

# Starving or not: What's the sink bug's hunger level I need for my bioassay?

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## Introduction

The green stink bug, *Nezara viridula*, is a cosmopolitan pest that threatens soybean yield and quality. Its feeding behavior, from host location to host acceptance and consumption, is closely influenced by nutritional cues. Understanding its feeding behavior is essential to optimize experimental studies.

## Aims

Optimize bioassays to evaluate soybean damage and *Nezara viridula* feeding behavior.

Develop a starvation protocol to standardize feeding behavior in bioassays.

Analyze how the insect's nutritional status impacts feeding preference between induced and non-induced pods.

## Conclusion

Our results indicate that a starvation period of 48–72 hours is optimal for damage assessment assays, as it standardizes *N. viridula*'s response time regardless of the plant's prior defense status. Principal Component Analysis (PCA) of plant chemical defenses, such as isoflavonoids and protease inhibitors, clearly distinguished induced from uninduced pods, highlighting the role of these compounds in shaping feeding behavior. Overall, these findings refine bioassay protocols and enhance our understanding of how soybean defense induction influences stink bug responses, offering valuable insights for designing more realistic and effective pest management experiments.

## Results

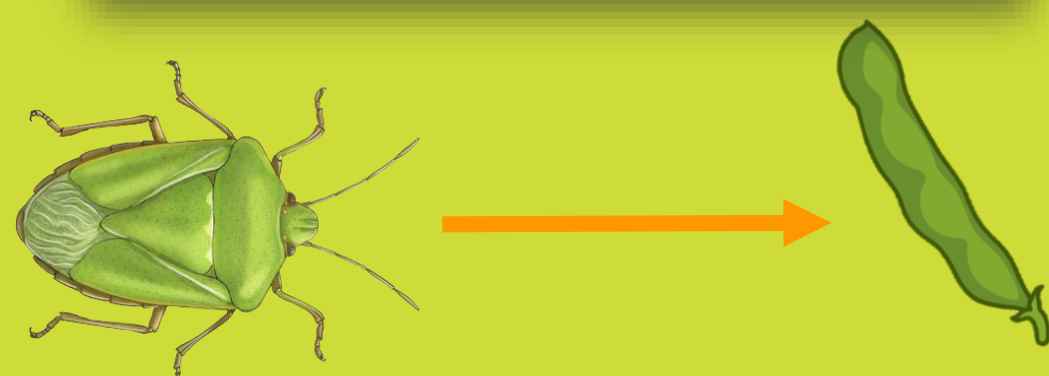
### Reference

UD: Undamage

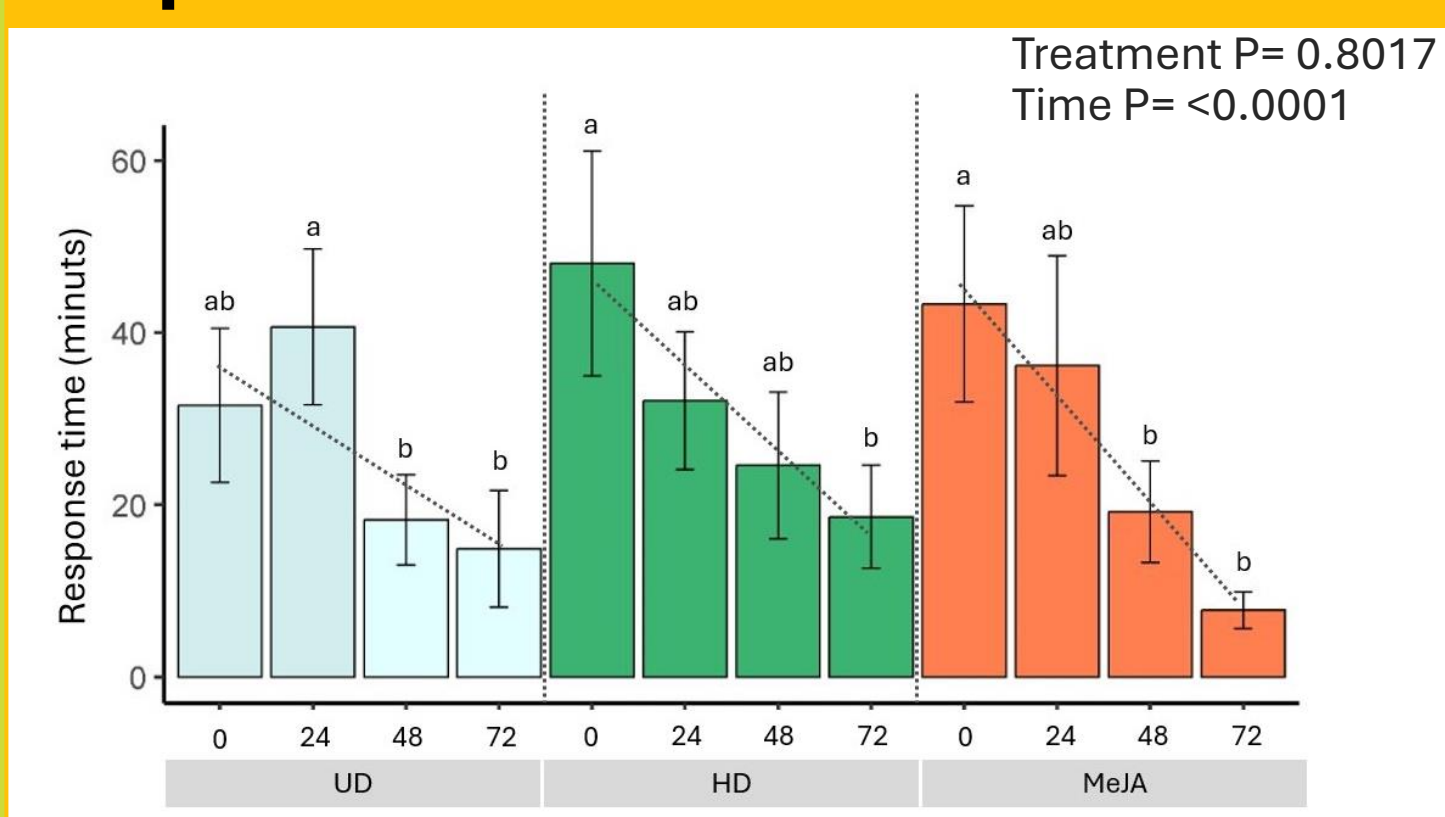
HD: Herbivory-damage

MeJA: methyl jasmonate induced

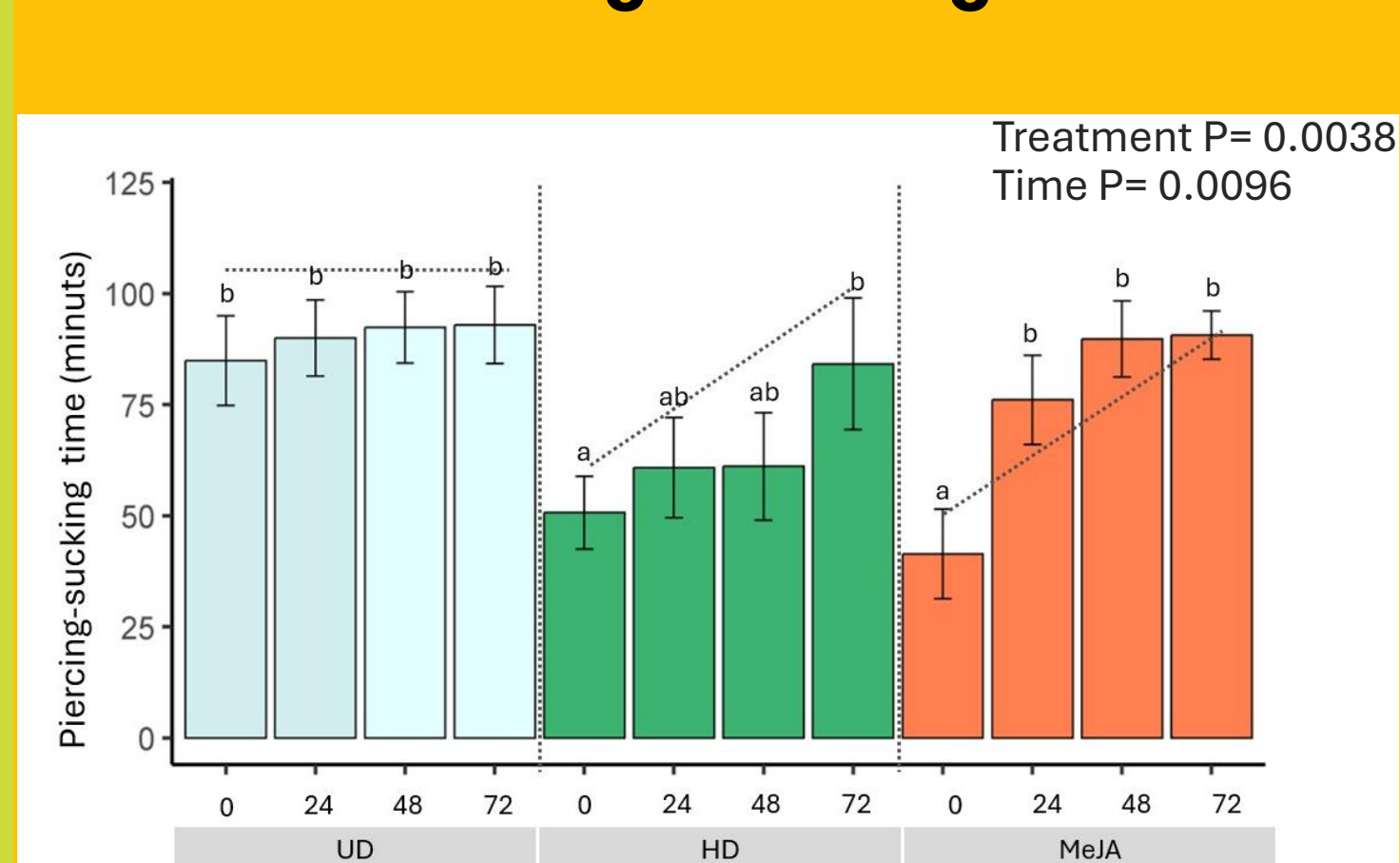
\*Different letters indicate significant differences.



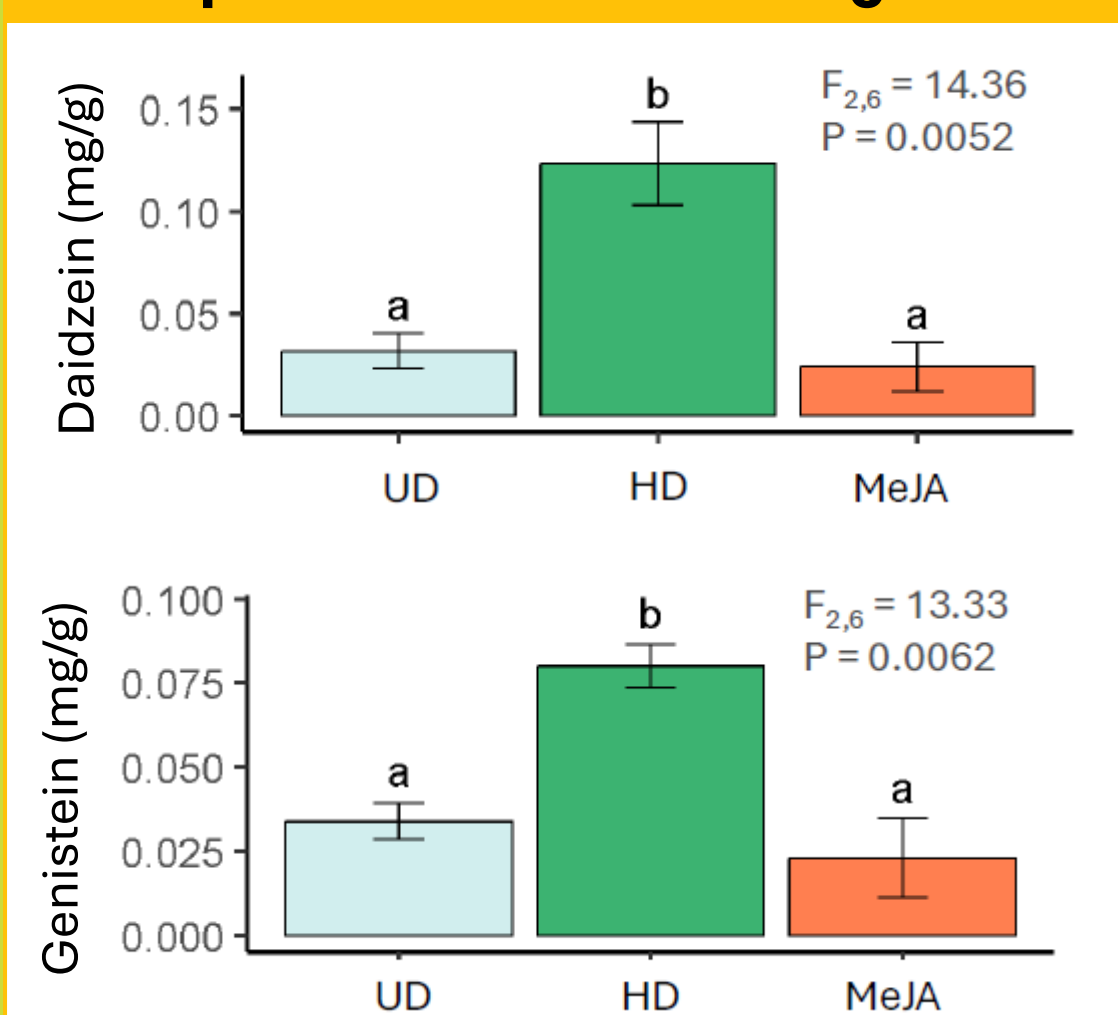
### Time it takes for the stink bug to reach the pod.



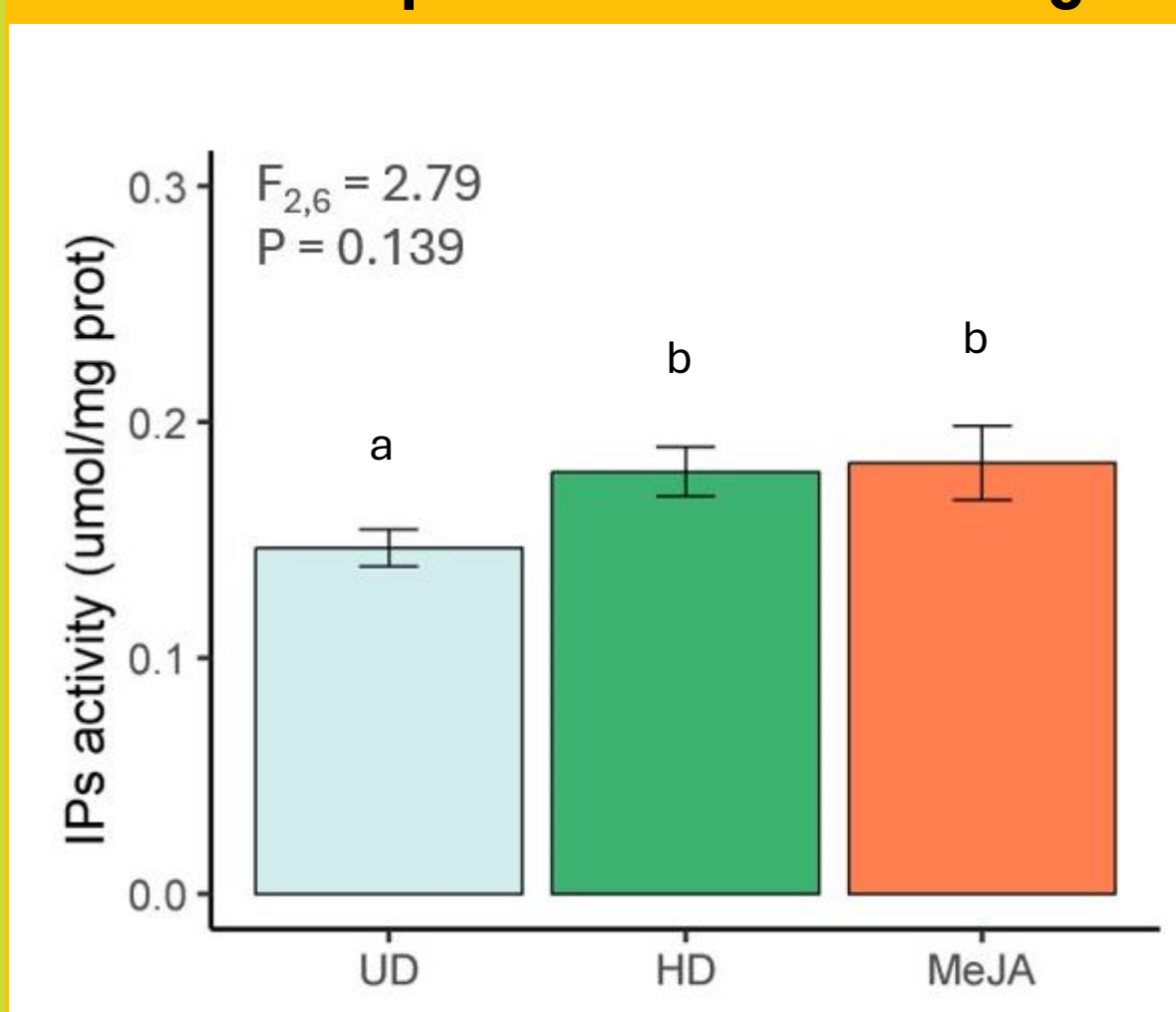
### Time the stink bug is feeding.



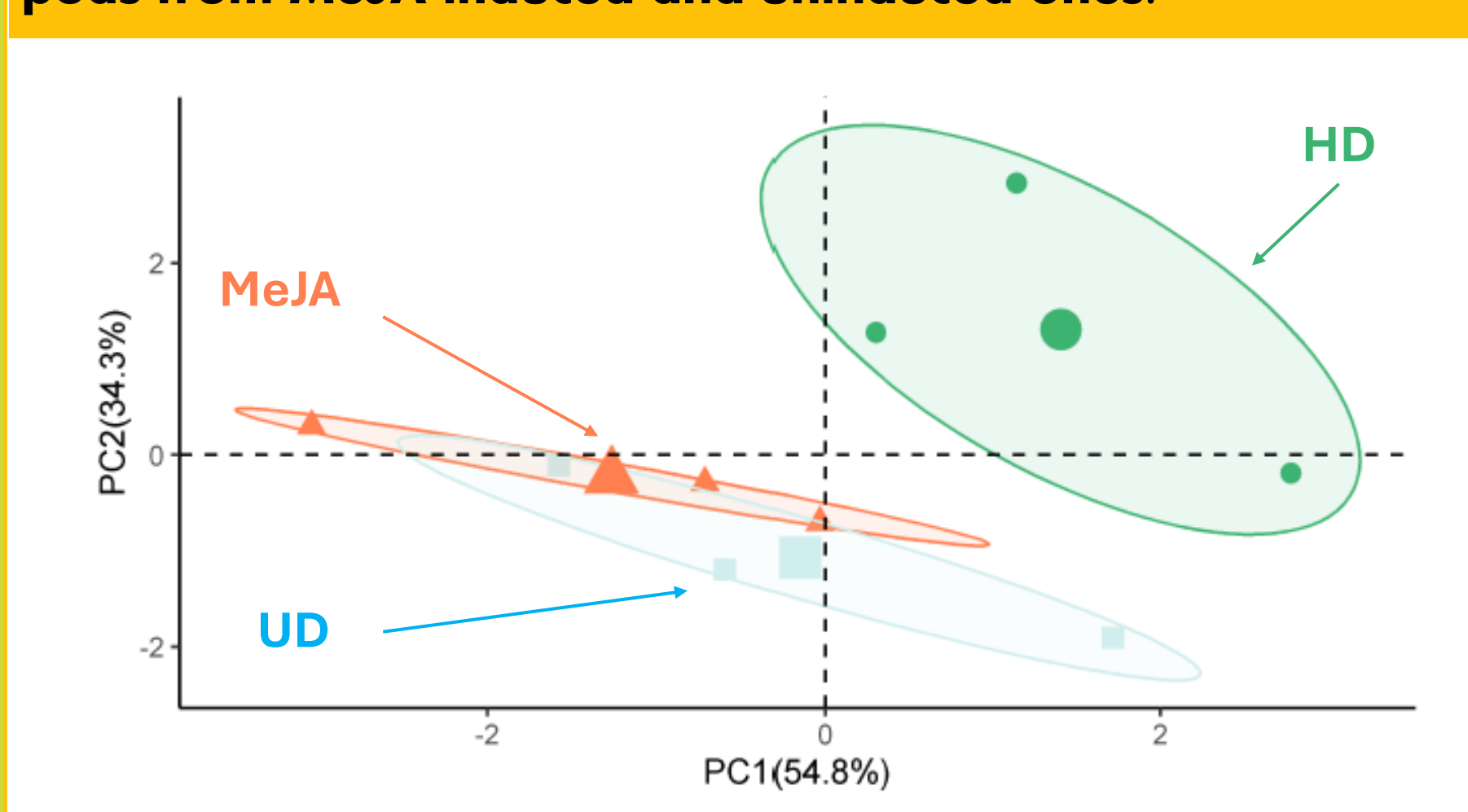
### Isoflavonoids detected in pod before the experiments with stink bug.



### Activity of protease inhibitors in pod before the experiments with stink bug.



### PCA of chemical defenses separates herbivory-induced pods from MeJA-induced and uninduced ones.



## Materials and Methods

### Insect collection and setup

Experiments were conducted in the Biochemistry Lab (FAUBA) using adult *Nezara viridula* individuals, sexed prior to the assays.

### Plant material

Fresh soybean pods (*Glycine max*, cv. Williams 82, R6 stage) were used as food source. Their levels of isoflavonoids and protease inhibitors were previously determined.

### Starvation treatments



Insects were subjected to different starvation periods: 0, 24, 48, and 72 hours.

### Plant defense treatments

Pods were classified into three defense levels: control (UD), herbivory-damage (HD), and methyl jasmonate (MeJA)-induced.

### Feeding behavior assays

No-choice experiments were performed. Feeding behavior was recorded and analyzed using BORIS software.