

Systemic defenses of soybean seeds within pods: the role of Protease Inhibitors against *Nezara viridula* (Hemiptera: Pentatomidae)

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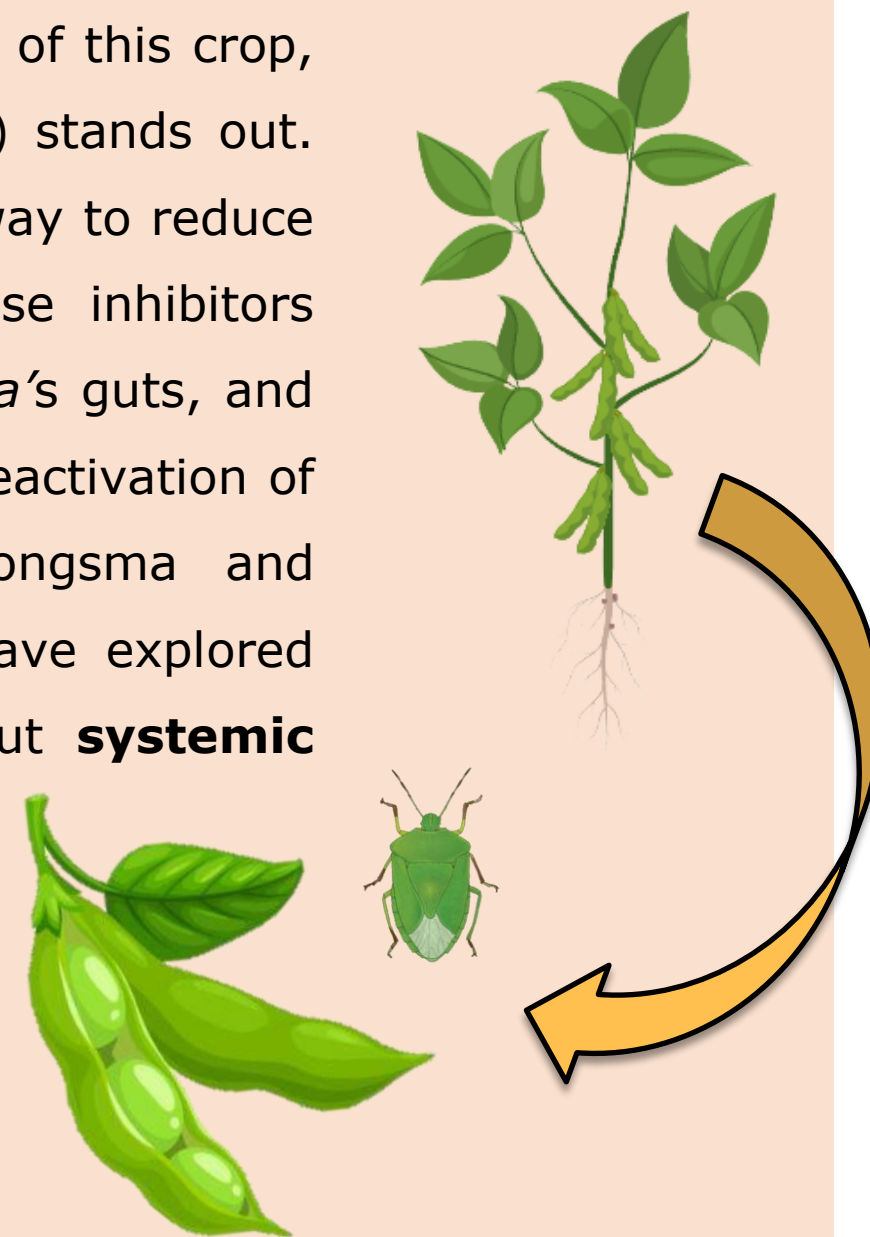
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Soybean is the main oilseed cultivated in Argentina, constituting nearly half of the country's production. The stink bug complex is the main pest of this crop, among which the Southern Green Stink Bug (*Nezara viridula* L.) stands out. Leveraging the natural resistance of plants can be an important way to reduce insecticide applications. Among soybean's defenses are protease inhibitors (PIs), which deactivate digestive cysteine proteases in *N. viridula*'s guts, and are induced in pods and seeds following herbivory, causing the deactivation of the digestive enzymes cysteine proteases of the bugs (Jongsma and Beekwilder, 2008; Sardoy *et al.*, 2021). While many studies have explored local defense responses to insect damage, less is known about **systemic** mechanisms that enhance resistance in undamaged seeds.



In this study we evaluated systemic PIs activity of seeds from the same pod of field-grown soybean and the impact of these compounds on the preference of *Nezara Viridula*.

CONTEXT

CONCLUSIONS

- Herbivory increased PIs activity in both attacked and unattacked seeds within the same pod. Soybean pods respond systemically to herbivory by synthesizing PIs, even in undamaged seeds.
- Preference choice experiments showed that *N. viridula* preferred to feed on unattacked seeds. The systemic response of soybean alters feeding preferences of *N. viridula* likely by affecting the activity of their gut proteases, highlighting the potential of PIs in pest management strategies.

METHODS

- Adults of *Nezara viridula* (laboratory reared + field collected)
- Soybean Williams'82 (isogenic line)
- Measurement of protease inhibitors' activity in soybean pods and gut cysteine proteases in *N. viridula* (Zavala *et al.*, 2008).

No-choice experiment.

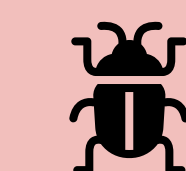
- Aluminum foil covered two of the three seeds in randomly selected pods to identify the targeted seed. A starved adult stink bug was confined to each pod (excluding controls - undamaged) within a mesh bag to feed on the uncovered seed. Insects were allowed to feed for 24h or 72 hs.

Three treatments:

- Treatment A:** Seed 1 (closest to the node) free, without aluminum.
- Treatment B:** Seed 3 (furthest from the node) free, without aluminum.
- Control (undamaged)** without insects.

- Seeds were taken from each pod at 24 and 72h.

- Protease inhibitors' activity was measured from the chromogenic substrate p-Glu-Phe-Leu-pNA.



Preference choice experiment.

- Aluminum foil covered two of the three seeds in selected pods to identify the targeted seed. A starved adult stink bug was confined to each pod (excluding controls - undamaged) within a mesh bag to feed on the uncovered seed. After 24 h, this stink bug and the foil were removed, and a second starved stink bug was introduced. The first seed pierced by this stink bug was recorded.

- Two possible options:

- Seed previously damaged by another bug.**

- Undamaged seeds from the same pod.**

Activity of cysteine protease inhibitors in seeds: 24h of damage

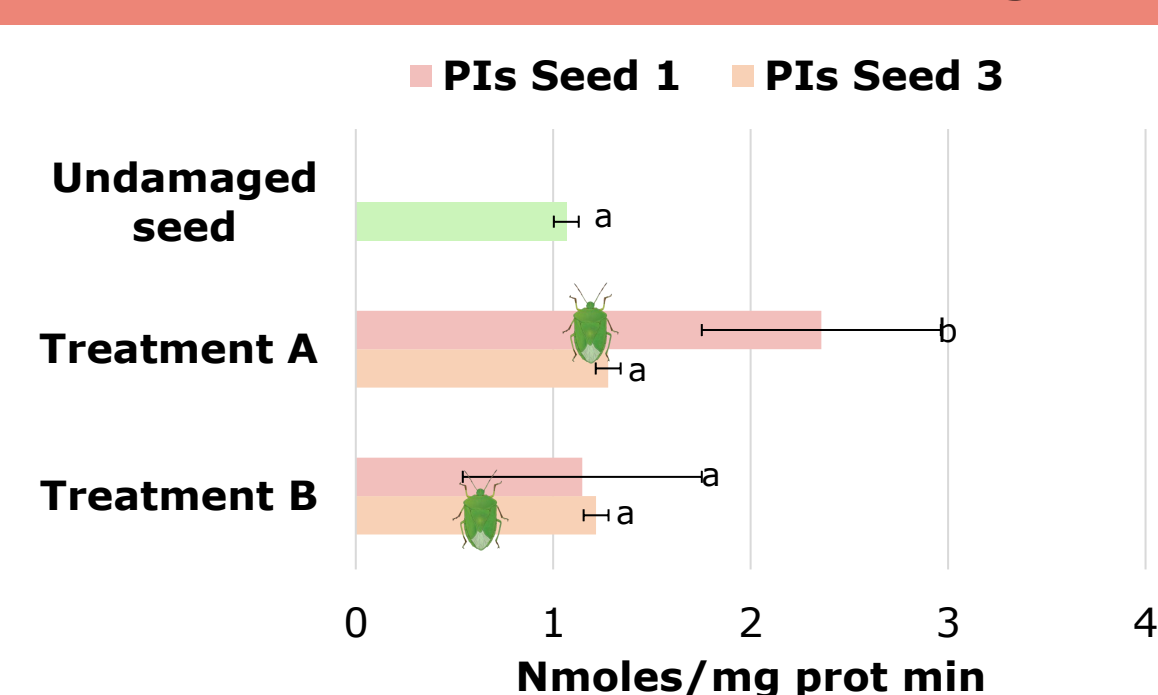


Figure 1: PIs' activity in seeds from pods subjected to damage treatments (A y B) and Undamaged ones (control, green bar). **Seed 1: closest to the node (pink bars). Seed 3: furthest from the node (orange bars).** *The stink bug image was used to demarcate the directly damaged seed (local response).*

Activity of cysteine protease inhibitors in seeds: 72h of damage

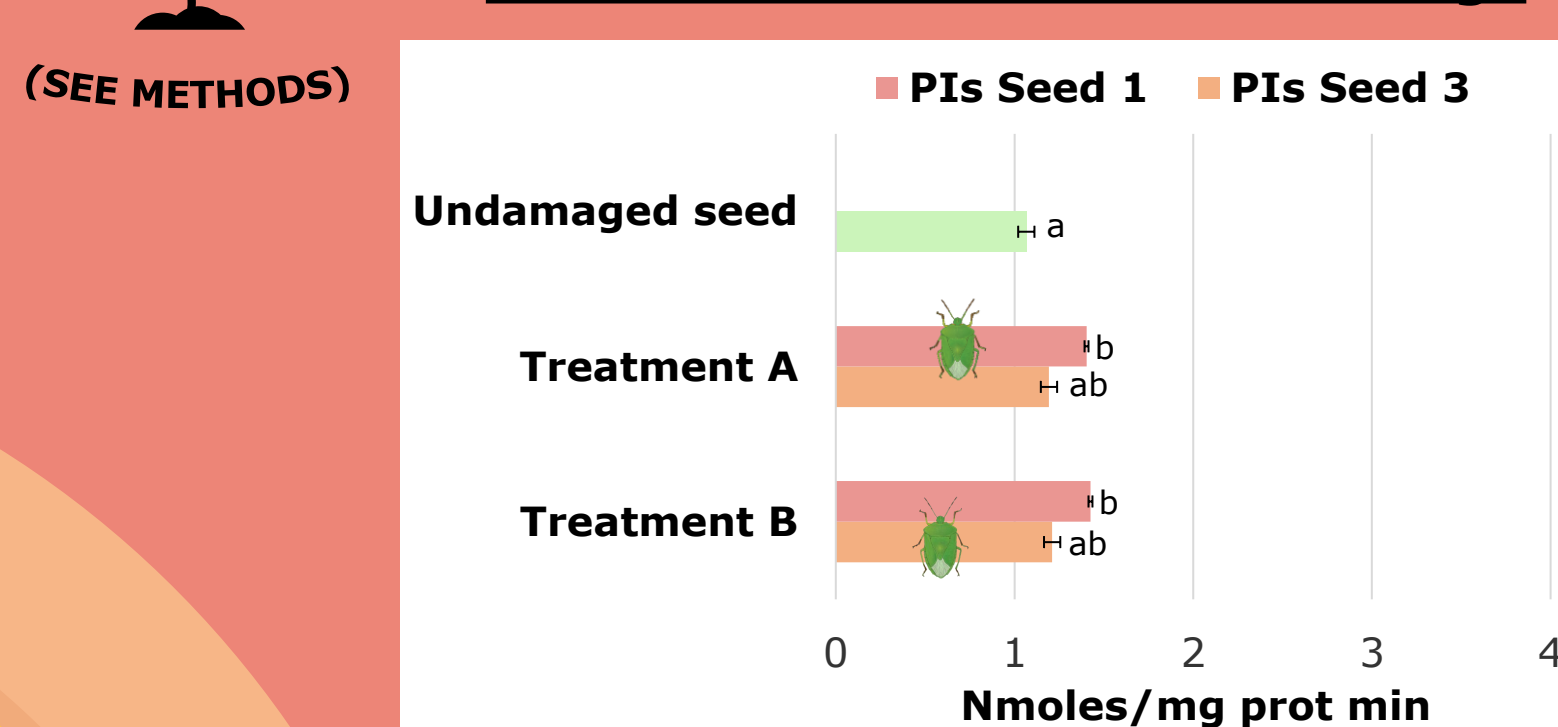


Figure 2: PIs' activity in seeds from pods subjected to damage treatments (A y B) and Undamaged ones (control, green bar). **Seed 1: closest to the node (pink bars). Seed 3: furthest from the node (orange bars).** *The stink bug image was used to demarcate the directly damaged seed (local response).*

Preference choice experiment: 24h of damage

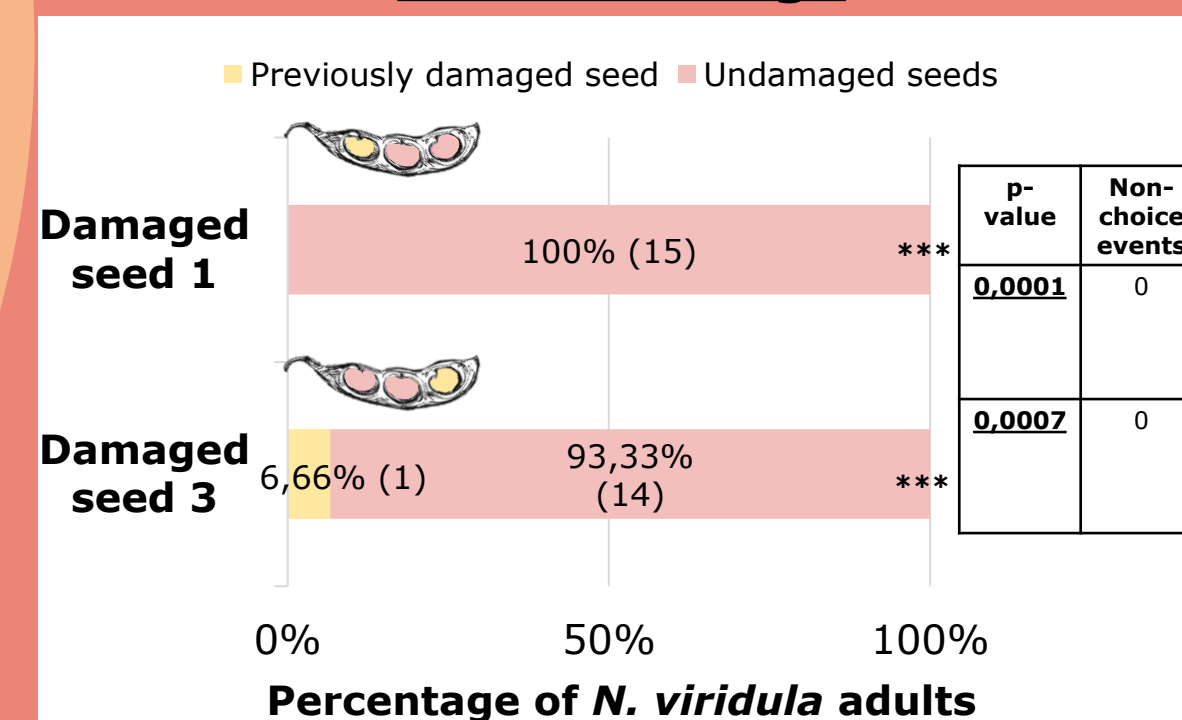


Figure 3: Percentage of *Nezara viridula* adults in choice-experiment with different seeds (1 or 3) previously damaged by another bug for 24h. Values in parentheses indicate number of individuals (N=15). The table shows the p-values (chi-square analysis) and the number of individuals who did not choose for each treatment.

Preference choice experiment: 72h of damage

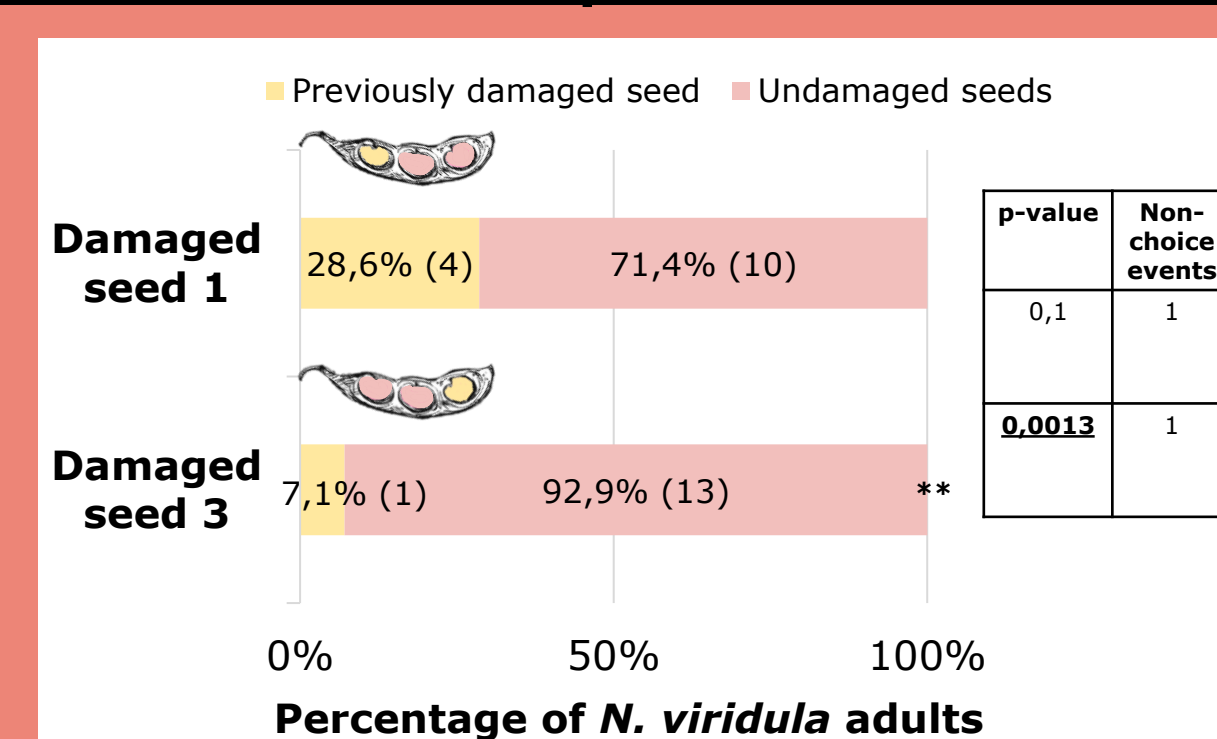


Figure 4: Percentage of *Nezara viridula* adults in choice-experiment with different seeds (1 or 3) previously damaged by another bug for 72h. Values in parentheses indicate number of individuals (N=15). The table shows the p-values (chi-square analysis) and the number of individuals who did not choose for each treatment.

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