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Research Article

Dinotefuran toxicity on predators and egg parasitoids of *Piezodorus guildinii* and *Dichelops furcatus* (Hemipetra: Pentatomidae) under field conditions[1]

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ABSTRACT

Piezodorus guildinii and *Dichelops furcatus* stink bug populations are naturally regulated by predators and egg parasitoids. However, these natural enemies can be affected by the application of non-selective insecticides commonly employed for stink bug chemical control. This study aimed to evaluate the dinotefuran toxicity on predators and egg parasitoids associated with *P. guildinii* and *D. furcatus* (Hemimpera: Pentatomidae) populations under field conditions. Predators and egg parasitoids were recorded weekly from V2 to R7, in dinotefuran-treated and untreated soybean plots. *Telenomus podisi* (Hymenoptera: Scelionidae) was present on 100 % of the egg masses parasitized on both stink bugs species and treatments. No significant differences were found in parasitism proportion and sex ratio between treatments for either species. The egg mass emergence proportion showed significant differences among the treatments for *P. guildinii*, but not for *D. furcatus*. The dinotefuran application did not affect the presence of generalist predators as Araneae, Syrphidae and Chrysopidae. Dinotefuran can be considered selective for these predators, but not for *P. guildinii*.

KEYWORDS: *Telenomus podisi*, stink bug, biological control.

RESUMO

Toxicidade de dinotefurano em predadores e parasitoides de ovos de *Piezodorus guildinii* e *Dichelops furcatus* (Hemipetra: Pentatomidae) em condições de campo

Populações de percevejos *Piezodorus guildinii* e *Dichelops furcatus* são naturalmente reguladas por predadores e parasitoides de ovos. No entanto, esses inimigos naturais podem ser afetados pela aplicação de inseticidas não seletivos comumente utilizados no controle químico dos percevejos. Objetivou-se avaliar a toxicidade de dinotefurano em predadores e parasitoides de ovos associados a populações de *P. guildinii* e *D. furcatus* (Hemiptera: Pentatomidae) em condições de campo. Predadores e parasitoides de ovos foram registrados semanalmente de V2 a R7, em parcelas de soja tratadas e não tratadas com dinotefurano. *Telenomus podisi* (Hymenoptera: Scelionidae) estava presente em 100 % das massas de ovos parasitados em ambas as espécies de percevejos e tratamentos. Não foram encontradas diferenças significativas na proporção de parasitismo e na razão sexual entre os tratamentos para nenhuma das espécies. A proporção de emergência da massa de ovos mostrou diferenças significativas entre os tratamentos para *P. guildinii*, mas não para *D. furcatus*. A aplicação de dinotefurano não afetou a presença de predadores generalistas como Araneae, Syrphidae e Chrysopidae. Dinotefurano pode ser considerado seletivo para esses predadores, mas não para *P. guildinii*.

PALAVRAS-CHAVE: *Telenomus podisi*, percevejo, controle biológico.

INTRODUCTION

The *Piezodorus guildinii* (Westwood) and *Dichelops furcatus* (Fabricius) (Hemiptera: Pentatomidae) stink bugs rank among the most important soybean cultivation hemipteran pests in southern South America (Zerbino & Panizzi 2019). Particularly, *P. guildinii* is considered the most important species in several Argentine provinces (Gamundi & Sosa 2008, Barakat et al. 2022), whereas, in recent years, it has colonized new territories in Argentina, such as Catamarca, La Rioja and Río Negro (Dellape 2021). *D. furcatus* abundance has significantly increased, making it currently the second most important species affecting soybean in Argentina (Zerbino & Panizzi 2019) and in the neotropical region (Panizzi et al. 2022).

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These insects cause damage to plant tissues by inserting their stylets and sucking nutrients. They induce fruit and seed abortion and formation of deformed grains, affecting yield and quality, and can transmit pathogens during this process, thereby increasing their harm (Panizzi 1997). Severity degree depends on penetration frequency, duration of activity and introduction of toxic salivary secretions that can cause tissue necrosis (Depieri & Panizzi 2011). Nowadays, it is widely recognized that *P. guildinii* exhibits a greater aggressiveness, when compared to other pentatomid species, even at similar population levels (Corrêa-Ferreira & Azevedo 2002). In this regard, Depieri & Panizzi (2011) reported that the greater damage of *P. guildinii* arises from its ability to inflict greater damage to soybean seeds, if compared to other stinks bugs, primarily attributed to the higher degree of seed tissue injury caused by the chemical dissolution resulting from its saliva.

In the neotropics, the main natural regulators of stink bug populations are the egg parasitoids (Zerbino & Panizzi 2019). In Argentina, the reported species include *Te. Podisi* (Ashmead), *Tr. urichi* (Crawford), *Tr. teretis* (Johnson), *Tr. basalis* (Wollaston) and *Gryon scutellatum* (Masner) (Hymenoptera: Platygastridae) (La Porta et al. 2013, Cingolani et al. 2014a, Zerbino & Panizzi 2019). On the other hand, among the predatory arthropods associated with soybean cultivation are spiders (Araneae), Geocoridae (Hemiptera: Lygaeidae), Chrysopidae (Neuroptera), Nabidae (Hemiptera: Nabidae), Orius spp. (Hemiptera: Anthocoridae), *Eriopis connexa* (Coleoptera: Coccinellidae) and *Harmonia axyridis* (Coleoptera: Coccinellidae) (Ribeiro & Castiglioni 2008).

At present, the management of stink bugs relies exclusively on chemical control with broad-spectrum insecticides (Baur et al. 2010) and the use of high doses due to their low susceptibility to chemicals, especially *P. guildinii* and *D. furcatus*, which have been reported for their differential susceptibility towards insecticides (Temple et al. 2013). The intensive use of broad-spectrum insecticides adversely affects non-target species such as parasitoids and predators, which play an important role in regulating populations (Bentancourt & Scatoni 2001, Abbate et al. 2022), decreasing its populations, in terms of richness and abundance (Altieri & Nichols 2000). Furthermore, pesticides may cause adverse sub-lethal effects to natural enemies, compromising their survival and the biological control of insect pests (Silva & Bueno 2015).

Although they are inside the host, parasitoids in the preimaginal stage, such as egg, larvae and pupa, could also be exposed to pesticides, what threatens their survival in host eggs (Zantedeschi et al. 2018a). In microhymenopterans, the sex ratio has direct implications on the performance of a parasitoid, in terms of biological control, since the females exert such control by regulating the sex of their progeny, maximizing their fitness for a given host density (Yu et al. 2003). However, it can exhibit a significant variation, both among and within species, influenced by multiple factors. Among these factors are the host species type (La Porta et al. 2013), the phenological condition of the egg mass during parasitism (Temple et al. 2016) and the age of the adult parasitoid (Cingolani et al. 2014b).

Several authors have studied the *P. guildinii* and *D. furcatus* susceptibility to various neonicotinoids and their mixtures with pyrethroids, as well as their effects on stink bug egg parasitoids (Baur et al. 2010, Temple et al. 2013), and classified the substances based on their laboratory performance according to the standards of the International Organization for Biological Control (Turchen et al. 2016, Zantedeschi et al. 2018a, Zantedeschi et al. 2018b, Abbate et al. 2022).

Studies on broad-spectrum insecticides reporting lethal effects have been extensively tested on predators (Schneider et al. 2006, Benamú et al. 2013). However, in recent years, the interest in the study of sub-lethal effects on parasitoids and predators have increased. Some authors reported physiological and behavioral effects that negatively impact their performance, even when using insecticides considered selective (Schneider et al. 2004, Rimoldi et al. 2008, Rimoldi et al. 2012, Sgarbi et al. 2023). It is of utmost importance to evaluate the toxicity of insecticides commonly used in stink bug management towards natural enemies, especially egg parasitoids closely associated with them, for implementing them in integrated management programs (Zantedeschi et al. 2018b, Abbate et al. 2022).

There is extensive information about the toxicity of insecticides to egg parasitoids and predators of sting bugs in the laboratory, but field studies in producer-level plots are scarce (Rakes et al. 2022). In this sense, this study aimed to evaluate the toxicity of dinotefuran, a third-generation neonicotinoid insecticide, on egg parasitoids and predators associated with populations of *Piezodorus guildinii* and *Dichelops furcatus* (Hemiptera: Pentatomidae) under field conditions.

MATERIAL AND METHODS

The trial was conducted over 10 ha of ACA 3939 GR soybean group III variety, in El Triunfo (35º05'S and 61º31'W), Lincoln, Buenos Aires, Argentina, during the 2016-2017 crop season.

Two treatments were applied: control (without insecticide application) and treated (with application of dinotefuran, STARKLE 20 % WDG) with third-generation neonicotinoid insecticide. The plant-to-plant distance was 35 cm, with a 70 m buffer zone distance between T1 and T2 to mitigate the effect of spray drift. Twelve samples were collected weekly per treatment (n = 24). The samples were taken on both treatments for 14 weeks during the soybean growth stages. The first sampling point was randomly selected and the remaining points determined over a zigzag transect every 30 m. Then, the vertical beat cloth (1 m width) method for sampling insect populations in soybean fields was used (Boyer & Dumas 1969). At each point, five plants were completely registered, searching for stink bug eggs, which were placed in labeled containers and taken to the laboratory, where they were individually placed in Petri dishes and maintained in a climate-controlled chamber at 25 ± 2 ºC, 60 % of relative humidity and photoperiod of 16:8 to continue their growth. The samplings began on December 15, 2016 (V2) and extended until March 17, 2017 (R7).

The following calculations were performed: parasitized egg mass proportion - number of egg mass parasitized out of the total egg mass collected; parasitism proportion - number of emerged parasitoids plus the number of pupae or fully developed adults that died inside the host (observed by dissecting the material); emergence proportion - number of emerged wasps out of the total number of parasitized eggs; and sex ratio - number of emerged females out of the total number of adult wasps.

The data were analyzed with the generalized linear mixed model for repeated measures (MGLMs) and differences between means with the Tukey test ($p < 0.05$), using the InfoStat software, version 2019 (Di Rienzo et al. 2019).

RESULTS AND DISCUSSION

The dinotefuran application was effective for the control of *Nezara viridula*, *Piezodorus guildinii* and *Edessa meditabunda*, while *Dichelops furcatus* proved to be insensitive to this neonicotinoid, which provided an effective control for the majority of the stink bug species without negatively affecting the predators present at the time of application (Table 1). On this sense, Abbate et al. (2022) found that mixtures including neonicotinoids as thiamethoxam + lambda-cyhalothrin provided a high level of stink bug control, but negatively affected predators.

The predators recorded during the growing crop cycle were spiders (Araneae), *Eriopis connexa* (Coleoptera, Coccinellidae), hoverflies (Diptera), *Crysopa* sp. (Chrysopidae) and predatory bugs such as *Nabis* sp. and *Orius* sp. At the time of the dinotefuran application, no records of *Eriopis connexa* were documented, thereby impeding the assessment of the effects of its application.

However, for the Araneae taxon, Chrysopidae and Syrphidae, it was observed that the application of dinotefuran showed no significant differences between the treatments (Table 1). Additionally, there were no differences observed in the pattern of their population dynamics before and after the application (Figure 1). Meanwhile, Sgarbi et al. (2023) reported that the Araneae taxon was susceptible to anthranilic diamides because the growth curve of the arachnid population was affected, as compared with the untreated field plots.

Sub-lethal effects on laboratory application of different modes of action of insecticides over other generalist predators have been reported (Schneider et al. 2006, Rimoldi et al. 2012).

Table 1. Average arthropods/m surveyed in treatments with and without dinotefuran application.

Species	Control	Dinotefuran
Nezara viridula	0.24 a	0.06 b
Dichelops furcatus	0.09 a	0.15 a
Piezodorus guildinii	0.18 a	0.05 b
Edessa meditabunda	0.10 a	0.06 b
Araneae	0.33 a	0.35 a
Chrysopidae	0.37 a	0.24 a
Syrphidae	0.06 a	0.07 a

* Different letters in the same column indicate significant differences between means by the Tukey test ($p < 0.05$).

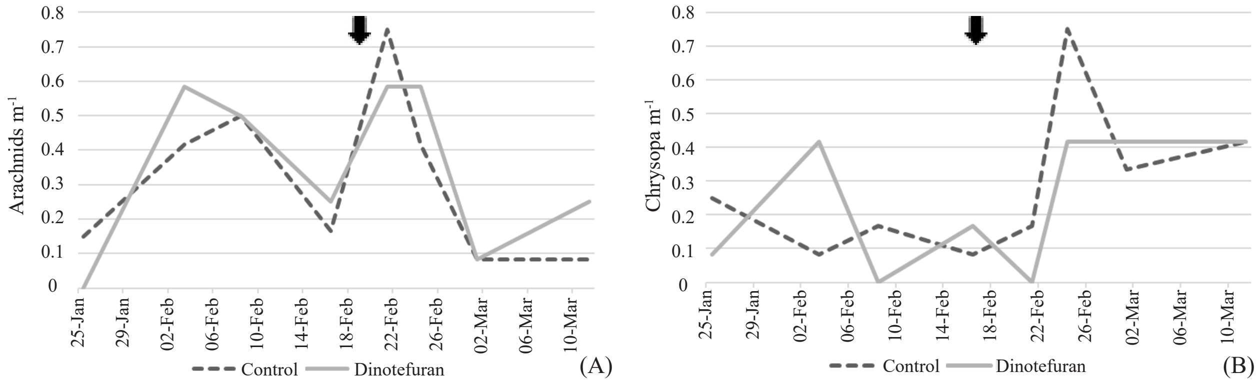

Figure 1. Population dynamics of predators before and after the dinotefuran application. A) Araneae; B) Chrysopidae. The arrow indicates the moment of insecticide application during the soybean growth stages.

However, further studies are needed to assess the impact of these insecticides on the population dynamics in the field.

The results of the present study indicate that *Telenomus podisi* was present on 100 % of the egg mass parasitized on both stink bug species and treatments. In Argentina, Cingolani et al. (2014a) also reported *Tr. urichi* and *Tr. basalis* for *P. guildinii*, while, for *D. furcatus*, La Porta et al. (2013) reported *Tr. basalis*, *Tr. urichi* and *Tr. teretis*, with predominance of *T. podisi* for both species. The low diversity of parasitoids found agrees with the results of Ribeiro et al. (2008), who reported the predominance of *T. podisi* (99.6 %), followed by *T. brochymenae* (0.31 %) and *Tr. basalis* (0.04 %), over *P. guildinii*, in soybean crops in Uruguay. This poor biodiversity could be associated with the high use of agrochemicals characterized in the productive areas of the core region of the Buenos Aires province (Pengue & Rodriguez 2018), which could reduce beneficial fauna, in terms of richness and abundance.

The *D. furcatus* egg mass was recorded starting on Dec. 28, 2016, and those of *P. guildinii* from Feb. 16, 2017, when the crop was at the V6 and R5 stages, respectively. Parasitized egg masses were registered from Jan. 6, 2017 (R1) and Feb. 21, 2017 (R5), respectively for *D. furcatus* and *P. guildinii*. These results agree with Ribeiro & Castiglioni (2008), who also observed parasitism even with low egg densities. The spatial and temporal abundance of parasitized egg masses was variable. For *P. guildini*, the parasitized egg mass percentage fluctuated between 0 and 80 % for both treatments over the monitoring period (Figure 2). The *D. furcatus* variation on parasitism percentage ranged from 0 to 65 % for the control, and from 0 to 80 % for the dinotefuran plots (Figure 3). La Porta et al. (2013) reported similar

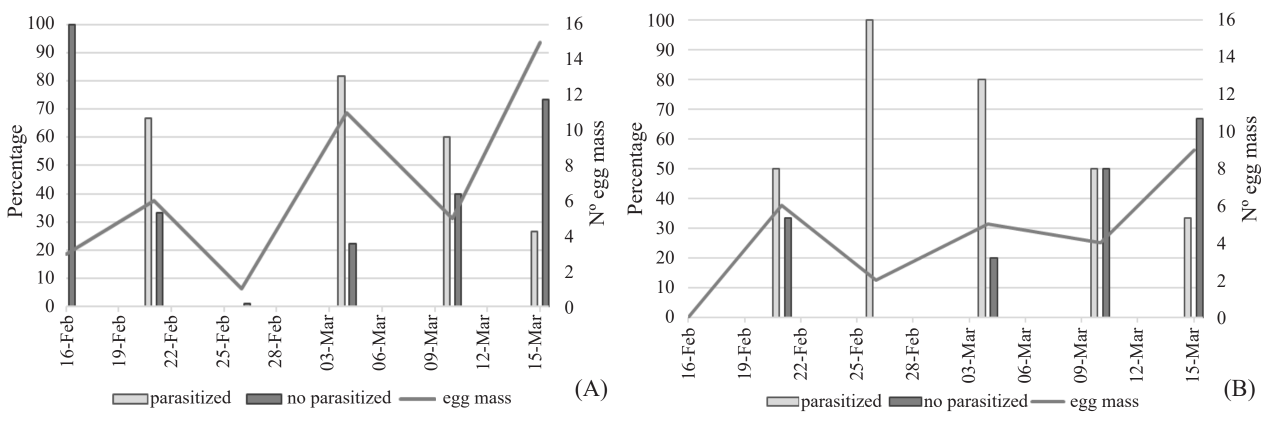

Figure 2. *Piezodorus guildinii* egg mass dynamics registered on the control (A) and for dinotefuran (B). Dinotefuran application: Feb. 16, 2017.

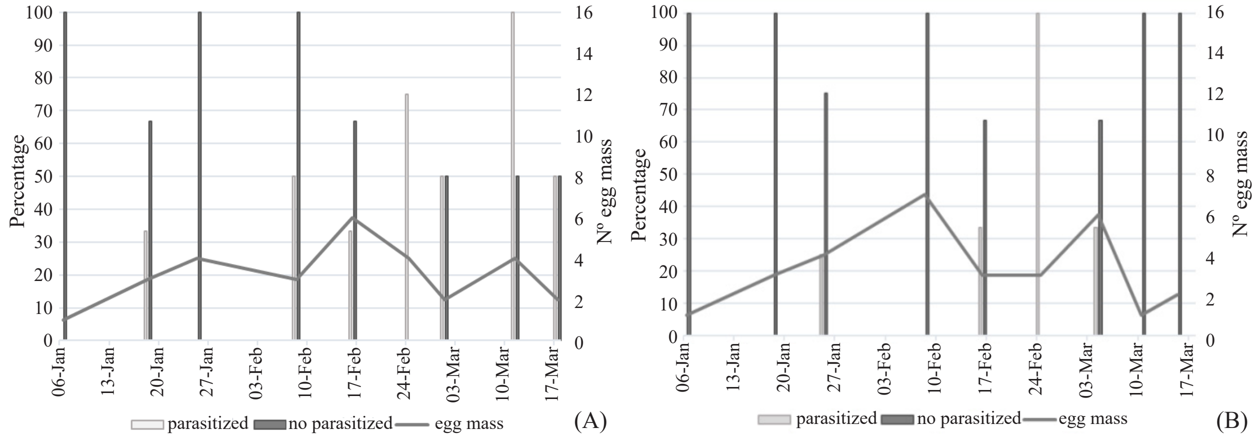

Figure 3. *Dichelops furcatus* egg mass dynamics registered on the control (A) and for dinotefuran (B). Dinotefuran application: Feb. 16, 2017.

natural parasitism percentage values for *P. guildinii* eggs mass across three evaluated campaigns.

A reduction in parasitized egg mass percentage for *D. furcatus* was observed in the weeks following the application (Figure 3). This could indicate *a priori* that, under field conditions, the insecticide did not affect the parasitoid's performance over *P. guildinii*, but affected it over *D. furcatus*. In this regard, Turchen et al. (2016) reported a reduction in the *T. podisi* parasitism percentage over *Euchistus heros* eggs previously treated with neonicotinoids.

No statistically significant differences were observed in the parasitism proportion between the treatments for either species after the field dinotefuran application (Table 2). Under laboratory conditions, Zantedeschi et al. (2018a) classified the application of mixtures integrated by neonicotinoids as slightly harmful (class 2) to *T. podisi* when adults were exposed to egg masses that had been previously pulverized with thiamethoxam + lambda-cyhalothrin (neonicotinoid + pyrethroid), while the same mixture was considered as selective during post-parasitism spraying. The direct application of thiamethoxam + lambda-cyhalothrin onto pupae and adults of *T. podisi* resulted in severe negative effects on the parasitoids, impacting over their performance and reducing subsequent parasitism percentages (Silva et al. 2022). In laboratory studies, neonicotinoid/pyrethroid mixtures reduced the parasitism rates of *T. podisi* on *E. heros* by more than 90 %. However, the application of imidacloprid alone resulted in a reduction of 32 % in parasitism (Feltrin-Campos et al. 2018).

Some authors have reported that the adult sex ratio can be influenced by various factors, including host quality (Cingolani et al. 2014b), soybean phenological stage (Temple et al. 2016) and egg mass size provided by the host (La Porta et al. 2013). In the present study, no significant differences were recorded for sex ratio from adults emerged by egg masses of both *P. guildinii* and *D. furcatus* between the control and dinotefuran treatment (Table 2). This agrees with Rakes et al. (2022), who reported that thiamethoxam, another neonoicotinoid, had no significant effects on the sex ratio of the emerged *T. podisi* adults, while cyhalofop-butyl showed a higher proportion of emerged males.

Table 2. Parasitism proportion (Pp), parasitized egg mass proportion (P), emergence proportion (Ep) and sex ratio (S) for *Telenomus podisi* on *Piezodorus guildinii* and *Dichelops furcatus* host, for the control and the dinotefuran treatments.

Host	Treatment	Pp	P	Ep	S
P. guildinii	Control	0.48	0.95 a	0.869 a	0.803 a
	Dinotefuran	0.53	0.98 a	0.722 b	0.826 a
D. furcatus	Control	0.42	0.92 a	0.830 a	0.818 a
	Dinotefuran	0.23	0.98 a	0.880 a	0.861 a

* Different letters in the same column indicate significant differences between means by the Tukey test ($p < 0.05$).

Photos: Sgarbi Carolina

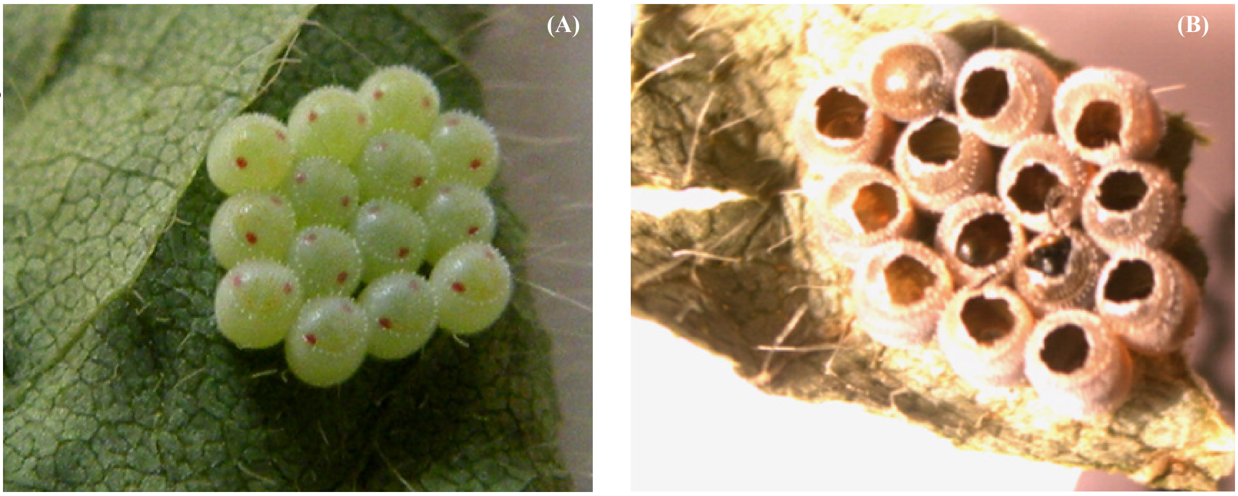

Figure 4. *Dichelops furcatus* egg mass without parasitoidism (A) and parasitized (B) by *Telenomus podisi*, where unmerged adults of the parasitoid can be observed.

La Porta et al. (2013) highlighted that the adult emergence proportion varies among agroclimatic sites for *D. furcatus*, but not for *P. guildinii*. Here, the adult emergence proportion exhibited significant differences among the treatments for *P. guildinii*, whereas no significant differences were observed for the *D. furcatus* egg masses (Table 2; Figure 4). Feltrin-Campos et al. (2018) reported significant differences in the emergence percentage of *T. podisi* from *E. heros* egg masses previously treated with mixtures of thiamethoxam + lambda-cyhalothrin, but not when treated with imidacloprid alone.

CONCLUSIONS

1. The application of dinotefuran proved to be effective for controlling *Nezara viridula*, *Piezodorus guildinii* and *Edessa meditabunda* in field plots, but not for *Dichelops furcatus*;
2. Dinotefuran showed no significant effects on predators such as Araneae, Syrphidae and Chrysopidae, and their population curves exhibited no variation after the insecticide application;
3. The sex ratio and parasitism proportion of *Telenomus podisi* were not affected by the dinotefuran application on *D. furcatus* and *P. guildinii*;
4. Dinotefuran affected the *T. podisi* proportion emergence from *P. guildinii*, but not from *D. furcatus* eggs.

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