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Podisus nigrispinus (Heteroptera: Pentatomidae) and IAC-24 Soybean Cultivar Are Compatible

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Abstract

The main tactics of soybean integrated pest management combine plant resistance and biological control. The impact of insect resistant IAC-24 soybean cultivar [caterpillars of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) in UFVS-2006 soybean cultivar susceptible to insects and water compared to *A. gemmatalis* in IAC-24 soybean cultivar resistant to insects and water] on the nymphal and adult stages of the predator *Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae) was evaluated. The duration and survival of the instars and nymphal stage and the body mass of nymphs, males and females of this predator were similar between treatments. The periods of pre-oviposition, oviposition, post-oviposition and egg incubation; interval between egg clutch; number of egg clutch per female, eggs per egg clutch, eggs per female, nymphs per female, nymphs per egg clutch and percentage of nymph eclosion and male and female longevity were also similar between treatments. The use of insect resistant IAC-24 soybean cultivar on *P. nigrispinus* was found to be safe because that legume did not have any deleterious effects on the referred predator.

Keywords: Asopinae, insect resistant, Lepidoptera, predator, soybean plants, stinkbug, *Tenebrio molitor*, velvetbean caterpillar

1. Introduction

Anticarsia gemmatalis Hübner, 1818 (Lepidoptera: Noctuidae) is the main soybean pest in Brazil, causing defoliation and reducing the productivity of this crop (Fugi et al., 2005). This pest has been controlled with insecticides (Pereira et al., 2002), but the chemical control can cause pest resistance, death of natural enemies, environmental contamination and toxicity to those who apply insecticides (Zanuncio et al., 1998; Tavares et al., 2009, 2010a). Thus, the tactics that combines host plant resistance and biological control in soybean integrated pest management (IMP) may be of considerable importance (Matos Neto et al., 2002, 2004; Tavares et al., 2010b, 2011).

The antibiosis mechanism related to the deleterious effects on survival, development and reproduction can occur in soybean plants because the new varieties possess toxic to insects, as protease inhibitors that act on defoliators and flavonoids (Kraemer, 2001). However, the soybean transgenic line expressing the gene *cry Ac* from *Bacillus thuringiensis* Berliner, 1915 (Bacillales: Bacillaceae), cultivated mainly in the USA, can confer resistance to defoliating caterpillars (Macrae et al., 2005). On the other hand, Brazil has cultivated transgenic soybean resistant to glyphosate and used in no-tillage systems, which, however, was found to have no effect on soybean pests. This crop system increased, in some cases, the number of pests because of its high toxicity to natural enemies by herbicide (Berman et al., 2010).

Natural enemies are abundant in soybean crops, but those of the genus *Podisus* (Heteroptera: Pentatomidae) efficiently reduce flying pests to levels that do not affect the economy. Moreover, these predators are polyphagous and migrate between forest and agricultural crops to feed on Lepidoptera pests, especially those of

A. gemmatalis (Zanuncio et al., 1994; De Clercq et al., 1998; Ferreira et al., 2009).

Poorly nutritious prey, such as larvae of *Musca domestica* L., 1758 (Diptera: Muscidae) and caterpillars of *Trichoplusia ni* Hübner, 1803 (Lepidoptera: Noctuidae) reduced the growth and the fertility and increased the development and mortality of predators (Wittmeyer et al., 2001; Zanuncio et al., 2001), but this effect was reduced for *Podisus nigrispinus* Dallas, 1851 (Heteroptera: Pentatomidae) when branches of *Eucalyptus cloeziana* F. Muell or *Psidium guajava* L. (Myrtaceae) were added to the diet of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) pupae (Lemos et al., 2009a). However, the mortality rate of *Geocoris punctipes* Say, 1832 (Hemiptera: Lygaeidae) was higher with caterpillars of *A. gemmatalis* in insect resistant soybean cultivar (Rogers & Sullivan, 1986) and *Nabis roseipennis* Reuter, 1872 (Hemiptera: Nabidae) had slow development and reduced fertility with caterpillars of *Pseudoplusia includens* Walker, 1857 (Lepidoptera: Noctuidae) in insect resistant soybean cultivar (Pfannenstiel & Yeargan, 1998), indicating that such crops may have indirect impact on polyphagous insects.

The present study was aimed to assess the duration, survival, body mass, reproductive characteristics and the longevity of nymphs and adults of *P. nigrispinus* with caterpillars of *A. gemmatalis* in insect resistant soybean cultivar IAC-24 compared to UFVS-2006 cultivar susceptible to insects.

2. Materials and Methods

2.1 Soybean Cultivar

Seeds of the soybean cultivars IAC-24 (resistant to insects) and UFVS-2006 (susceptible to insects) were obtained at Agronomic Institute of Campinas (IAC) and at the Federal University of Viçosa (UFV), respectively. These cultivars were sown in pots in the experimental area of the Insectarium - Department of Animal Biology (DBA) - UFV in Viçosa, Minas Gerais State, Brazil. The fertilization was done on plants at stages V7 or V6 (vegetative) up to R6 (reproductive), according to technical recommendations of the Soil Fertility Commission of the State of Minas Gerais (Corrêa-Ferreira, 2005).

2.2 Caterpillars of Anticarsia Gemmatalis

Caterpillars of third or fourth instar larvae of *A. gemmatalis* were obtained by intensively nourishing this prey on artificial diet (125.0g - black-eye bean; 62.4g - yeast; 100.0g - wheat germ; 100.0g - soybean protein; 50.0g - casein; 35.0g - Agar; 5.0g - Nipagin; 6.0g - ascorbic acid; 3.0g - sorbic acid; 6.0mL - formaldehyde 40.0% and 10.0mL - vitamin solution) and nutrient solution (10.5g - honey; 1.05L - distilled water; 350.0mL - beer; 60.0g - sucrose; 1.05g - ascorbic acid and 1.05g - Nipagin) of the Laboratory of Biological Control of Insects (LCBI) - DBA - UFV and soybean leaflets of UFVS-2006 or IAC-24 cultivars.

2.3 Pupae of Tenebrio Molitor

Pupae of *T. molitor* were obtained from intensive nourishment this prey on wheat bran and yeast of the LCBI - DBA - UFV. Also, these insects are often given sliced chayote and sugarcane as a source of moisture and sheets on the substrate to prevent luminosity and the stress of the animals.

2.4 Nymphs of Podisus Nigrispinus

Egg clutch of *P. nigrispinus* were obtained by intensively nourishing this predator on pupae of *T. molitor*, branches of *Eucalyptus* spp. and distilled water of the LCBI - DBA - UFV.

2.5 Description of the Experiment

Egg clutch of *P. nigrispinus* were individualized in Petri dishes (9.0 ± 1.2cm) with a cotton swab moistened with distilled water within them, to prevent the drying of the eggs. The second instar nymphs of this predator were fed on caterpillars of *A. gemmatalis* reared in UFVS (T1) or IAC-24 (T2) cultivars or soybean leaflets (T3) in the Petri dishes. These nymphs were transferred to 500.0mL transparent plastic pots in groups of 10 individuals from the beginning of the third stage, according to the treatment (Zanuncio et al., 2011).

The indirect impact of insect resistant soybean cultivar IAC-24 on the nymphal development, body mass, reproductive characteristics and longevity of *P. nigrispinus* was assessed with caterpillars of *A. gemmatalis* in UFVS-2006 or IAC-24 cultivars, though without soybean plants. This trial was performed in laboratory with individuals of *P. nigrispinus* and caterpillars of third or fourth instars (1.5 to 2.0cm long) of *A. gemmatalis* in transparent plastic pots 500.0mL in the treatments: T1- *P. nigrispinus* and caterpillars of *A. gemmatalis* in UFVS-2006 and T2- *P. nigrispinus* and caterpillars of *A. gemmatalis* in IAC-24, with 11 repetitions, each one in a 500.0mL transparent plastic pot, with 10 individuals of *P. nigrispinus*. The prey were provided *ad libitum* and replaced as necessary.

Eleven couples of *P. nigrispinus* were individualized per treatment after the nymphs reach adulthood. Each couple placed in a 500.0mL transparent plastic pot was considered a repetition.

2.6 Variables Analyzed

The duration and survival of the I, II, III, IV and V instars and of the nymphal stage; body mass of nymphs in the first day of the III, IV and V instars and of newly emerged males and females; periods of pre-oviposition (emergence of the female to day before the first egg clutch), oviposition (first to last egg clutch), post-oviposition (last egg clutch to death) and of incubation of eggs; interval between egg clutch; number of egg clutch per female, eggs per egg clutch, eggs per female, nymphs per female, nymphs per egg clutch; percentage of nymph eclosion and male and female longevity of *P. nigrispinus* were evaluated. The body mass of this predator was obtained with Coleman FA1604 precision balance with a readability of 0.1mg.

2.7 Statistical Analysis

The design was entirely casualized and the data were submitted to variance analysis at 5% probability. The data on the survival and body mass of nymphs were submitted to nonparametric analysis by Wilcoxon test at 5% probability since they did not meet the assumptions of variance homogeneity and normal distribution. The data on body mass of nymphs of III instar and of males were transformed into log (x + 1) because they did not adjust to the conditions of variance homogeneity and normal distribution and were submitted to variance analysis at 5% probability. Analyses were processed using the software SAS (Supplier: UFV) (SAS Institute, 1989).

3. Results

3.1 Duration and Survival

The duration and survival of the instars and the nymphal stage of *P. nigrispinus* were similar between treatments (F, $P > 0.05$) (Table 1).

Table 1. Duration and survival (average ± standard error of mean) of the I, II, III and IV instars and of the nymphal stage of *Podisus nigrispinus* (Heteroptera: Pentatomidae) with caterpillars of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars UFVS-2006 (T1) susceptible or UFVS-2006 (T2) resistant to insects. Viçosa, Minas Gerais State, Brazil

	Duration (Days)		Survival (%)	
Instars	T1 (UFVS-2006)	T2 (IAC-24)	T1 (UFVS-2006)	T2 (IAC-24)
I[1]	3.18 ± 0.10	3.19 ± 0.14	98.18 ± 1.22	99.09 ± 0.91
II[1]	4.65 ± 0.18	4.28 ± 0.16	86.06 ± 4.13	91.72 ± 2.27
III[1]	4.79 ± 0.19	4.75 ± 0.17	88.11 ± 3.16	85.91 ± 2.57
IV[1]	5.11 ± 0.18	5.02 ± 0.24	88.76 ± 4.14	77.69 ± 5.91
V[1]	6.88 ± 0.30	6.91 ± 0.28	73.23 ± 6.34	76.54 ± 4.56
Nimphal stage[1]	24.61 ± 0.18	24.14 ± 0.21	47.27 ± 4.69	44.55 ± 2.91

[1] No significant at 5% by F test

3.2 Body Mass

The body mass of nymphs and of male and female adults of *P. nigrispinus* were similar between treatments (F, $P > 0.05$) (Table 2).

Table 2. Body mass of nymphs (average ± standard error of mean) in the first day of the III, IV and V instars after ecdysis and of adult newly emerged of *Podisus nigrispinus* (Heteroptera: Pentatomidae) with caterpillars of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars UFVS-2006 (T1) susceptible or UFVS-2006 (T2) resistant to insects. Viçosa, Minas Gerais State, Brazil

	Body Mass (mg)	
Instars	T1 (Susceptible)	T2 (Resistant)
III[1, 2]	3.50 ± 0.22	3.32 ± 0.19
IV[1]	9.72 ± 0.59	9.66 ± 0.40
V[1]	22.87 ± 0.97	22.00 ± 0.88
Male[1, 2]	33.00 ± 0.41	34.53 ± 0.93
Female[1]	49.85 ± 1.10	48.05 ± 0.86

[1] No significant to 5% by F test; [2] Data transformed in log (x + 1), for statistical analysis

3.3 Pre-oviposition, Oviposition and Post-oviposition

Prey of *A. gemmatalis* with soybean cultivars resistant or susceptible to insects did not affect the pre-oviposition (t= 0.53, gl= 20, and P> 0.05), oviposition (t= 0.93, gl= 20, and P> 0.05), and post-oviposition (t= 1.19, gl= 20, and P> 0.05) periods of females of *P. nigrispinus* (Table 3).

Table 3. Reproductive characteristics (average ± standard error of mean) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) with caterpillars of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars UFVS-2006 (T1) susceptible or UFVS-2006 (T2) resistant to insects. Viçosa, Minas Gerais State, Brazil.

Characteristics evaluated	T1 (UFVS-2006)	T2 (IAC-24)	Prob.[1]
Pre-oviposition period (days)	6.18 ± 0.42	5.91 ± 0.28	0.30
Oviposition period (days)	32.55 ± 4.17	27.55 ± 3.40	0.18
Post-oviposition period (days)	0.73 ± 0.47	1.82 ± 0.78	0.12
Interval between egg clutch (days)	2.35 ± 0.23	2.01 ± 0.17	0.13
Egg incubation period (days)	4.79 ± 0.05	4.75 ± 0.03	0.23
Number of egg clutch/female	15.91 ± 2.63	14.55 ± 1.50	0.33
Number of eggs/female	248.00 ± 44.03	248.00 ± 32.89	0.50
Number of eggs/egg clutch	15.89 ± 0.88	16.93 ± 1.34	0.26
Percentage of nymph's eclosion (%)	93.94 ± 2.04	90.41 ± 2.07	0.12
Number of nimphs/female	230.36 ± 40.41	227.91 ± 32.54	0.48

[1] "t" test of Student

3.4 Parameters

The interval between egg clutch (t= 1.16, gl= 20, and P> 0.05), egg incubation period (t= 0.74, gl= 20, and P> 0.05), percentage of nymph eclosion (t= 1.21, gl= 20, and P> 0.05) and number of egg clutch per female (t= 0.45, gl= 20, and P> 0.05), eggs per female (t= 0,00, gl= 20, and P> 0.05), eggs per egg clutch (t= 0.64, gl= 20, and P> 0.05) and of nymphs per female of *P. nigrispinus* (t= 0.04, gl= 20, and P> 0.05) were similar between treatments (Table 3).

3.5 Longevity

The longevity of males (t= 0.79, gl= 20, and P> 0.05) and females (t= 0.75, gl= 20, and P> 0.05) of *P. nigrispinus* was similar between treatments (Figure 1), but with greater initial mortality of females of this predator in the insect resistant IAC-24 soybean cultivar (Figure 2).

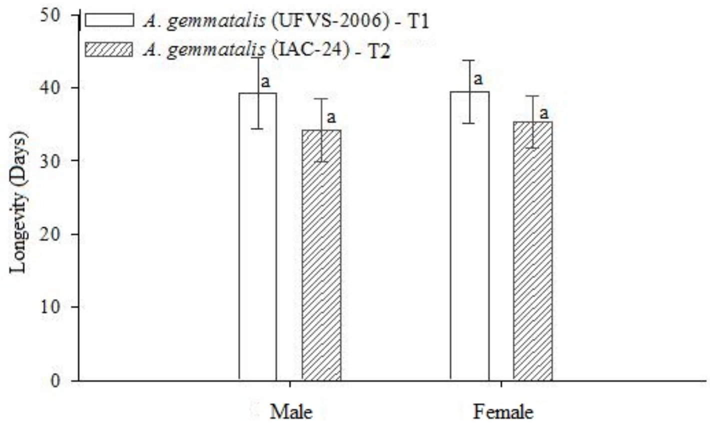

Figure 1. Longevity (average ± standard error of mean) of males and females of *Podisus nigrispinus* (Heteroptera: Pentatomidae) with caterpillars of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars UFVS-2006 (T1) susceptible or UFVS-2006 (T2) resistant to insects. Viçosa, Minas Gerais State, Brazil. Columns with same letter by sex do not differ by "t" test of Student ($P > 0.05$).

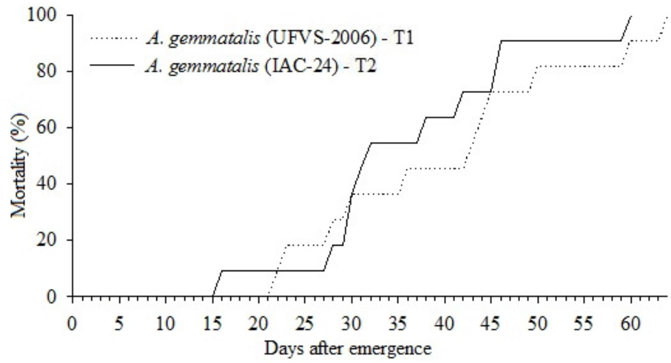

Figure 2. Mortality (%) of females of *Podisus nigrispinus* (Heteroptera: Pentatomidae) with caterpillars of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars UFVS-2006 (T1) susceptible or UFVS-2006 (T2) resistant to insects. Viçosa, Minas Gerais State, Brazil.

4. Discussion

A similar duration of the instars and nymphal stage of *P. nigrispinus* with caterpillars of *A. gemmatalis* in IAC-24 or UFV-2006 soybean cultivars was similar regarding the lack of impact of the *Bt* transgenic cotton on *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) in the nymphal stage of the predator *Orius sauteri* Poppius, 1909 (Heteroptera: Anthocoridae), suggesting that agricultural crops can be genetically engineered to increase productivity, but they have no impact on natural enemies (Zhang et al., 2008). This differs from the longer duration of the nymphal stage of males and females of *P. nigrispinus* with caterpillars of *A. gemmatalis* in insect resistant IAC-17 soybean cultivar (Matos Neto et al., 2002), indicating that the IAC-24 and IAC-17 cultivars may have different degrees of insect resistance. This can be explained by the fact that plant cultivars have different levels of trichome density, chemical composition and nitrogen and potassium concentrations in the leaves, as observed for the different impacts of *Thrips palmi* Karny, 1925 (Thysanoptera: Thripidae) and *A. gossypii* in cultivars of *Solanum melongena* L. (Solanaceae) in two Brazilian regions (Leite et al., 2006a).

A similar survival rate of *P. nigrispinus* with caterpillars of *A. gemmatalis* in IAC-24 soybean cultivar is consistent with the fact that this parameter is not affected by insect resistant soybean cultivars, as observed for this predator with this prey in IAC-17 cultivar (Matos Neto et al., 2002) and for *N. roseipennis* with caterpillars of *P. includens* in PI229358 (Pfannenstiel & Yeargan, 1998). On the other hand, survival rate is indirectly affected by insect resistant PI171451 and PI229358 soybean cultivars for the *G. punctipes* predator with caterpillars of *P. includens*, suggesting that insect resistance studies should be conducted for each variety of agricultural crops (Rogers & Sullivan, 1986). This occurs because predators of stink bugs do not suck in the cell cytoplasm of plants, but they can ingest the xylem fluids of plants and, therefore, are not directly exposed to the secondary compounds or toxic materials expressed in cells of biotech varieties and neonicotinoid insecticides, but they can be affected by substances of resistant cultivars (Torres & Ruberson, 2004; Torres et al., 2010).

A similar body mass of *P. nigrispinus* with caterpillars of *A. gemmatalis* in insect resistant IAC-24 soybean cultivar suggests that it is harmless to the reproductive system of this predator, because heavier females of the stink bug reproduced more successfully, as observed in the higher fertility rate of those heavier females of *P. nigrispinus*, *S. cincticeps* and *Podisus rostralis* Stäl, 1860 (Heteroptera: Pentatomidae) compared to lower weight females (Mohaghegh et al., 1999; Zanuncio et al., 2002; Zanuncio et al., 2005). On the other hand, females with greater body mass may have higher survival rates at times of shortage of prey, which would not affect their reproduction (Molina-Rugama et al., 1998). Furthermore, the combination of prey with branches of *E. cloeziana* or *P. guajava* increased the weight of 21-d-old females of *P. nigrispinus* compared to the predator with branches of *Eucalyptus urophylla* S. T. Blake (Myrtaceae) and pupae of *T. molitor*, or with pupae of this prey only, improving reproduction efficiency (Lemos et al., 2009a). In addition, the weight of females of *P. maculiventris* with *Galanthus nivalis* L. (Amaryllidaceae) agglutinin (GNA) transgenic proteins and cowpea trypsin inhibitor (CpTI) in caterpillars of *Lacanobia oleraceae* L., 1758 (Lepidoptera: Noctuidae) injected (Bell et al., 2003) and of males and females of *N. roseipennis* with caterpillars of *Pseudoplusia includens* Walker, 1857 (Lepidoptera: Noctuidae) in the insect resistant PI229358 soybean cultivar (Pfannenstiel & Yeargan, 1998) was lower than that of *P. nigrispinus* with caterpillars of *A. gematalis* in IAC-24 soybean cultivar, suggesting that the effect may depend on the combination of resistant cultivars and natural enemies.

The similar pre-oviposition period of *P. nigrispinus* is consistent with the one observed for this predator with caterpillars of *A. gemmatalis* in soybean cultivars IAC-17 and UFV-16 (Matos Neto et al., 2002) and for *P. maculiventris* with caterpillars of *P. includens* in PI 227687 and Davis soybean cultivars (Orr & Boethel, 1986). Moreover, the insect resistant IAC-17 soybean cultivar indirectly caused deleterious effects on the oviposition of *P. nigrispinus* with caterpillars of *A. gemmatalis*, indicating varying levels of resistance in soybean cultivars for this parameter (Matos Neto et al., 2002). This was also reported for most small and large mines and mined leaves by *Tuta absoluta* Meyrick, 1917 (Lepidoptera: Gelechiidae) on 57 access and three commercial cultivars (Santa Clara, Moneymaker, and TOM-601) of *Lycopersicon esculentum* Mill. (Solanaceae) in greenhouse, although those of HGB-674 and HGB-1497 were found to be promising for cultivars resistant to insects (Oliveira et al., 2009). The similar post-oviposition period of *P. nigrispinus* with caterpillars of *A. gemmatalis* in UFV-16 soybean cultivar is not consistent with the lowest value of the referred parameter for this predator with caterpillars of this prey in insect resistant IAC-17 soybean cultivar (Matos Neto et al., 2002). Nevertheless, this latter value would be interesting for the lower survival of females of *P. nigripinus* after its last egg clutch, which would reduce the number of infertile females in the field. These would keep on feeding on prey, though, which would be desirable for the biological control and reduction of multiplication cost of this predator (Saavedra et al., 1997).

The similar number of egg clutch and eggs per egg clutch by female of *P. nigrispinus* with caterpillars of *A. gemmatalis* in insect resistant IAC-24 soybean cultivar differs from the indirect deleterious effect of the IAC-17 soybean cultivar in the number of egg clutch and eggs per egg clutch by females of this predator (Matos Neto et al., 2002), but shows the compatibility of that cultivar in soybean farming systems. This finding corroborates the fact that the particularities of agricultural crops may affect the biological parameters of insects, as reported for the different impacts of *Bemisia tabaci* Gennadius, 1889 biotype B (Hemiptera: Aleyrodidae) on cultivars of *Cucumis sativus* L. (Cucurbitaceae). The different levels of resistance of these plants may have been caused by temperature, incidence of predators and parasitoids, plant age, leaf chemical composition, leaf of nitrogen and potassium concentration and density of trichomes (Leite et al., 2006b).

The similar number of eggs per female of *P. nigrispinus* shows the compatibility of the soybean cultivars IAC-24 (resistant to insects) and UFVS-2006 (susceptible to insects) for this predator that indirectly fed on these plants, as observed for the insect resistant IAC-17 soybean cultivar (357.79 ± 103.43 eggs) (Matos Neto et al., 2002) and for *P. maculiventris* that indirectly fed on insect resistant PI227687 soybean cultivar (Orr & Boethel, 1986). On the other hand, the number of eggs per female of the *N. roseipennis* predator was lower with caterpillars of *P. includens* in insect resistant PI229358 soybean cultivar (Pfannenstiel & Yeargan, 1998) and greater for *P. nigrispinus* with caterpillars of *A. gemmatalis* in UFV-16 soybean cultivar susceptible to insects (554.00 ± 70.47

eggs) (Matos Neto et al., 2002). This can be explained by the fact that *P. nigrispinus* has not fed on plant material, only on caterpillars of *A. gemmatalis*, while in the latter paper these predators have been offered caterpillars of *A. gemmatalis* and soybean leaflets. The offering of plant material (branches of *Eucalyptus* spp.), alternative prey (*T. molitor* pupae or caterpillars of *A. argillacea*), salts and amino acid solution increased the number of eggs per female of *Brontocoris tabidus* Signoret, 1852 (Heteroptera: Pentatomidae) (Zanuncio et al., 2000) and of *P. nigrispinus* (Lemos et al., 2001; Freitas et al., 2006), because predators of the stink bug feed on plants to increase their capacity of postures in habitats with lower prey availability (Coll & Guershon, 2002; Zanuncio et al., 2004; Lemos et al., 2009b).

A similar longevity of *P. nigrispinus* is consistent for this predator with caterpillars of *A. gemmatalis* in insect resistant IAC-17 soybean cultivar (Matos Neto et al., 2002) and *P. maculiventris* with caterpillars of *P. includens* in PI 227687 and Davis soybean cultivars (Orr & Boethel, 1986), but the largest initial mortality of females of *P. nigrispinus* with those caterpillars in that cultivar was similar to that reported for the insect resistant IAC-17 soybean cultivar (Matos Neto et al., 2002), which could negatively impact the population dynamics of this predator.

The insect resistant IAC-24 soybean cultivar has shown indirect deleterious effect on the early mortality of females of *P. nigrispinus*, but did not affect the reproduction and longevity of this predator and, therefore, this crop could be combined with the referred predator in regions of soybean cultivation.

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