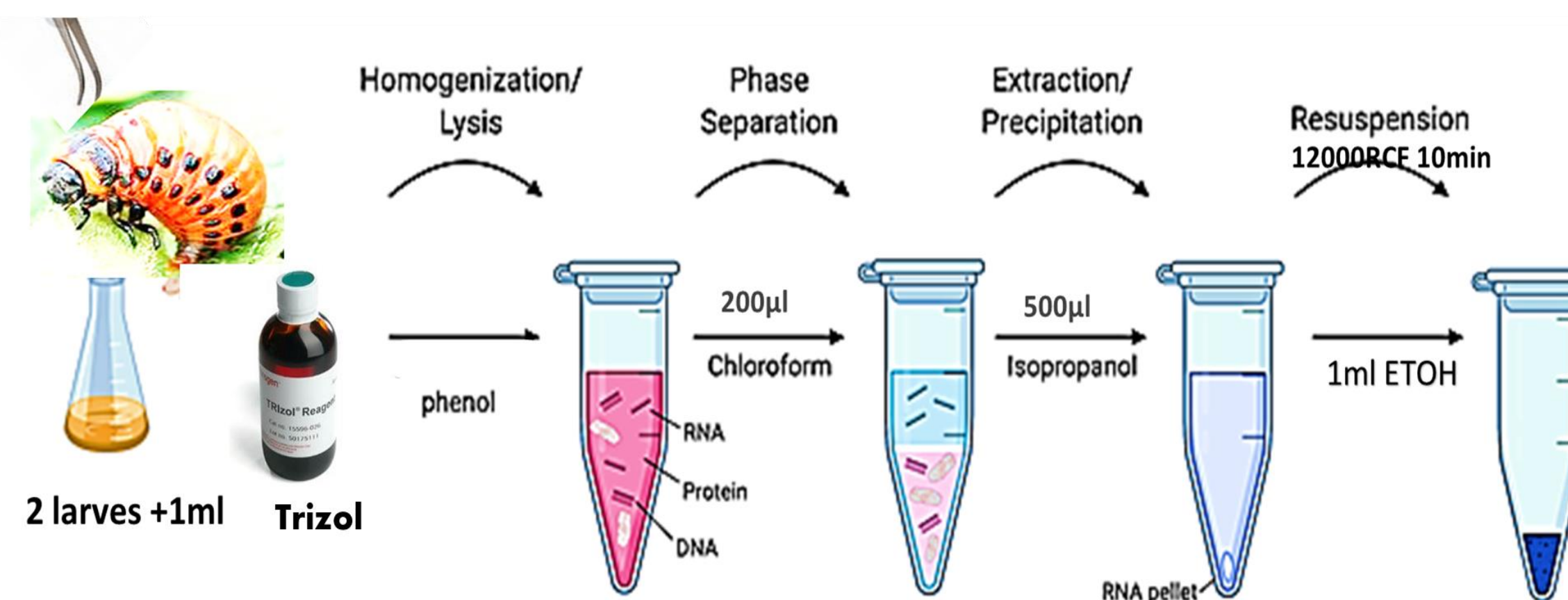
This study was conducted to:

- Determine the expression profile of transcripts coding for certain **cytochromes P450** with a potential involvement for resistance in *L. decemlineata* larvae treated with cyantraniliprole or thiamethoxam.
- Characterize the expression profile of transcripts coding for select **glutathione S-transferases** with a possible role for resistance in *L. decemlineata* larvae exposed with cyantraniliprole or thiamethoxam.
- Explore the signature of these transcripts in *L. decemlineata* larvae populations that exhibit differences in cyantraniliprole or thiamethoxam susceptibility.

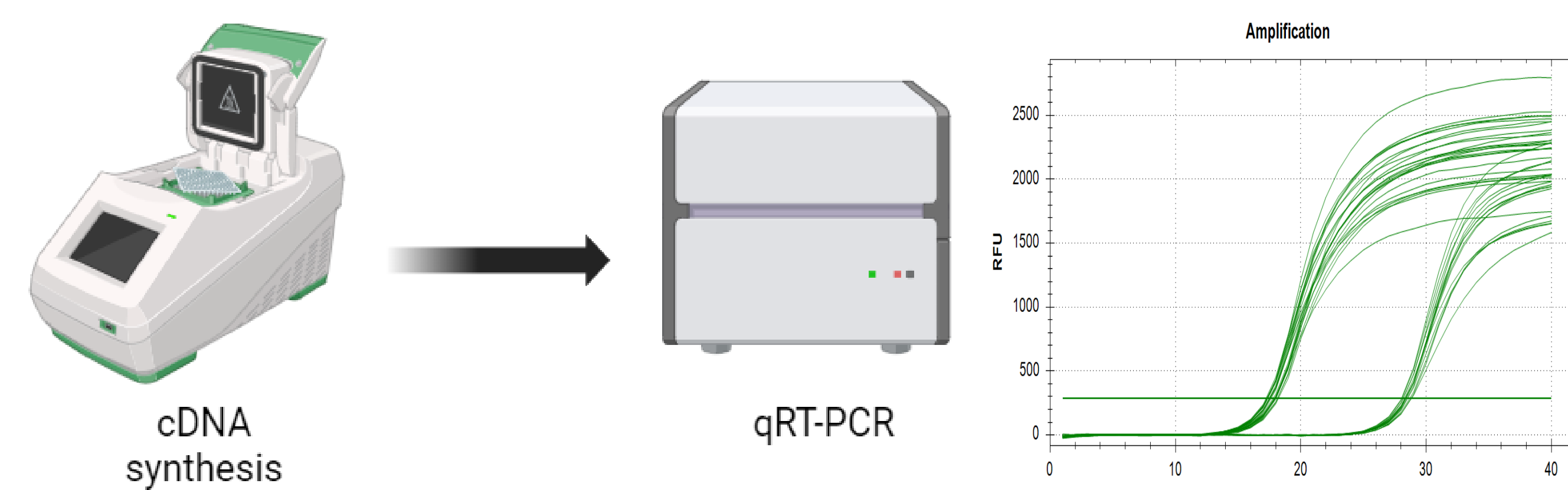


Methods

RNA isolation :

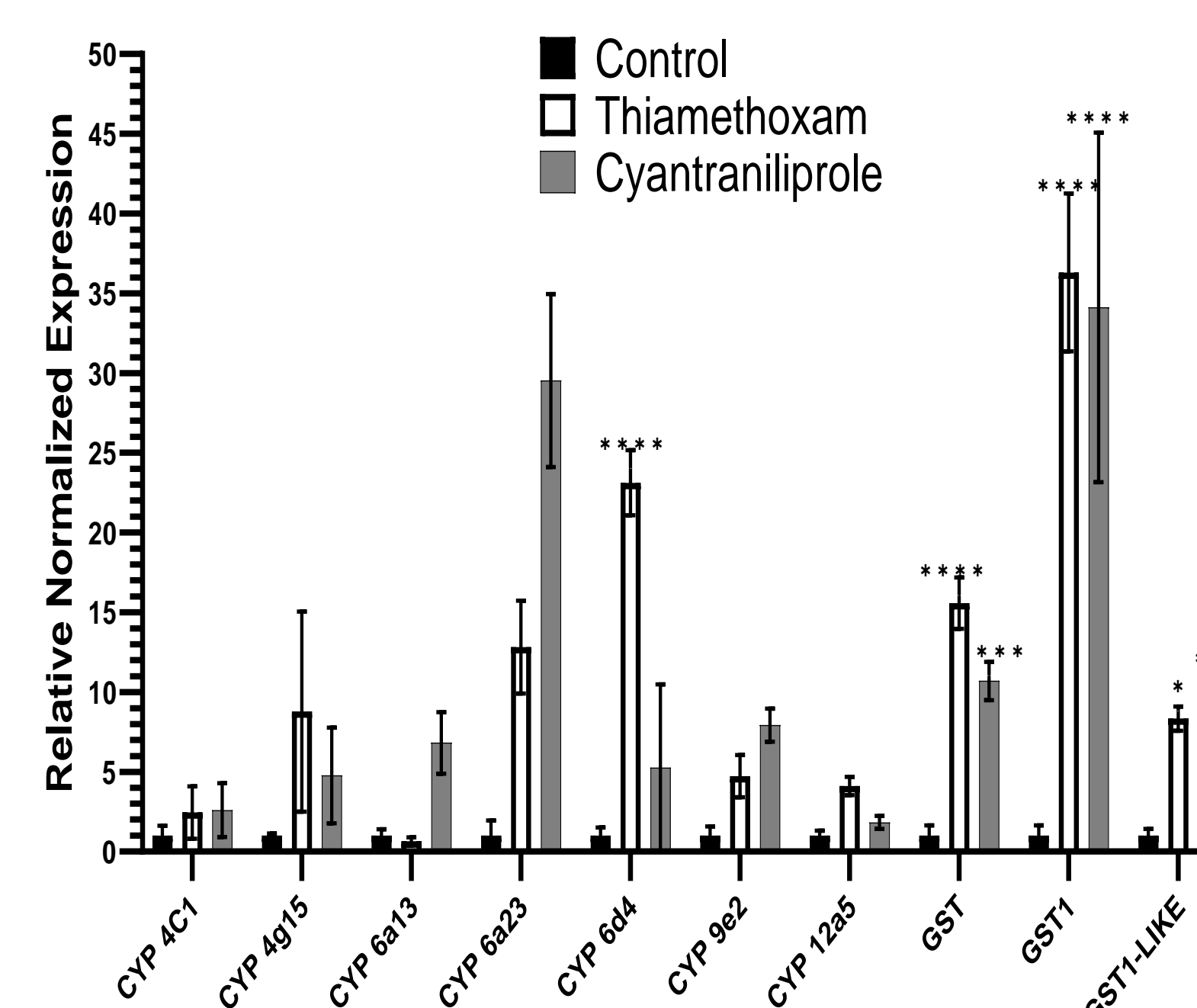


cADN synthesis & quantification of transcript levels using qRT-PCR:



Results

Fig.1: Transcript expression in a CPB larvae population exposed to thiamethoxam or cyantraniliprole deemed susceptible to these compounds



Overexpression of :

- **CYP6d4** (23.1-fold), **GST** (15.6-fold), **GST1** (36.3-fold) and **GST1-like** (8.3-fold) upon thiamethoxam treatments.
- **GST** (10.7-fold), **GST1** (34.1-fold) and **GST1-like** (9.7-fold) in CPB sensitive to cyantraniliprole

Fig.2: Transcript expression in a CPB population exposed to thiamethoxam or cyantraniliprole deemed resistant to these compounds

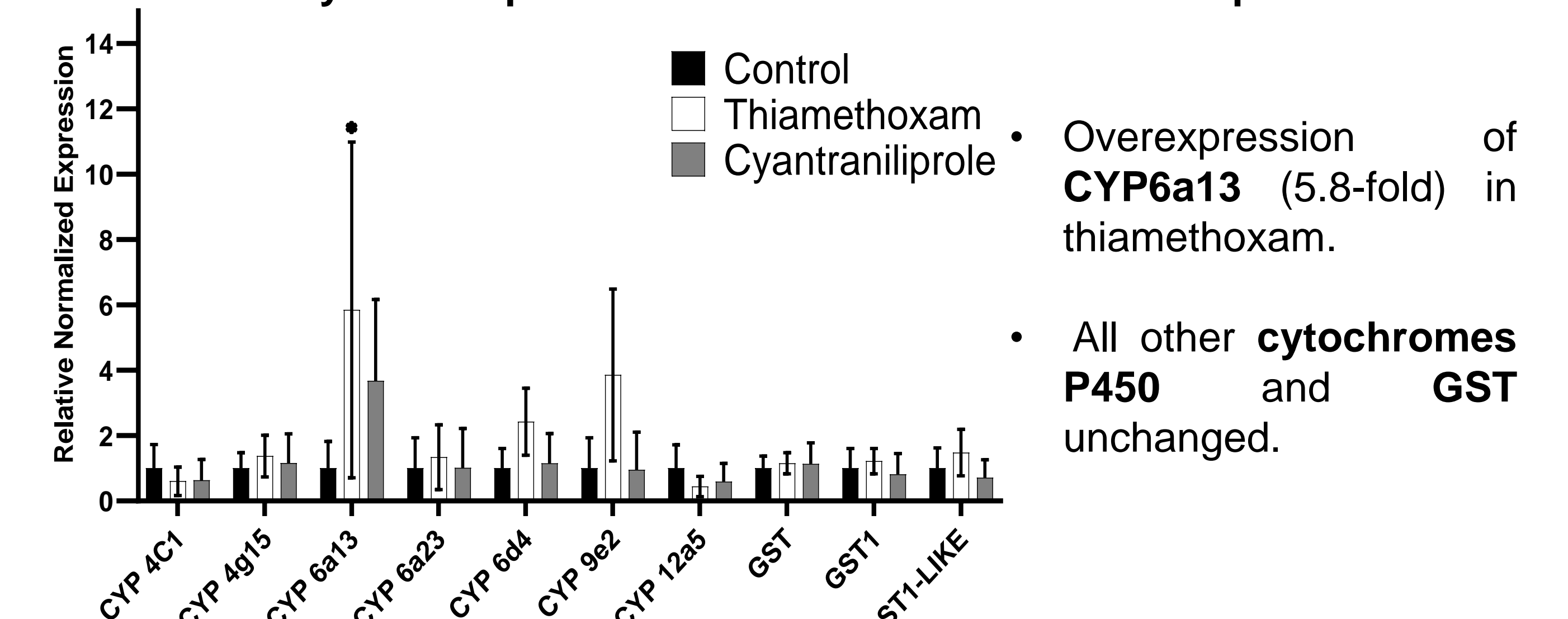
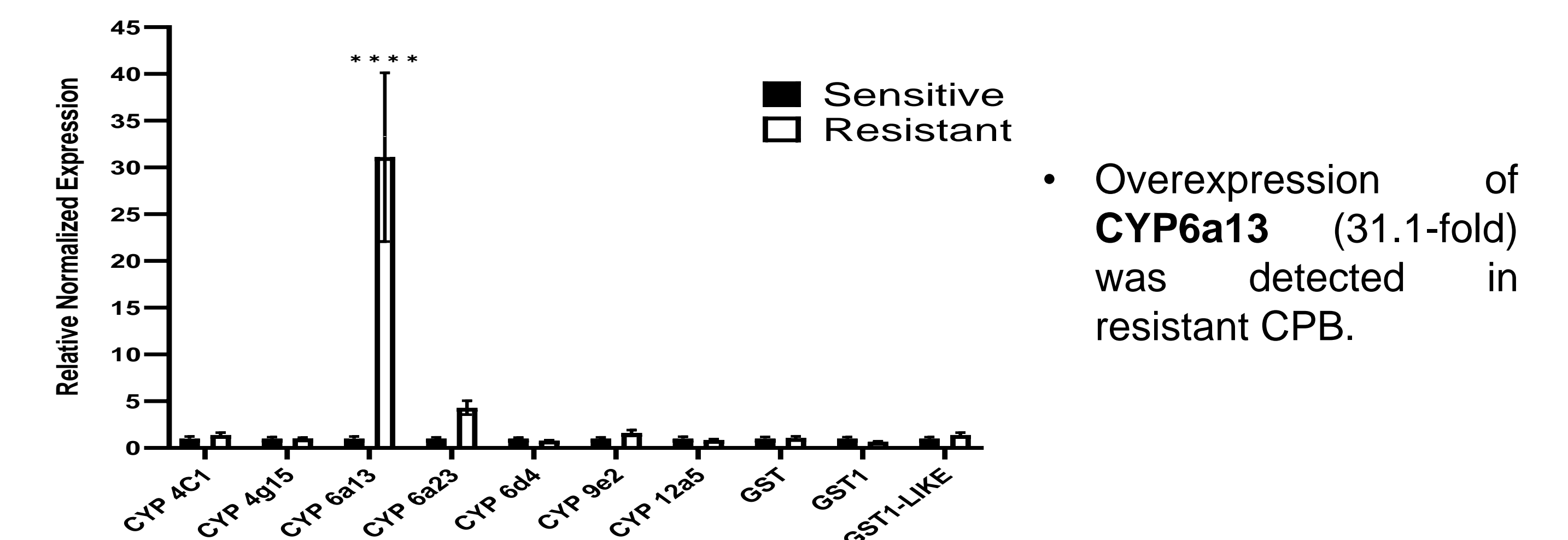


Fig.3: Transcript expression in CPB larvae treated with thiamethoxam : comparison between sensitive versus resistant populations



Conclusions

In conclusion, the expression of transcripts coding for cytochrome P450 and glutathione S-transferases was conducted in *L. decemlineata* larvae displaying differences in susceptibility towards two compounds. Multiple changes were detected including an overexpression of **CYP6d4**, **GST**, **GST1** and **GST1-like** in a thiamethoxam-treated population of larvae that was susceptible to the compounds investigated. Overexpression of **CYP 6a13** was identified in larvae that displayed resistance to thiamethoxam. RNA interference targeting these genes can next be envisioned in *L. decemlineata* to silence these genes and explore the impact on resistance.

References

- Chen YH, Cohen ZP, Bueno EM, Christensen BM, Schoville SD. Rapid evolution of insecticide resistance in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Curr Opin Insect Sci.* 2023 Feb;55:101000. doi: 10.1016/j.cois.2022.101000. Epub 2022 Dec 13. PMID: 36521782.
- Bastarache P, Bouafoura R, Omakele E, Moffat CE, Vickruck JL, Morin PJ. Spinosad-associated modulation of select cytochrome P450s and glutathione S-transferases in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Arch Insect Biochem Physiol.* 2023 Mar;112(3):e21993. doi: 10.1002/arch.21993. Epub 2022 Dec 22. PMID: 36546461.