Effects of Feeding Fluorescent Brightener 28 and Blue Dextran to European Corn Borer Larvae

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Abstract

The European corn borer (ECB), *Ostrinia nubilalis*, is a common pest for corn crops in the United States and most of Europe (Hudan and LeRoux, 1986). Use of traditional pesticides to control ECB has resulted in the development of resistance in pest populations and significant loss of important biological control species. As such, novel methods, such as use of RNA interference (RNAi), are necessary to overcome resistance to traditional pesticides and protect non-target insects.

RNAi takes advantage of intrinsic pathways that use long double-stranded RNAs (dsRNAs) to suppress the expression of specific genes (Zhang et al., 2010), however, many insects do not produce an efficient RNAi response, at least partially as a result of the presence of double-stranded ribonucleases (dsRNases) that degrade the dsRNAs prior to incorporation into the RNAi pathway (Kim et al., 2015). These dsRNases are present in the guts of many species, including ECB and are a powerful factor limiting the efficacy of RNAi. We hypothesize that applying dsRNA when expression of dsRNases is low (such as in young ECB larvae) and degrading the gut lining will minimize contact between dsRNAs and dsRNases and increase RNAi efficiency.

Fluorescent Brightener 28 (FB28) is a chemical that has been used previously to damage insect gut linings and so is a good candidate for performing these experiments (Zhang et al., 2010). In addition, blue dextran (BD) is also necessary as a marker to demonstrate successful weakening and increased permeability of the gut, however, it is still unclear what concentrations of these chemicals ECB larvae will tolerate without significant adverse effects. Accordingly, these experiments were designed to identify the optimum levels of FB28 and BD needed in the diet of larval ECB for clear visualization of gut disruption.

Figure 1. European corn borer larva (left) and adult (right)

Photographs by John L. Capinera, University of Florida

Questions, Hypotheses, and Predictions

Question: What are the optimum levels for oral administration of FB28 and BD so that disruption of the gut lining of ECB larvae is clearly visualized without causing adverse effects on ECB?

Hypothesis: A diet consisting of FB28 levels above 0.5% will cause adverse effects on ECB larvae but a diet FB28 levels below 0.375% will be ineffective in successfully rupturing the gut lining of ECB. Likewise a BD level in the ECB diet of around 5% should be sufficient to visualize gut lining integrity for any level of FB28 without causing any visible adverse effects.

Prediction: We expect that 0.375% FB28 and 5% BD will be sufficient to visualize disruption of the gut lining in ECB larvae.

Purpose

The purpose of this research is to discover an optimum level of FB28 and BD to maximize visualization of gut disruption without causing adverse effects. These results may help to enhance the efficacy of RNAi by improving dsRNA uptake and limiting contact with dsRNases.

Study System

The European corn borer is a Lepidopteran in the Crambidae family. It is common to corn crops and can be a serious pest in agriculture. Lepidopterans are typically refractory to oral or topical treatment with dsRNAs.

Methods and Experimental Design

Larvae were divided into four groups of 25 each (1, 2, 3, 4). Larvae were fed 25 mg of diet containing FB28 and/ or BD according to the treatment protocol below. Diet was prepared and dried using N_2 gas (Fig. 2A). Larvae were then kept at 25 °C for 3 days in small containers (Figs. 2B and C). Larvae were then transferred (Fig. 2D) to new containers with 50 mg of diet containing the same amount of FB28 and/or BD as before. After 3 days, larvae were then analyzed for mortality, and to determine if gut lining integrity was reduced as a result of FB28 treatment. Significant differences ($p < 0.05$) were determined using a one way ANOVA with Tukey's post hoc analysis.

Control	1) corn diet + water + blue food coloring
BD	2) corn diet + 5% BD + water
FB 0.375%	3) corn diet +0.375% FB28 + 5% BD + water
FB 0.5%	4) corn diet + 0.5% FB28 + 5% BD + water

Figure 2. Experimental preparation of diet and larvae. A. Diet preparation and drying with N_2 gas. B. Containers for ECB larvae and diet. C. Larvae in containers feeding on diet. D. Transfer of larvae to new containers/diet on day 3.

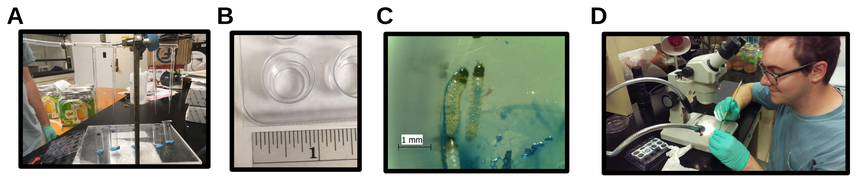
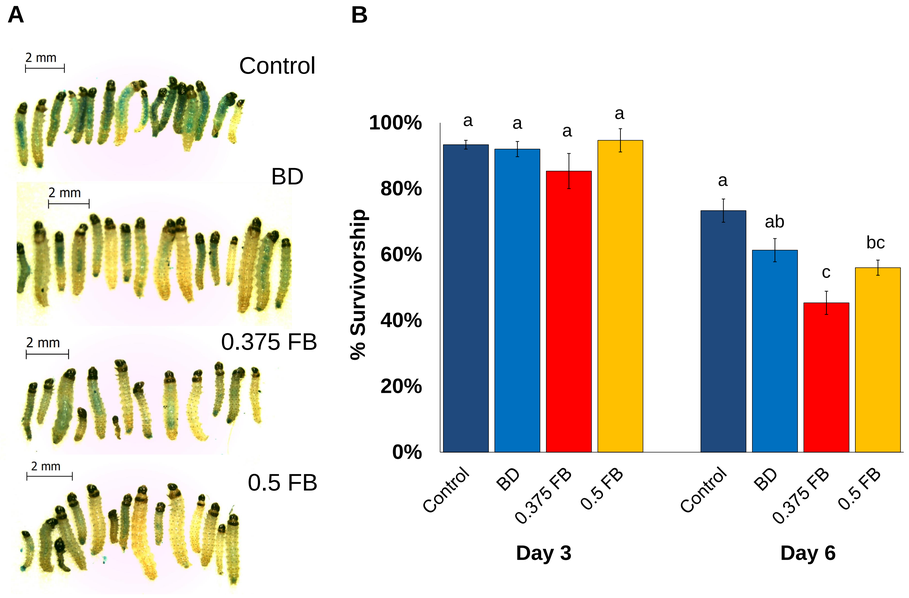


Figure 3. A. Blue staining in ECB larvae after 6 days fed BD and/or FB28. B. Percent survivorship of ECB larvae fed BD and/or FB28 on Day 3 and 6.



Conclusions

Incorporation of 5% BD appeared to be sufficient to visualize the integrity of gut lining in ECB larvae, and did not cause mortality in ECB larvae. However, treatment of larvae with FB28 seemed to increase mortality on day 6 in ECB larvae without obviously affecting escape of BD from the gut. Therefore both 0.5% and 0.375% FB28 are unsuitable for gut disruption in RNAi experiments.

Future Directions

In future experiments I would like to explore additional concentrations of FB28 (0.1, 0.5, 0.75, 1.0, and 1.25%) to determine the concentration that can disrupt the gut lining with minimal effects on larval survival. In addition, I would also implement a new and effective technique I discovered while transferring the larvae from one container to another. By placing the containers on a lighted surface, ECB larvae were drawn toward the bottom of the container as opposed to the overhead lights and out of the container. This method made transferring larvae much faster and allowed for less unpredictable larvae escaping.

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