Effects of EDTA feeding on survival and development of European corn borer larvae

Mylah Knight[1,2], Anastasia Cooper[1], Kristopher Silver[1], and Kun Yan Zhu[1]

[1]Department of Entomology, College of Agriculture, Kansas State University
[2]Department of Animal Science, College of Agriculture, Kansas State University

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Abstract

RNA interference (RNAi) is a process that uses double-stranded RNA (dsRNA) to target and suppress the expression of specific genes (Zhang et al., 2010). It is a powerful tool that has been used to understand the function of many genes and has great potential to control insect pests. However, differences in sensitivity to RNAi among insects have limited the use of RNAi (Kim et al., 2015). In some insects, such as European corn borer (ECB), RNAi efficiency is limited by degradation of dsRNA in insect gut. Strategies to overcome this limitation are necessary. Some studies have suggested that high temperatures or treatment with EDTA can inhibit nuclease activity (Garbutt et al., 2014), however the effects of treatment of insects with EDTA are unknown. The objective of this study was to determine if feeding different concentrations of EDTA to ECB larvae will have any effect on larval survival, weight, or development. Our results showed that neither 6 nor 10 mM EDTA had any significant effect on larval survival or development from 2nd instar to 3rd instar larvae. In contrast, average larval weight was suppressed by treatment with 10 mM EDTA. These data show that larvae can be safely treated with up to 6 mM EDTA without adverse effects on development or larval survival, suggesting that 6 mM EDTA can be used in future experiments to test the ability of EDTA to inhibit nuclease activity in ECB gut and possibly improve RNAi efficiency.

Purpose

To determine the concentration of EDTA to feed ECB larvae without affecting survival or development in preparation for future experiments evaluating the ability of EDTA to inhibit nuclease activity in the ECB gut.

Questions, Hypotheses, and Predictions

Question: Will feeding EDTA to ECB larvae affect larval development or survival?

Hypothesis: Feeding small amounts of EDTA to ECB larvae will not affect development or survivorship.

Prediction: We anticipate no difference in larval survival, development, or weight following feeding with EDTA.

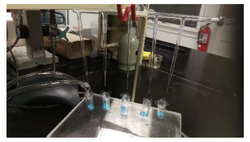
Study System

European corn borer (*Ostrinia nubilalis*)

- Found in all states east of the Rocky Mountains
- Larvae feed inside in the corn stalk causing lodging
- Adults are small moths that do not damage corn crops

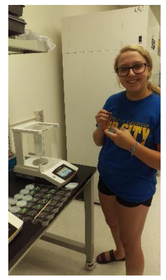
Methods and Experimental Design

Food was weighed and placed in a plastic container.



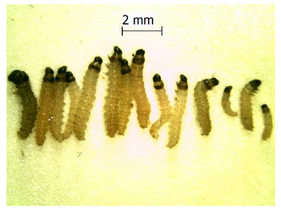
EDTA solutions were added and food was dried with N_2 gas.

25 larvae were placed in each container with 25 mg food for 3 days (3 biological replicates).



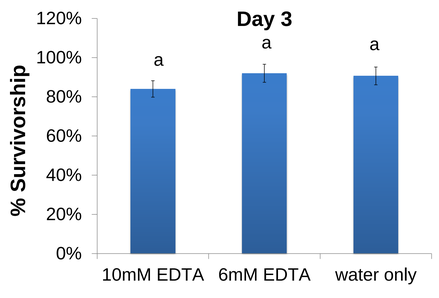
On day 3, survivorship was recorded and larvae were transferred to a newt container with treated food (50 mg) for 3 days.

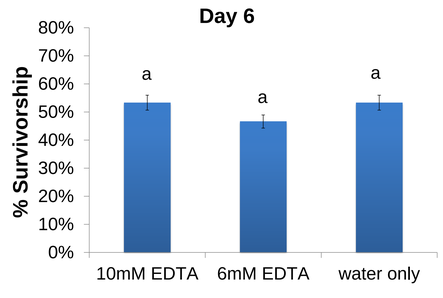
On day 6, larvae were weighed and survivorship was recorded.

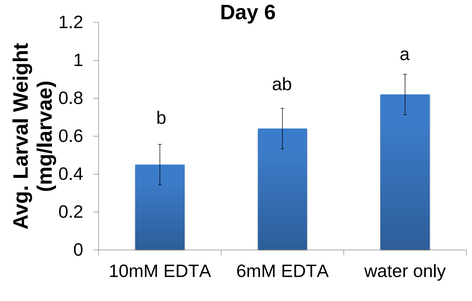


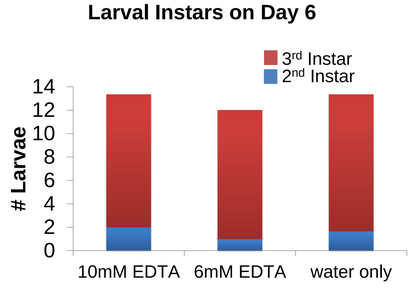
On day 6, larvae were also photographed under a dissecting microscope.

Results









Conclusions

- Feeding higher concentrations of EDTA resulted in reduced larval body weight.
- Feeding EDTA did not affect survivorship or cause larval death. Therefore, it is not likely to affect survivorship when used in future RNAi experiments.
- Larval development was similar across all treatments, showing that EDTA does not affect larval development.
- Our results provide a solid foundation for further experiments directed at overcoming nuclease activity as an obstacle to an efficient RNAi response.

Future Directions

Future research in this area could include testing these concentrations of EDTA in ECB larvae to see if they inhibit nuclease activity in gut contents and/or increase RNAi efficiency. Additionally, we could also test higher concentrations of EDTA, should the current levels prove ineffective. Testing other larval instars and/or adults for improvements in RNAi efficiency following EDTA treatment could also prove fruitful.

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