Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

ECOLOGY, BEHAVIOR AND BIONOMICS

Duration of Feeding and Superficial and In-Depth Damage to Soybean Seed by Selected Species of Stink Bugs (Heteroptera: Pentatomidae)

RA Depieri[1], AR Panizzi[2]

[1]Programa de Pós-Graduação em Ciências Biológicas, Univ Federal do Paraná, Depto de Zoologia, Curitiba, PR, Brasil
[2]Lab de Bioecologia de Percevejos, Embrapa Soja, Londrina, PR, Brasil

Keywords
Insecta, stink bug, soybean, seed damage, stylet penetration

Correspondence
Antônio R Panizzi, Lab de Bioecologia de Percevejos, Embrapa Soja, Londrina, PR, Brasil; Present address: Lab de Entomologia, Embrapa Trigo, Rod BR 285, km 294, CP 451, 99001-970, Passo Fundo, RS, Brasil; panizzi@cnpt.embrapa.br

Edited by Wesley A C Godoy – ESALQ/USP

Received 18 February 2010 and accepted 08 July 2010

Abstract

Laboratory studies were conducted to compare duration of feeding and superficial and in-depth damage to soybean (*Glycine max*) seeds by the Southern green stink bug, *Nezara viridula* (L.), the Neotropical brown stink bug, *Euschistus heros* (F.), the red-banded stink bug, *Piezodorus guildinii* (Westwood), and the green-belly stink bug, *Dichelops melacanthus* (Dallas). Results indicated that feeding time was significantly longer for *N. viridula* (≈ 133 min) compared to *E. heros* and *D. melacanthus* (≈ 70 min), but not different from *P. guildinii* (≈ 103 min). There was a positive correlation between feeding time and the resulting damage for *E. heros, N. viridula* and *P. guildinii* ($R^2 > 0.80$, $P < 0.0001$), but not for *D. melacanthus* ($R^2 = 0.1011$, $P = 0.1493$). The deepest seed damage (2.0 mm) was made by *P. guildinii* and the shallowest (0.5 mm) by *D. melacanthus*. The depth of the seed damage by *E. heros* and *N. viridula* (0.8, 1.2 mm, respectively) was intermediate in comparison to the other species studied. Feeding damage to the seed endosperm caused variable cell disruption and protein body dissolution, particularly when *P. guildinii* fed on seeds, suggesting that the deleterious action of salivary enzymes was greater for this bug compared to the others.

Introduction

Stink bugs are major pests of soybean in various parts of the world (Turnipseed & Kogan 1976, Panizzi & Slansky 1985, Kogan & Turnipseed 1987). Over 50 species are known to cause damage to this crop worldwide, and the Southern green stink bug, *Nezara viridula* (L.), is perhaps the most studied and economically important species. Other species such as the Neotropical brown stink bug, *Euschistus heros* (F.), and the red-banded stink bug, *Piezodorus guildinii* (Westwood), are also important pests in the neotropics (Panizzi *et al* 2000). A fourth species, the green-belly stink bug, *Dichelops melacanthus* (Dallas), occasionally found on soybeans, has recently become an important pest in Brazil damaging wheat and other summer crops such as corn (Chocorosqui & Panizzi 2004, 2008).

Pentatomid bugs inject salivary secretions into seeds and ingest the food slurry that salivary enzymes create. The impact of stink bug feeding on soybean seed has been studied by many authors worldwide (e.g., Miner 1966, Todd & Turnipseed 1974, Vicentini & Jimenez 1977, Panizzi *et al* 1979, Brier 1993), but only a few (Daugherty *et al* 1964, McPherson *et al* 1993, Sosa-Gómez & Moscardi 1995, Corrêa-Ferreira & Azevedo 2002) have compared the damage to soybean seeds by different species of stink bugs. To the best of our knowledge, none have studied the possible relationship between the duration of stink bug feeding and the amount of damage in (histological disruption) or on the seed.

Therefore, we conducted this study to determine if there is a relationship between insect feeding time and soybean seed damage and to compare superficial and internal damage to soybean seeds by four species of stink bugs.

Material and Methods

Insect colony

During November 2006 to March 2007 and from December 2007 to March 2008, adults of *D. melacanthus*, *E. heros*, *N. viridula*, and *P. guildinii* were collected at the Embrapa (Empresa Brasileira de Pesquisa Agropecuária) Farm in Londrina Co., Paraná State, Brazil (23º 11' S latitude, 51º 11' W longitude) from soybean fields. In the laboratory, mating pairs (n = 30) of each stink bug species were placed individually in clear plastic boxes (25 x 20 x 20 cm), and provided every other day with fresh food consisting of pods of green beans, *Phaseolus vulgaris*, raw shelled peanuts, *Arachis hypoagea* (Leguminosae), and berries of privet, *Ligustrum lucidum* (Oleaceae). Insects were reared to adults in a walk-in chamber at controlled conditions (25 ± 1°C;60 ± 10% RH; 14:10 h L:D).

Pre-feeding and feeding times and superficial damage to soybean seeds

To study pre-feeding and feeding time and superficial damage to soybean seeds, *D. melacanthus*, *E. heros*, *N. viridula*, and *P. guildinii* adults (n = 50 for each species) that had been provided only water for 24h were placed individually in a plastic Petri dish (6 x 2 cm). Each insect was offered a water-soaked mature soybean seed (cv. BRS-257) and feeding activity was observed continuously for 6h at 25 ± 1°C. Pre-feeding time was characterized as the interval of time from seed offering to the beginning of the first feeding session. This trait is important because it indicates the suitability of the food, i.e., the smallest the pre-feeding time the greater the acceptability of the food. Feeding time (feeding session) was characterized as the interval of time from the start of vertical head movements after the insertion of mouthparts into the seed to the withdrawal of the stylets. The sucked seed was kept in a germination chamber (25 ± 1ºC) for 24h to allow bioactivity of the salivary compounds. Tetrazolium (2,3,5-triphenyl tetrazolium chloride) (França-Neto *et al* 1998) was added to the entire seed to obtain color contrast of the seed damaged area. The seed was photographed into 20 x 20 mm square for measurement of the superficial damaged area using Photop software (IAPAR, Londrina, PR, Brazil).

In-depth damage to soybean seeds at a fixed feeding time

To compare the in-depth damage to soybean seeds for a set feeding time, adults (n = 250 for each species) of *D. melacanthus*, *E. heros*, *N. viridula*, and *P. guildinii* were fasted in the presence of water for 24h and placed individually in a plastic Petri dish (6 x 2 cm). Each insect was offered a water-soaked mature soybean seed (cv. BRS-257). In this test, each insect that showed feeding activity was allowed to feed on a seed continuously for 60 min, and then the feeding session was discontinued. To measure the in-depth damage to the seeds, each seed was thinly sliced under the stereomicroscope using a razor blade. When the center of the damaged area was reached, the depth of the damage was measured (mm) using an ocular micrometer.

Histological seed damage

For histological studies, the section of any damaged seed fed upon by an insect was obtained and prepared according to the following procedure. Sections were cut into small cubes (1 mm^3) and fixed by immersion overnight into a mix of EM grade 3% glutaraldehyde and 2% paraformaldehyde in a sodium cacodylate buffer (0.2M, pH 7.2). Samples were rinsed thrice (15 min) with 0.1M sodium cacodylate buffer to remove all residual fixative. Post-fixation was made in 1% cacodylate-buffered osmium tetroxide. Seed cubes were dehydrated in an ethanol graded series, diaphanized in EM grade propylene oxide (1,2-epoxy propane) and gradually infiltrated with mixtures of Araldite 502® epoxy resin and propylene oxide followed by polymerization at 60º C during 24h in silicone flat embedding block mold.

The blocks (n = 5) containing portions of the seeds damaged by each species tested were razor blade trimmed and sectioned (70 nm) on a Leica Ultracut UCT® (Wetzlar, Germany). Sections were collected on 200 mesh grids and stained with 2% uranyl acetate in methanol and Reynolds lead citrate for contrast formation in bright field mode on a Fei Tecnai T12® (Eindhoven, Netherlands) electron transmission microscope. Histological damage was photographed. Eight damaged seeds were examined for each insect species studied.

Data analysis

The experimental design for all trials was completely randomized. The data on pre-feeding and feeding durations, superficial seed damage in variable and fixed times, depth of the seed damage were submitted to ANOVA (SAS Institute Inc. 1998). When appropriate, means were compared using the Tukey test ($P \leq 0.05$). Feeding time and the resulting damage for all stink bug species were analyzed using regression analysis calculating the coefficient of correlation (R^2).

Results and Discussion

Pre-feeding and feeding times and superficial damage to soybean seeds

Duration of pre-feeding time varied from 103 min (*N. viridula*) to 158 min (*E. heros*), but it was not significantly different among stink bug species ($P > 0.05$) (Table 1). Considering that the bugs were not fed for 24h before the test and food were offered in a small arena, these pre-feeding times were relatively long. The observed "delay" for all species to engage in feeding could be due to the lack of a prompt recognition of this food source, since stink bugs in nature feed mostly on immature seeds inside pods.

Feeding time was significantly longer for *N. viridula* (≈ 133 min) compared to *E. heros* and *D. melacanthus* (≈ 70 min), but similar to *P. guildinii* (Table 1). Little information is found in the literature regarding feeding time of adult pentatomids. Simmons & Yeargan (1988) reported that nymphs and adults of the Green stink bug *Acrosternum hilare* (Say) fed ≈ 90 min on water-soaked mature soybean seeds. Similarly, starved *N. viridula* fed for a mean time of 92.5 min (Panizzi 1995). In addition, mean feeding time (71.1 min) for *Euschistus servus* (Say) nymphs and adults feeding on shelled green beans (Tillman & Mullinix Jr 2004) was very similar to that found for *E. heros*. The resulting superficial damage (area) to the seeds varied from 4.0 mm^2 caused by *P. guildinii* down to 0.3 mm^2 caused by *D. melacanthus* (Table 1).

There was a positive correlation between feeding time and the resulting damage for *E. heros*, *N. viridula* and *P. guildinii* ($R^2 > 0.80$, $P < 0.0001$, Fig 1b-d). However, this correlation was not significant for *D. melacanthus* ($R^2 = 0.1011$, $P = 0.1493$, Fig 1a). This laboratory feeding behavior of *D. melacanthus* is consistent with known crop preferences for this stink bug species, as it is primarily a pest of corn and wheat, and, only occasionally, a pest of soybean (Ávila & Panizzi 1995, Chocorosqui & Panizzi 2004).

In-depth damage to soybean seeds at a fixed time

In-depth feeding damage to mature soybean seeds caused by the different species of pentatomids studied showed that *P. guildinii* feeding resulted in the deepest seed damage, while *D. melacanthus* feeding resulted in the shallowest seed damage in comparison to the other species. Feeding by *E. heros* and *N. viridula* yielded intermediate values of seed damage compared to other stink bug species (Table 2).

Histological seed damage

For each pentatomid species, feeding on soybean seeds damages the endosperm (Figs 2 a-h). The histological damage is characterized by the disruption of cells caused by the boring of stylets into the seed and by the fusionation (PBf in Fig 2b) and dissolution (PBd in Fig 2h) of protein bodies caused by the salivary digestive enzymes. The salivary digestive enzymes disturbs the physiology and biochemistry of the tissues surrounding the "pierce" canal (PC in Figs 2c-g) as described by Hori (2000). The saliva spreads (Nuorteva & Reinius 1953), and cell contents can be damaged as far as 3.5 mm from the penetration point without mechanical damage, suggesting either general maceration of tissues or withdraw of cytoplasmatic content by osmotic pump feeding (Miles 1987).

The disruption of endosperm cells is illustrated

Table 1 Mean (± SEM) duration of pre-feeding and feeding times, and area of superficial damage to mature soybean seed by four species of pentatomids (initial number of bugs = 50).

Species	Pre-feeding duration (min) (mean ± SE)^{ns}	Feeding duration (min) (mean ± SE)	Superficial damage (mm^2) (mean ± SE)
Dichelops melacanthus	150.2 ± 22.58 (22)	71.4 ± 7.37 b (22)	0.3 ± 0.08 d (22)
Euschistus heros	157.9 ± 23.58 (22)	69.8 ± 7.47 b (22)	1.0 ± 0.12 c (22)
Nezara viridula	103.0 ± 14.21 (32)	133.3 ± 13.75 a (32)	2.4 ± 0.27 b (32)
Piezodorus guildinii	113.2 ±17.41 (30)	102.7 ± 13.02 ab (30)	4.0 ± 0.57 a (30)

Means in each column followed by the same letter do not differ significantly using the Tukey test ($P > 0.05$); ^{ns}non significant.

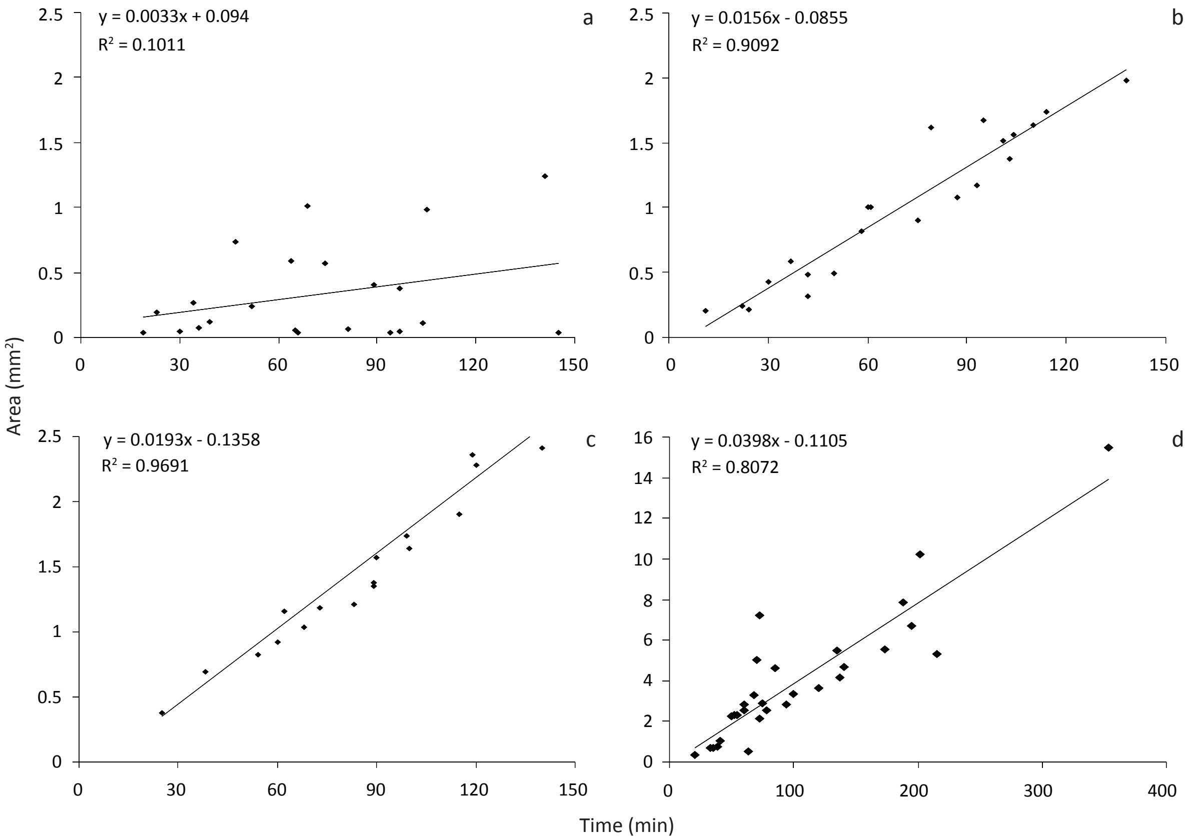

Fig 1 Correlation of feeding time and damaged area on mature soybean seed caused by different species of pentatomids in the laboratory. *Dichelops melacanthus* (a); *Euschistus heros* (b); *Nezara viridula* (c); and *Piezodorus guildinii* (d).

in detail in Fig 3. The undamaged seed has an intact protein body (PB) and cellulose wall (CW) (Fig 3a). On the remaining figures (seeds damaged by the bugs) the protein bodies are shown in different degrees of dissolution. Note that for *P. guildinii* (Figs 3e,f) the protein bodies are completely dissolved, suggesting that the deleterious action of salivary enzymes to seed tissues is greater for this bug compared to that for the other species. Salivary pectinases are possibly responsible for the start of tissue damage (Miles & Taylor 1994, Frati *et al* 2006). Pectinases are known to disrupt the middle lamella of plant cell walls (Batemann & Miller 1966) and cause softening and death of cells surrounding the injured area (Hori 2000).

Table 2 Mean (± SEM) in-depth soybean seed damage caused by four species of pentatomids after 60 min of feeding (initial number of bugs = 250).

Species	In-depth seed damage (mm) (mean ± SE)	% of cohort fed (mean ± SE)[1]
Dichelops melacanthus	0.5 ± 0.07 d (99)	44.4 ± 6.27 b
Euschistus heros	0.8 ± 0.06 c (133)	52.4 ± 2.93 b
Nezara viridula	1.2 ± 0.05 b (109)	68.4 ± 3.87 a
Piezodorus guildinii	2.0 ± 0.08 a (101)	41.2 ± 1.85 b

Means in each column followed by the same letter do not differ significantly using the Tukey test (P > 0.05).
[1] Mean of five replicates of 50 insects.

Piezodorus guildinii is known to cause greater damage to soybean compared to other common species of pentatomids (Sosa-Gómez & Moscardi 1995, Corrêa-Ferreira & Azevedo 2002). The greater damage caused by *P. guildinii* is not related to the length of the stylets, since this species have shorter mouthparts than *N. viridula* and *E. heros* (Panizzi & Machado Neto 1992). Our data indicate that the greater damage is due to the higher level of seed tissue damage observed for this species in comparison to the other three stink bug species.

Results of these studies demonstrate that with the exception of *D. melacanthus*, seed damage is proportional to feeding time. Also, the red-banded stink bug, *P. guildinii*, is the most harmful bug to soybean seeds among the stink bug species studied, and this is due to the chemical dissolution of seed tissues caused by its saliva. *Dichelops melacanthus* is the least damaging of all four stink bug species.

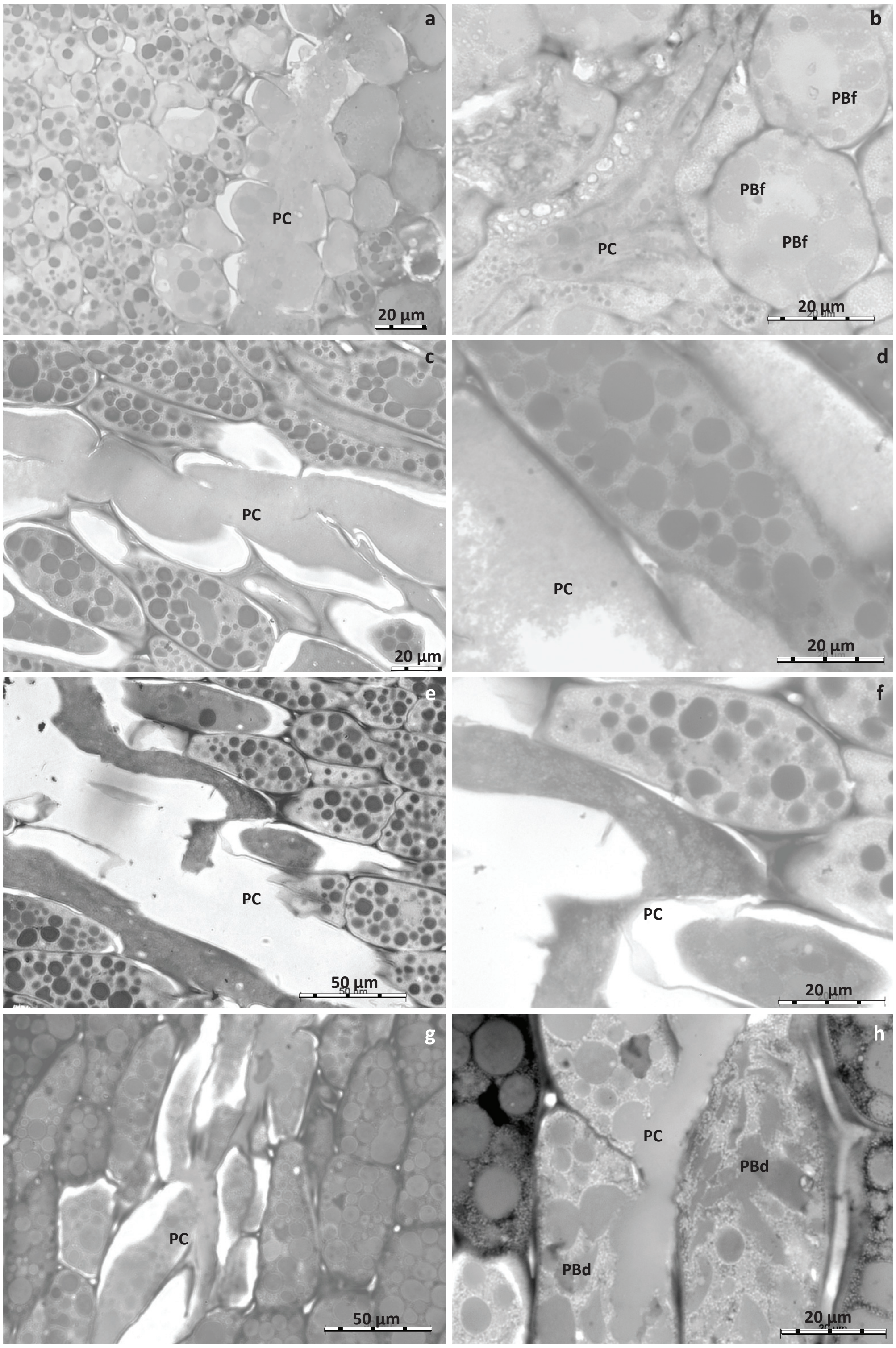

Fig 2 Endosperm tissue of mature soybean seeds damaged by stink bugs. *Dichelops melacanthus* (a, b); *Euschistus heros* (c, d); *Nezara viridula* (e, f); and *Piezodorus guildinii* (g, h). Pierce canal (PC); Protein body fusion (PBf); and dissolved protein body (PBd).

Acknowledgments

We thank Glynn Tillman, Norman Neumaier, and Daniel R. Sosa-Gómez for critically reading the manuscript. This research was supported by a scholarship to RAD from the National Research Council for Scientific and Technological Development (CNPq), and through laboratory facilities provided by the Embrapa National Soybean Research Center, and Paraná State University at Londrina, PR, Brazil. This paper was approved for publication by the Editorial Board of Embrapa Soja as manuscript number 18/2009.

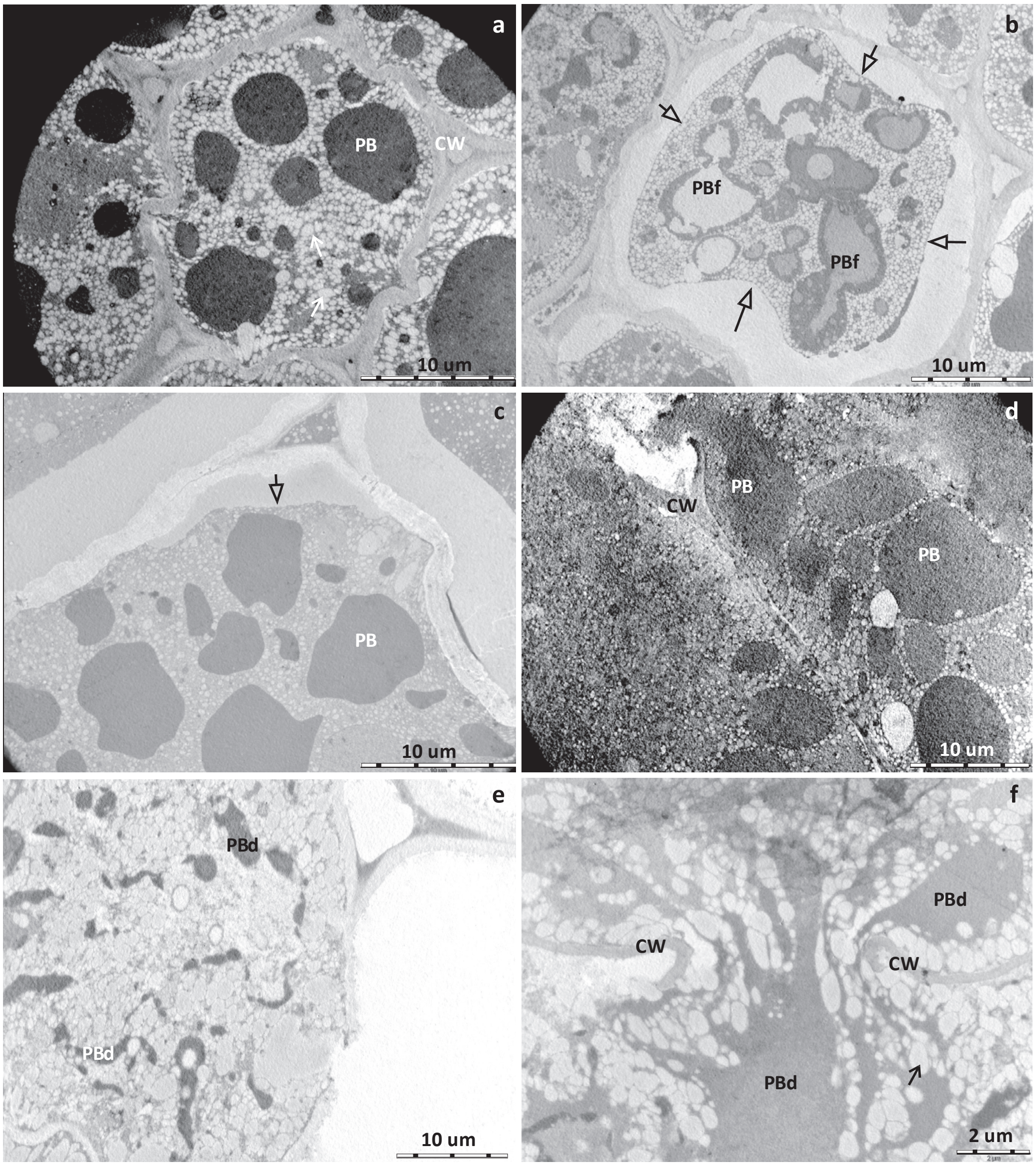

Fig 3 Endosperm cells of mature soybean seeds. No damaged seed (a); Endosperm cells of seed sucked by *Dichelops melacanthus* (b); *Euschistus heros* (c); *Nezara viridula* (d); and *Piezodorus guildinii* (e, f). Cellulose wall (CW); Protein body (PB); Protein body fusion (PBf); and protein body dissolved (PBd). Open arrows indicate spherosomes; closed arrows indicate shrinkage of the cell membrane; see text for explanation.

References

Ávila CJ, Panizzi AR (1995) Occurrence and damage by *Dichelops* (*Neodichelops*) *melacanthus* (Dallas) (Heteroptera: Pentatomidae) on corn. An Soc Entomol Brasil 24: 193-194.

Batemann DF, Miller RL (1966) Pectic enzymes in tissue degradation. Annu Rev Phytopathol 4: 119-146.

Brier H (1993). Extent of bug damage in soybeans dependent on species. Northern Focus 4-5.

Chocorosqui VR, Panizzi AR (2004) Impact of cultivation systems on *Dichelops melacanthus* (Dallas) (Heteroptera: Pentatomidae) population and damage and its chemical control on wheat. Neotrop Entomol 33: 487-492.

Chocorosqui VR, Panizzi AR (2008) Nymph and adult biology of *Dichelops melacanthus* (Dallas) feeding on cultivated and non-cultivated host plants. Neotrop Entomol 37: 353-360.

Corrêa-Ferreira BS, Azevedo J (2002) Soybean seed damage by different species of stink bugs. Agric Forest Entomol 4: 145-150.

Daugherty DM, Neustadt MH, Gehrke CW, Cavanah LE, Williams LF, Green DE (1964) An evaluation of damage to soybeans by brown and green stink bugs. J Econ Entomol 59: 719-722.

França Neto JB, Krzyzanowski FC, Costa NP (1998) O teste de tetrazólio em sementes de soja. Londrina, EMBRAPA-CNPSo, Documentos 116, 72p.

Frati F, Galletti R, De Lorenzo G, Salerno G, Conti R (2006) Activity of endo-polygaracturonases in mirid bugs (Heteroptera: Miridae) and their inhibition by plant cell wall proteins (PGIPs). Eur J Entomol 103: 515-522.

Hori K (2000) Possible causes of disease symptoms resulting from the feeding of phytophagous Heteroptera, p.11-35. In Schaefer CW, Panizzi A R (eds) Heteroptera of economic importance. Boca Raton, FL, CRC Press, 828p.

Kogan M, Turnipseed SG (1987) Ecology and management of soybean arthropods. Annu Rev Entomol 32: 507-538.

McPherson RM, Douce GK, Hudson RD (1993) Annual variation in stink bug (Heteroptera: Pentatomidae) seasonal abundance and species composition in Georgia soybean and its impact on yield and quality. J Entomol Sci 28: 61-72.

Miles PW (1987) Plant sucking bugs can remove the content of cells without mechanical damage. Experientia 43: 937-939.

Miles PW, Taylor GS (1994) Osmotic pump feeding by coreids. Entomol Exp Appl 73: 163-173.

Miner FD (1966) Biology and control of stink bugs on soybeans. Ark Agric Expt Stn Bull 708, 40p.

Nuorteva P, Reinius L (1953) Incorporation and spread of C14-labeled oral secretions of wheat bugs in wheat kernels. Ann Entomol Fenn 19: 95-104.

Panizzi AR (1995) Feeding frequency, duration and preference of the southern green stink bug (Heteroptera: Pentatomidae) as affected by stage of development, age, and physiological condition. An Soc Entomol Brasil 24: 437-444.

Panizzi AR, Machado Neto E (1992) Development of nymphs and feeding habits of nymphal and adult *Edessa meditabunda* (Heteroptera: Pentatomidae) on soybean and sunflower. Ann Entomol Soc Am 85: 477-481.

Panizzi AR, McPherson JE, James DG, Javahery M, McPherson RM (2000). Stink bugs (Pentatomidae), p.421-474. In Schaefer CW, Panizzi AR (eds) Heteroptera of economic importance. Boca Raton, FL, CRC Press, 828p.

Panizzi AR, Slansky Jr F (1985) Review of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybean in the Americas. Fla Entomol 68: 184-203.

Panizzi AR, Smith JG, Pereira LAG, Yamashita J (1979) Efeitos dos danos de *Piezodorus guildinii* (Westwood, 1837) no rendimento e qualidade da soja. Anais I Semin Nac Pesq Soja, Londrina, PR, Vol. II, p. 59-78.

Simmons AM, Yeargan KV (1988) Feeding frequency and feeding duration of the green stink bug (Hemiptera: Pentatomidae) on soybean. Environ Entomol 81: 812-815.

SAS Institute Inc. (1998) SAS user's guide: statistics. Version 6.12. SAS Institute, Cary, NC, USA.

Sosa-Gómez DR, Moscardi F (1995) Retenção foliar diferencial em soja provocada por percevejos (Heteroptera: Pentatomidae). An Soc Entomol Brasil 24: 401-404.

Tillman PG, Mullinix Jr BG (2004) Comparison of susceptibility of pest *Euschistus servus* and predator *Podisus maculiventris* (Heteroptera: Pentatomidae) to selected insecticides. J Econ Entomol 97: 800-806.

Todd JW, Turnipseed SG (1974) Effects of southern green stink bug damage on yield and quality of soybeans. J Econ Entomol 67: 421-426.

Turnipseed SG, Kogan M (1976) Soybean entomology. Annu Rev Entomol 21: 247-282.

Vicentini R, Jimenez HA (1977) El vaneo de los frutos en soja. INTA Serie Tecnica 47, 30p.