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Original Article

Nymph developmental time and survivorship, adult body weight, and adult damage of *Nezara viridula* (L.) feeding on canola seeds at different stages of maturation

Tempo de desenvolvimento e sobrevivência da ninfa, peso corporal adulto e danos causados por *Nezara viridula* (L.) em sementes de canola em diferentes estágios de maturação

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Abstract

This study investigated nymph developmental time and survivorship, adult body weight, and the impact of the southern green stink bug *Nezara viridula* (L.) on seeds of canola at different stages of maturation. Tests were conducted under controlled conditions (25 ± 1 °C, 65 ± 10% relative humidity, and L14:D10 h photoperiod) in the laboratory using rearing cages. Treatments were set at random and statistically analyzed using program 'R'. Results indicated that total nymph developmental time was significantly longer on green seed stage and early maturation seeds compared to advanced maturation seeds. Survivorship was high on all three food sources (≥80%). Adults exhibited significantly higher fresh body weight when fed on advanced maturation seeds compared to those fed on green stage or early maturation seeds. Adult feeding caused significant seed damage and dry weight loss across all seed stages, with green stage seeds experiencing the highest levels of damage and dry weight loss. Early maturation seeds and advanced maturation seeds showed no significant difference between them. These information reveal that *N. viridula* feeds and cause damage to canola seeds and may help to improve management of this pest in the field.

Keywords: biology, Pentatomidae, seed damage, southern green stink bug.

Resumo

Este estudo investigou o tempo de desenvolvimento e sobrevivência das ninfas, o peso corporal adulto e o impacto do percevejo verde *Nezara viridula* (L.) em sementes de canola em diferentes estágios de maturação. Os testes foram conduzidos em condições controladas (25 ± 1 °C, 65 ± 10% umidade relativa, e fotoperíodo de L14:E10 h) em laboratório usando gaiolas de criação. Os tratamentos foram distribuídos aleatoriamente e analisados estatisticamente usando o programa 'R'. Os resultados indicam que o tempo total de desenvolvimento das ninfas foi significativamente maior em sementes verdes e de maturação média em comparação com sementes maduras. A sobrevivência foi alta em todas as três fontes de alimentos (≥80%). Adultos exibiram pesos corporais frescos significativamente maiores quando alimentados com sementes maduras em comparação com aqueles alimentados com sementes verdes ou de maturação média. A alimentação de adultos causou danos significativos às sementes e perda de peso seco em todos os estágios das sementes, com sementes imaturas apresentando os maiores níveis de danos e perda de peso seco. As sementes de maturação média e madura menos danificadas não mostraram diferença significativa entre elas. Essas informações revelam que *N. viridula* alimenta-se e causa danos nas sementes de canola, auxiliando para um melhor manejo desta praga a nível de campo.

Palavras-chave: biologia, Pentatomidae, danos às sementes, percevejo verde do sul.

1. Introduction

The canola plant, *Brassica napus* (L.) var. *oleifera*, stands as a prominent oilseed variety within the Brassicaceae. Originally developed through conventional genetic improvement of rapeseed, its cultivation in Brazil has been steadily increasing, particularly in the Southern Region, where canola serves as a valuable winter cultivation

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option in crop rotation systems. Its versatility extends to various applications, including oil production for human consumption, biodiesel, and animal feed (Tomm et al., 2009). The importance of cultivation of canola in Brazil is evidenced by the significant expansion of cultivated area in recent years. In 2023, it increased ca. 50% compared to the previous year, reaching ca. 83,000 hectares (Brasil, 2023).

Stink bugs are a potential threat to canola production. These pests are ubiquitous across continents, particularly in tropical regions, and are known to inflict damage on a wide range of cultivated plants of economic importance (Schaefer and Panizzi, 2000). Among the stink bug species affecting canola, *Nezara viridula* (Linnaeus, 1758) stands out as a notable threat. Initially reported by Dias (1992) for causing losses in seed yield and quality, *N. viridula*, along with *Euschistus heros* (Fabricius, 1798) and *Diceraeus furcatus* (Fabricius, 1775), are the most common pentatomids associated with canola (Bianchi et al., 2019).

A study carried out on soybean showed that the stages of seed ripeness affect the biology of *N. viridula*, such as the survival rate, which is higher when the nymphs feed on mature seeds rather than on green ones (Panizzi and Rossini, 1987). In canola, in addition to *N. viridula*, adults of *D. furcatus* and *E. heros* also survive feeding on siliques and nymphs reach adulthood feeding on green seeds (Possebom et al., 2020; Oliveira et al., 2023). These three species cause damage to the siliques (i.e., whitish spots with lesions in the form of starbursts, called rosetes) and the seeds become shrunken with rotten aspect (Oliveira et al., 2023). Despite the interaction of pentatomids with the canola crop in Brazil, there is little information on the biology and damage caused by them to seeds at different stages of maturity.

Therefore, the objectives of this study include evaluating nymph developmental time and survivorship of *N. viridula* on canola seeds at different stages of development; elucidating the influence of seed maturation on body weight gain of adults; and characterizing the resulting damage caused by adult feeding on seeds.

2. Materials and Methods

2.1. Stink bug colonies and canola plants

Adults of *N. viridula* were field-collected on plants of *Raphanus sativus* L. at the National Wheat Research Center, Embrapa Wheat, located in Passo Fundo, Rio Grande do Sul state, Brazil (28° 15' 46" S, 52° 24' 24" W), taken to the Laboratory of Entomology and placed in plastic rearing cages (20 × 20 × 24 cm). The cages were lined with filter paper and a strip of toilet paper was provided to serve as a substrate for oviposition. The rearing cages were kept in a walk-in chamber at 25 ± 1 °C, 65 ± 10% relative humidity, and L14:D10 h photoperiod.

A mixture of fresh pods of green beans (*Phaseolus vulgaris* L.), raw shelled peanuts (*Arachis hypogaea* L.) and dry soybean seeds (*Glycine max* (L.) Merrill) was provided as food source. The food was replaced, and eggs were removed, twice a week. The eggs were placed in plastic boxes (11 × 11 × 3.5 cm), lined with filter paper and wet foam to maintain humidity. After hatching, nymphs were fed with the same food provided to adults.

Canola plants were cultivated in a greenhouse under semi-controlled conditions. Seeds of the cultivar Hyola 575 CL were sown in plastic pots (2L) containing a mixture of soil + fertilizer. Sowing occurred every 21-days, so that there were plants at different phenological stages of development available throughout the experiments.

2.2. Nymphal biology and adult body weight at emergence

On the first day of the 2nd instar, nymphs were carefully collected from the colony and individually placed into Petri dishes (9.0 × 1.5 cm) lined with filter paper. The following treatments were tested (n = 15 nymphs/treatment): siliques containing seeds at different stages of maturation: 1) green stage (66% of water composition) (Figure 1A); 2) early maturation stage (41% water) (Figure 1B); and 3) advanced maturation stage (23% water) (Figure 1C). Levels of humidity were determined using the methodology of Brasil (2009). One canola silique was added to each Petri dish. To keep the plant tissue hydrated, moistened cotton

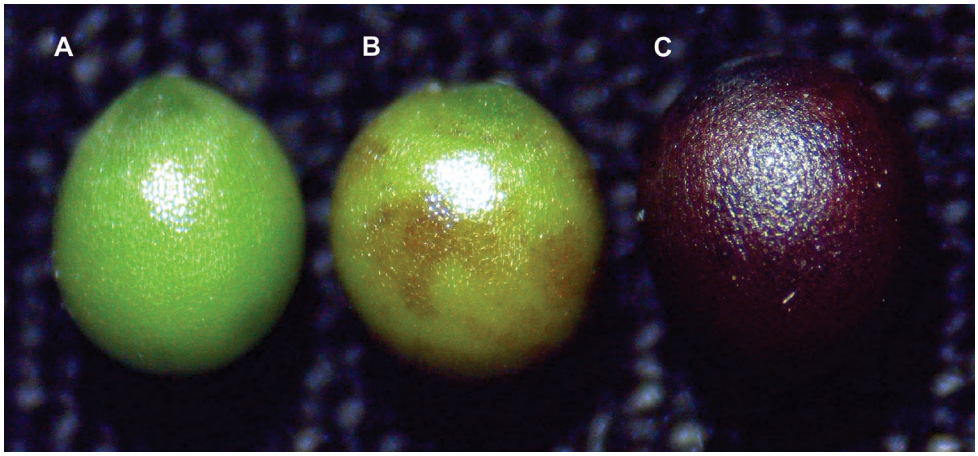

Figure 1. Canola seeds at different stages of maturation: green stage (A), early maturation (B) and advanced maturation (C). Photo: Alberto L. Marsaro Júnior.

was wrapped around the petiole of the silique and placed on a plastic lid (2 cm diameter). Siliques were replaced whenever necessary.

The Petri dishes were placed randomly in an environmental chamber maintained at 25 ± 1 °C and 65 ±5% RH and photoperiod of 14L:10D. Daily observations were made to check for nymph survivorship and instar change, to determine the following parameters: nymph developmental time, percentage survivorship during each stadium, and total developmental time (from 2nd instar to adult).

At adult emergence, the sex was determined, and fresh body weight was recorded using a precision electronic balance (Sartorius model BP210S, accurate to 0.0001 mg). In total, 40 nymphs reached adulthood, 13 adults on green stage seeds, 13 on advanced stage seeds, and 14 on early maturation seeds. For statistical analysis, we combined male and female weights to obtain more consistent results, because in the green stage treatment we obtained only 2 females.

2.3. Effect of stink bug feeding on different seed maturation stages

For this experiment, we used small plastic cages (11 × 11 × 3.5 cm) lined with filter paper, containing two canola siliques collected from plants with seeds at three different maturation stages: green stage, early maturation, and advanced maturation. The experiment was conducted in a completely randomized design with three treatments (maturation stages) and ten replicates each under laboratory conditions (25 ± 1 °C, 65 ± 10% RH, and 14 h photophase).

To keep the plant tissue turgid and prevent desiccation, the silique petiole was wrapped with moist cotton and placed inside a Petri dish (6.0 × 1.0 cm). One female *N. viridula* (ca. 20 days old) was added to each cage and allowed to feed for two days. After that, the siliques of each replicate were manually opened to obtain the seeds, which were individually examined under a stereomicroscope to determine the number of damaged and undamaged seeds. We considered damaged seeds those that showed signs of stylet insertion (i.e., presence of flanges, representing salivary sheath material outside the seed tissue) and softened endosperm with dark spots (Figures 2A-C). The data were used to calculate the percentage of damaged and undamaged seeds for each treatment (seed at green stage, early maturation, and advanced maturation).

Additionally, the dry weight of the seeds at different stages of maturation was taken. For each maturation stage (treatment), the seeds were separated into two groups, one containing undamaged seeds and the other with damaged seeds. After that, a sample containing 10 undamaged seeds and another containing 10 damaged seeds for each treatment was evaluated (six replications per group). Data were used to calculate the percentage of dry weight loss.

2.4. Statistical analysis

The data were subjected to the Bartlett test and Levene test to verify the homogeneity of variances and normality ($P < 0.05$), respectively, before ANOVA. If necessary, the data were transformed (see notation in the footnotes of tables and figures) to meet the requirements of ANOVA, using $\sqrt{x}$ or arcsine $\sqrt{(x/100)}$ for count or percentage data, respectively. The means of the treatments were separated by the Tukey test ($P < 0.05$). Statistical analyses were performed using the statistical program 'R', v.4.2.2 (R Development Core Team, 2022), applying native functions available in this program, including 'Bartlett.test' to check homogeneity of variances and 'aov' for the one-way ANOVA model. The Levene test was performed using the 'car' package (Fox and Weisberg, 2019), and the Tukey test was performed using the 'TukeyC' package (Faria et al., 2018).

3. Results

3.1. Nymphal biology and adult body weight at emergence

The majority (over 80%) of nymphs survived to reach adulthood while feeding on silique of canola containing seeds at different stages of maturation. Nymphs fed with seeds in the initial development stage (green stage) and advanced maturation seeds took less time to complete the 2nd stadium compared to early maturation seeds (f_{calc} = 5.207, df = 2;38, P = 0.01). For 3rd instars no significant difference was observed among treatments (f_{calc} = 1.314, df = 2;38, P = 0.281). During the 4th stadium, nymphs took longer to develop when fed with green stage seeds compared to advanced maturation seeds (f_{calc} = 7.632, df

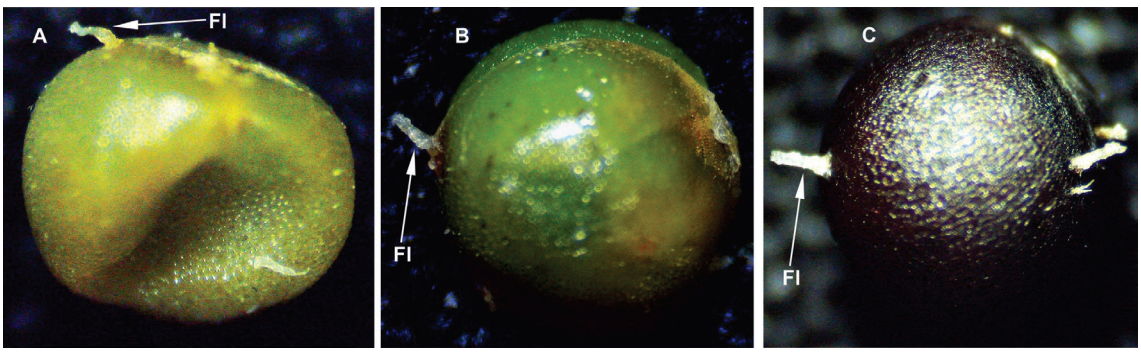

Figure 2. Canola seeds at different stages of maturation damaged by *Nezara viridula*: green stage (A), early maturation (B) and advanced maturation (C), with detail of the presence of flanges (Fl) - salivary sheath outside seed tissue. Photos: Alberto L. Marsaro Júnior.

= 2;38, P = 0.0016); on early maturation seeds, nymph developmental time was intermediate. For the 5th stadium and for total developmental time (from 2nd to adult), the duration was significantly longer on seeds at green stage and on early maturation seeds compared to advanced maturation seeds (5th instar: f_{calc} = 13.55, df = 2;36, P < 0.001; Total time: f_{calc} = 10.16, df = 2;36, P < 0.001) (Table 1).

On the first day of adult life, the fresh body weight of adults exhibited significant differences among the stages of seed maturation. Adults had a significantly higher fresh body weight when nymphs fed on seeds at the advanced maturation stage (145 mg), followed by those that consumed seeds at an early maturation stage (125 mg); those that fed on seeds in the green stage presented the lowest body weight (97 mg) (f_{calc} = 36.53, df = 2;36, P < 0.001) (Figure 3).

3.2. Effect of stink bug feeding on different seed maturation stages

Significant differences were observed in the percentage of seed damage and dry weight loss, underscoring the impact of stink bug attacks on seeds at these three maturation stages. The analysis revealed significant differences in both % seed damaged (F_{calc} = 64.24, df = 2;27, P < 0.001) and % dry weight loss (F_{calc} = 197.7, df = 2;15, P < 0.001) between damaged seeds compared to non-damaged seeds (Figures 4A-B).

Seeds at the green stage exhibited the highest values for both parameters, with approximately 80% of seeds being damaged and a 65% dry weight loss. In contrast, early and advanced maturation seeds displayed lower values, ca. 30% and 10%, respectively, for both % seed damaged and % dry weight loss. There was no significant difference between early and advanced maturation seeds in terms of these parameters (Figures 4A-B). The greater impact of damage by *N. viridula* to seeds at the green stage compared to those at the other stages of maturation can be seen by examining the inside of the seeds (Figure 5). The stylets damaged the seed radicle and the cotyledons.

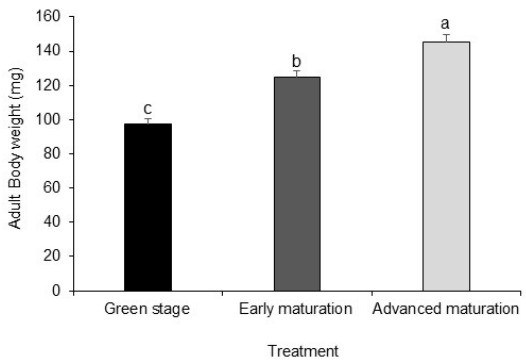

Figure 3. Fresh body weight (mg) of adult *Nezara viridula* on the first day of adult life feeding on siliques of canola at different stages of maturation under laboratory conditions. Means (± SE) followed by the same letter among treatments do not differ significantly (Tukey's test, P < 0.05).

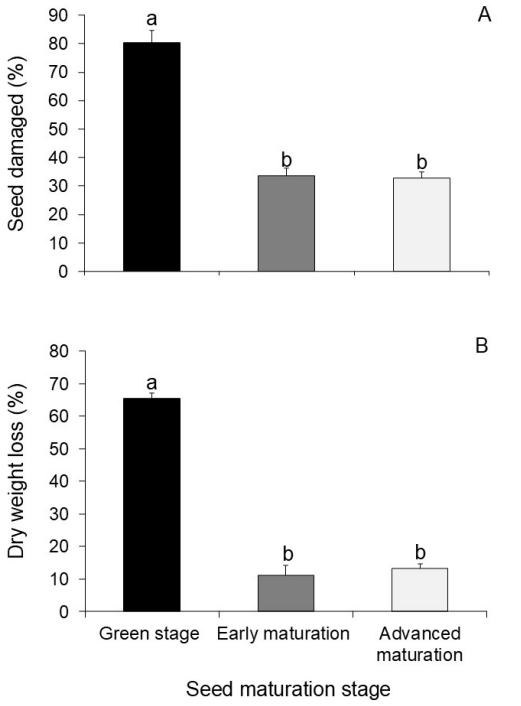

Figure 4. Percentage of seed damaged (A) and dry weight loss (B) caused by *Nezara viridula* adults on siliques of canola at different stages of maturation under laboratory conditions. Means (± SE) followed by the same letter within each variable do not differ significantly (Tukey's test, P < 0.05).

Table 1. Mean (±SE) developmental time and survivorship of *Nezara viridula* nymphs (n = 15) feeding on silique of canola at different stages of maturation under laboratory conditions.

Seed maturation stage	Instar duration (days)				Total developmental time (days)
	Second	Third	Fourth	Fifth	
Green stage	5.8 ± 0.4 b [14][a] (93%)[b]	5.3 ± 0.2 a [14] (93%)	6.3 ± 0.4 a [14] (93%)	11.0 ± 0.6 a [13] (80%)	27.8 ± 0.9 a
Early maturation	7.1 ± 0.4 a [14] (93%)	5.7 ± 0.4 a [14] (93%)	5.1 ± 0.5 ab [14] (93%)	10.6 ± 0.7 a [14] (93%)	28.5 ± 1.6 a
Advanced maturation	5.5 ± 0.4 b [13] (87%)	5.0 ± 0.2 a [13] (87%)	4.2 ± 0.2 b [13] (87%)	7.4 ± 0.1 b [13] (87%)	22.0 ± 0.4 b

Means (± SE) followed by the same letter in a column are not significantly different (Tukey's test, P < 0.05). [a]Number of nymphs; [b]Survivorship (%).

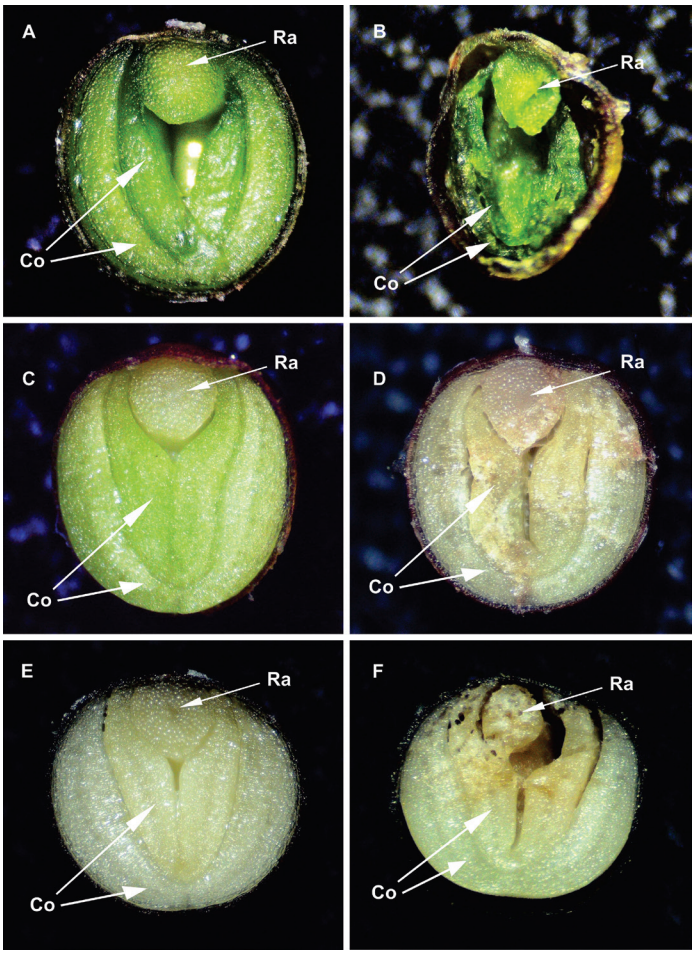

Figure 5. Canola seeds, in cross section, at different stages of maturity, without damage and damaged by *Nezara viridula*: seeds at green stage, without damage (A) and with damage (B); early maturation stage, without damage (C) and with damage (D); and advanced maturation seeds, without damage (E) and with damage (F). Details: Ra = radicle; Co = cotyledons. Photos: Alberto L. Marsaro Júnior.

4. Discussion

This study of stink bug behavior in relation to canola seed maturation stages offers valuable insights into the dynamics of pest-crop interactions. It reveals that *N. viridula* nymphs exhibited variable developmental times when feeding on seeds at different maturation stages (i.e., faster developmental time on advanced maturation seeds). In a similar way, adults achieved greater weight on advanced maturation seeds compared to seeds at the green stage. These results suggest that advanced maturation seeds provide better nutrition.

Oliveira et al. (2023) noted that nymphs of *N. viridula* survived and completed development on immature canola siliques without seeds (feeding only on the silique wall after manual seed removal), indicating the suitability of this food source for *N. viridula*. As canola plants progress to full flowering, the leaf count decreases, making stems and silique walls the predominant photosynthetic tissues (comprising over 65% of the total plant biomass) (Canola Council of Canada, 2024). Hence, this intriguing process may elucidate why nymphs can develop, albeit with delay, solely on immature silique walls.

Furthermore, Oliveira et al. (2023) observed that the *N. viridula* nymphs feeding on siliques containing green stage seeds completed their development in approximately 26 days with a high survival rate (93%), similar to the findings of our study. Therefore, their data suggest that silique walls provide sufficient nutrients to permit nymph development. In contrast, nymphs of *N. viridula* fed only vegetative structures of soybean, such as leaves and stems, did not complete development and all perished (Panizzi and Slansky Junior, 1991). On soybean, nymphs and adults of *N. viridula* do prefer immature soybean pods (with seeds) compared to immature seeds removed from the pods, and nymphs have lower mortality on soybean pods (Panizzi, 1987), which suggest the pod wall might play a role in their nutrition.

The greater fresh body weight of *N. viridula* adults fed as nymphs on advanced maturation seeds of canola compared to those fed on green and early seed maturation stages, further demonstrates that advanced maturation seeds are

a most suitable food source. Similarly to this study, Panizzi and Alves (1993) found that adult longevity, survivorship, oviposition, body weight, and body lipid gain were highest when reared on maturing soybean pods (R7). In addition, studying this same species of bug, Panizzi and Rossini (1987) found that the survival rate is higher when nymphs feed on mature soybean seeds rather than on green ones.

The fact that seeds at the green stage were more greatly damaged than early or advanced maturation seeds, might be related to the higher content of water and lower content of nutrients in the former compared to the latter food sources. This creates the need for higher frequency and longer duration of feeding events on the seeds at the green stage resulting in greater damage. A similar result, i.e., greater impact (62% of seeds damaged) was obtained with *N. viridula* feeding on immature siliques (Oliveira et al., 2023). As canola seeds mature there is an increase in the content of proteins and lipids (Borisjuk et al., 2013), which might explain the decreased seed damage observed in the early and advanced stages of maturation. Moreover, as canola seeds mature dry matter increases and water content decreases (Elias and Copeland, 2001; Castro et al., 2004).

The greater damage on seeds at the green stage might be further explained by the feeding strategies used by *N. viridula* (salivary sheath and laceration – Backus et al., 2005). Immature seeds are more susceptible to damage than mature seeds, because they are softer and easier to penetrate. Also *N. viridula* adults were demonstrated to use another strategy on immature siliques (rosetting), which consists in stylets penetrating the silique walls causing cell destruction and leaving whitish marks as illustrated by Oliveira et al. (2023).

5. Conclusions

This study adds relevant data on the biology of *N. viridula* nymphs and adults on canola seeds, and their damage, in particular with regard to the different stages of seed maturation. The fact that on advanced maturation seeds nymphs developed faster and yielded heavier adults, although causing less damage, demonstrates that this phenological stage can favor an increase in populations at the time of crop maturation in the field. This information is important and may be of use in the design of integrated pest management programs for *N. viridula* on this crop.

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