Differential responses of three stink bugs species to soybean defenses: Insights into the impact on digestive enzymes

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Background

The stink bugs *Diceraeus furcatus*, *Piezodorus guildinii*, and *Nezara viridula* (Hemiptera: Pentatomidae) are major pests of soybean (*Glycine max*; Fabales: Fabaceae) that feed on developing seeds.

In response to herbivory, soybean seeds synthesize **protease inhibitors (PI)** as a key defense mechanism. These inhibitors interact with digestive cysteine proteases in stink bug guts —such as **cathepsins**— thereby reducing nutrient assimilation.

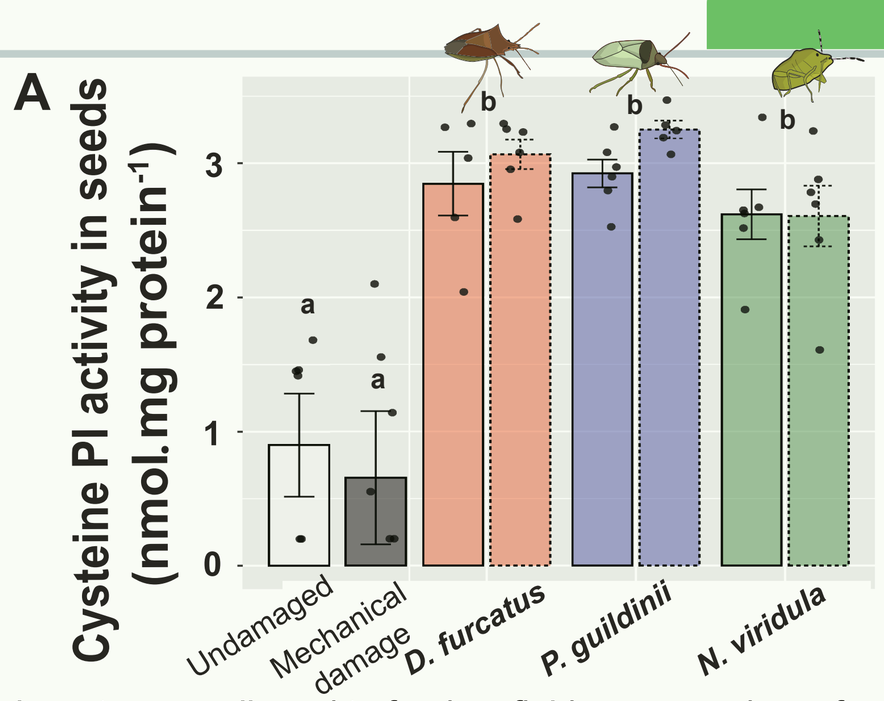
Although some information is available on the effects of cysteine PI on *N. viridula*, little is known about how different stink bug species, such as *Diceraeus furcatus* and *Piezodorus guildinii*, induce these defenses in field-grown soybean and the consequence inhibition of digestive proteases in the guts of stinkbugs.

In this study, we measured cysteine PI activity in field-grown soybean seeds following herbivory by the three insect species and analyzed cathepsin B and L activities in stink bug guts after feeding on growing seeds

Conclusions

1) Soybean induced cystein PI against the attack of the three stink bug species.
2) All three stink bugs species exhibited similar pod-piercing frequencies per pod.
3) Cathepsin L activity was inhibited in the gut of *D. furcatus* after feeding on field-grown soybean for 72 h.
4) Cathepsin B activity was inhibited in the gut of *P. guildinii* and *N. viridula* after feeding on field-grown soybean for 72 h.

Results



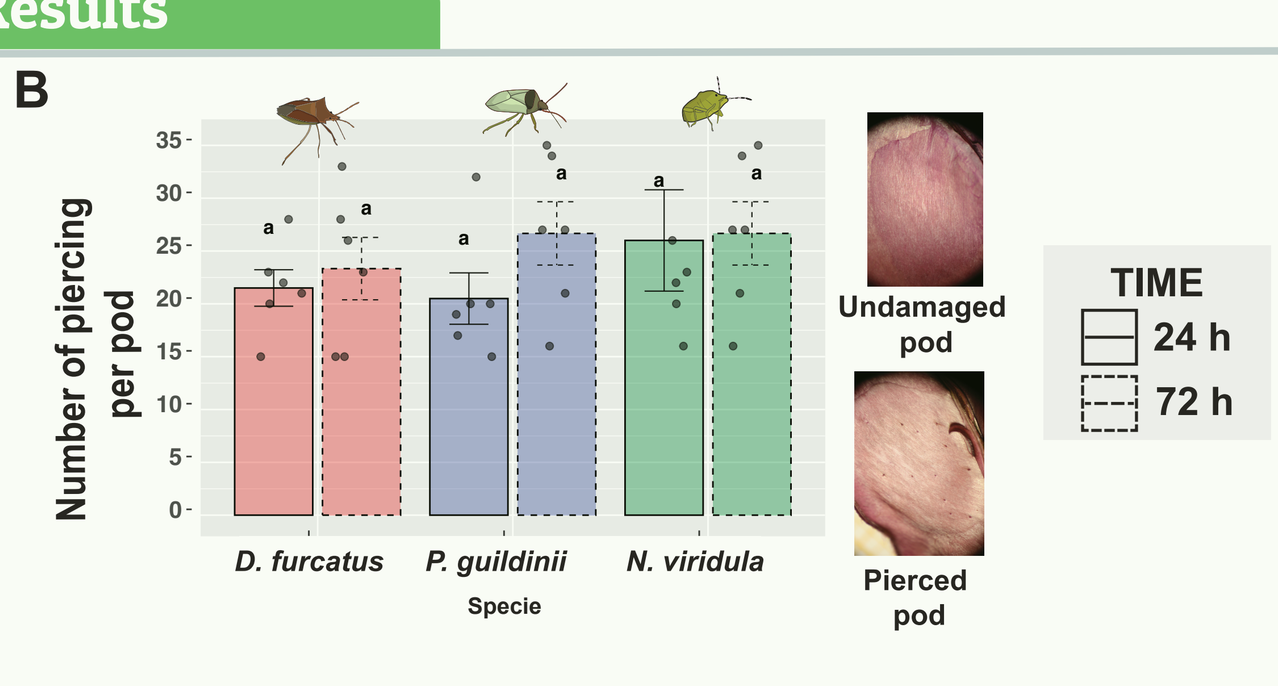


Figure 1: Insects were allowed to feed on field-grown soybean for 24 and 72 h. **A.** Herbivory induced the activity of PIs in soybean seeds but not in undamaged or mechanical damaged treatments One way ANOVA showed significant differences among groups [n=6, F= 3.02, P= <0.01]. **B.** All three stink bug species pierced the soybean pods 25 times each. One way ANOVA showed no significant differences among groups (n=6, F=1.69, P= 0.19).

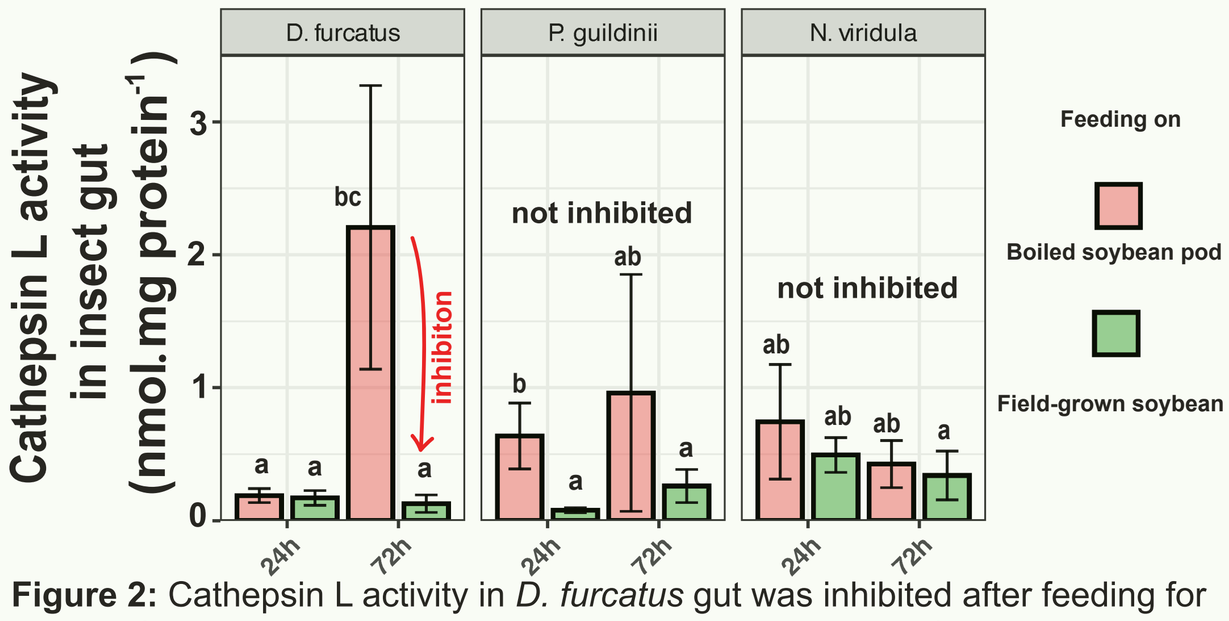


Figure 2: Cathepsin L activity in *D. furcatus* gut was inhibited after feeding for 72 h on field-grown soybean. Post hoc Dunn's test with Bonferroni correction showed significant differences between groups (72 h boiled soybean vs soybean, n=6, Z= 2.54, P<0.01).

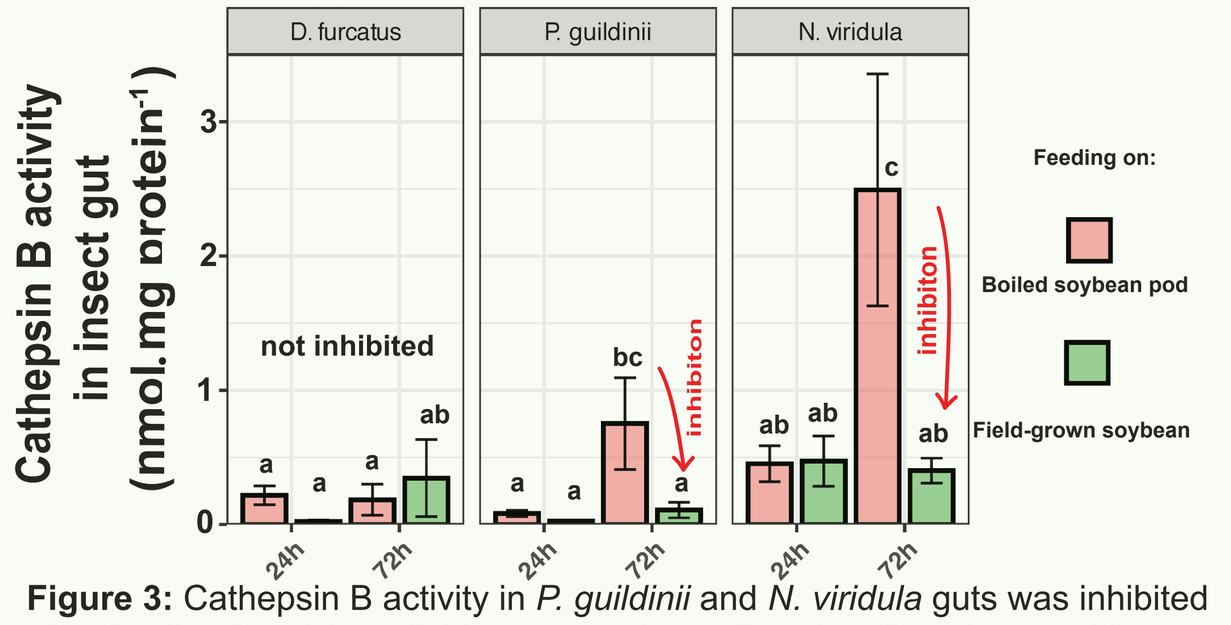


Figure 3: Cathepsin B activity in *P. guildinii* and *N. viridula* guts was inhibited after feeding for 72 h on field-grown soybean. Post hoc Dunn's test with Bonferroni correction showed significant differences between groups (72 h boiled soybean vs soybean, n=6, Z= 2.57, P<0.01).

Material and Methods

Protease inhibitors: PIs activity was measured using chromogenic substrate p-Glu-Phe-Leu-pNA. A unit of cystein-protease was defined as the quantity of required enzyme to produce 1mM of 4-nitroaniline/minat 37°C.

Cathepsin B and L activity: To determine cysteine proteases activity in the gut of stink bugs, a specific chromogenic substrate was used, p-Glu-Phe-Leu-pNA. The assay was carried out at 37 ºC in 100 µL of the reaction mixture. The activity was estimated in a reaction mixture containing buffer (0.1 M sodium phosphate pH 6.0, 0.3 M KCl, 0.1 mM EDTA, 3 mM DTT), gut extract, 0.38 mM p-Glu-Phe-Leu-pNA and 1 µM CA074. P-nitroaniline (pNA) release was followed at 405 nm every 30 s for 30 min. Initial rates of hydrolysis were estimated from the slopes of absorbance versus time graphs. One unit of cysteine protease activity was defined as the amount of enzyme required to produce 1 nM 4-nitroaniline per minute at 37 ◦C under given assay conditions. All reactions were carried out in triplicate, and appropriate blanks were run for all experiments.