## CROP PROTECTION

# Toxicity of Thiamethoxam and Imidacloprid to *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) Nymphs Associated to Aphid and Whitefly Control in Cotton

JORGE B. TORRES[1] AND JOHN R. RUBERSON[2]

[1]*DEPA-Fitossanidade, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros S/N, Dois Irmãos, 52171-900 Recife, PE, e-mail: jtorres@bugs.ent.uga.edu*
[2]*Dept. Entomology, CPES-University of Georgia, 31794, Tifton, GA, USA, e-mail: ruberson@tifton.uga.edu*

*Neotropical Entomology 33(1):099-106 (2004)*

Toxicidade de Tiametoxam e Imidaclopride para Ninfas de *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) em Associação ao Controle do Pulgão e da Mosca-Branca em Algodoeiro

RESUMO - Este trabalho avaliou a toxicidade de tiametoxam e imidaclopride para ninfas de *Podisus nigrispinus* (Dallas) e a eficiência desses inseticidas no controle da mosca-branca e do pulgão em algodoeiro. Em laboratório, os inseticidas foram 217,6 e 223,4 e 1435,2 e 346,8 vezes mais tóxicos ($CL_{90}$) por ingestão que por contato residual para ninfas de 2º e 5º instares do predador, respectivamente. A sobrevivência de ninfas de *P. nigrispinus* confinadas em plantas de algodoeiro em potes e tratadas com os inseticidas em concentrações acima de 1 mg (i.a.) por planta foi afetada até 52 dias após tratamento, porém no campo a sobrevivência de ninfas foi afetada somente até nove dias após tratamento com os inseticidas. Todas as concentrações dos inseticidas foram eficientes no controle de mosca-branca até 40 dias após tratamento de plantas em potes. A população da moca-branca apresentou baixa densidade no campo, sem diferenças entre tratamentos inicialmente, porém com maior população de mosca-branca em plantas não tratadas e tratadas com 0,5 mg (i.a.) do tiametoxam aos 64 dias de idade das plantas. Parcelas tratadas com concentrações de inseticidas inferiores a 1 mg (i.a.) por planta apresentaram infestação do pulgão abaixo de 10% até aos 61 dias de idade. Nessa idade das plantas, parcelas não tratadas ou tratadas com 0,5 mg do tiametoxam apresentaram infestações de 68,7 e 31,2%, respectivamente. A utilização de até 1 mg (i.a.) de tiametoxam e imidaclopride por planta objetivando o controle da mosca-branca e do pulgão do algodoeiro, vinculada à preservação de *P. nigrispinus*, apresenta maiores chances de sucesso devido ao menor efeito residual.

PALAVRAS-CHAVE: *Bemisia tabaci*, *Aphis gossypii*, fitofagia, percevejo predador

ABSTRACT - The toxicity of thiamethoxam and imidacloprid to *Podisus nigrispinus* (Dallas) nymphs, and their efficacy against whitefly and cotton aphid were studied. Thiamethoxam and imidacloprid were 217.6 and 223.4 and 1435.2 and 346.8 times more toxic ($LC_{90}$) by ingestion than by residual contact to 2nd- and 5th-instar nymphs of this predator, respectively. Nymphs caged on potted cotton plants and treated with either insecticide at 1 mg (a.i.) per plant or more had lower survival than those on untreated plants, up to day 52 after treatment. Thiamethoxam and imidacloprid reduced field survival of *P. nigrispinus* compared to untreated plants up to nine days after treatment. Thiamethoxam and imidacloprid showed significant control of whitefly in comparison with untreated plants up to 40 days after treatment in potted plants. Whitefly population had low density over time in the field with no differences between treatments and only at day 64 higher whitefly population was observed on untreated plants and plants treated with 0.5 mg (a.i.) of thiamethoxam per plant. Plots treated with thiamethoxam and imidacloprid at doses over 1 mg (a.i.) per plant retained aphid infestation lower than 10% up to 61 days of plant age. Untreated and treated plants with 0.5 mg of thiamethoxam showed infestation of 68.7 and 31.2%, respectively, at this time. Thiamethoxam and imidacloprid used in cotton for whitefly and aphid control aiming *P. nigrispinus* preservation can be more successful when they are used at doses bellow 1 mg (a.i.) per plant due to shorter residual effect.

KEY WORDS: *Bemisia tabaci*, *Aphis gossypii*, phytophagy, predatory stinkbug

Conservation of naturally occurring biological control agents is frequently limited by incompatibility between insecticides and natural enemies. Conventional use of insecticides can have deleterious effects on beneficial arthropod populations because beneficial species can have greater susceptibility to low concentrations of insecticides than their prey or host (Ruberson *et al.* 1998). The predatory stinkbug *Podisus nigrispinus* (Dallas) actively searches its prey on plant canopy (Torres *et al.* 2002b) where it can be exposed to insecticide residues during locomotion, self-cleaning or when feeding on contaminated prey (Torres *et al.* 2002a). *P. nigrispinus* is also a facultative plant feeder as many other important heteropteran predators in cotton (e.g., *Geocoris*, *Orius*, *Nabis*), what is useful in their survival during periods of low prey density. Because of this omnivorous behavior, *P. nigrispinus* can be exposed to systemic insecticides used on cotton for control of sucking pests.

Imidacloprid and thiamethoxam are considered first- and second-generation of neonicotinoid compounds belonging to the chloronicotinyl and thianicotinyl subclasses, respectively, with both having gut and contact activities (Mullins 1993, Maiensfisch *et al.* 2001). After foliar or soil application or seed treatment, thiamethoxam and imidacloprid have prolonged systemic and residual activity in several crop plants against a broad range of commercially important sucking insect pests. The use of thiamethoxam and imidacloprid as early cotton season treatment aims to suppress the whitefly and aphid populations hence reducing their direct and indirect injuries by virus transmission.

A key issue in a pest control program is to maximize control efficacy by diversifying control tactics, what requires information on possible side effects. Thiamethoxam and imidaclorpid have been recommended mainly as an initial protection against sucking pests, but they lack efficacy against most lepidopteran larvae (Elbert *et al.* 1991, Mullins 1993, Maiensfisch *et al.* 2001), whih are common after the crop reaches its mid cycle. The lack of efficacy of these insecticides against lepidotperan larvae combined with the complexity and costs of establishment of a program with seasonal inoculative release of pest-specific natural enemies, in a multi-pest ecosystem such as cotton, endorse the conservation of generalist predators as an increasingly recognized strategy. Generalist predators such as *P. nigrispinus* can attack many unrelated pest species, which are not target by thiamethoxam and imidacloprid in cotton. This fact supports the importance of the interaction between these insecticides commonly used for sucking pests control and the preservation of generalist predators such as *P. nigrispinus* that control lepidopteran larvae.

Predatory stinkbugs can be preserved as biological control agents of cotton defoliators, depending on time and dose of thiamethoxam applied under *ad libitum* prey condition. Torres *et al.* (2003b) reported that although *P. nigrispinus* nymphs had reduced survival on thiamethoxam-treated cotton plants during shorter periods after application, surviving individuals showed similar reproduction output compared to those reared on untreated plants. Therefore, the objectives of this study were to determine (1) the effect of thiamethoxam and imidacloprid to *P. nigrispinus* nymphs through residual and treated drinking water, (2) the effect of the insecticides on nymph survival in the absence of prey, simulating a situation where the host plant becomes an important diet supplement (Lemos *et al.* 2001, Oliveira *et al.* 2002), and (3) the efficacy of the insecticides on restraining whitefly and cotton aphid infestation, on potted and field drench-treated cotton plants.

## Materials and Methods

**Insecticides**. Thiamethoxam 250 g kg$^{-1}$ WG (Actara) was used in the laboratory at the concentrations 0, 16.25, 33.7, 66.2, 132.5 and 266.2 mg (a.i.) L$^{-1}$ for the residual assay, and 0, 0.125, 0.25, 0.375, 0.75 and 1.0 mg (a.i.) L$^{-1}$ for the ingestion assay. Likewise, imidacloprid 700 g kg$^{-1}$ WDGr (Confidor) was tested at the concentrations 0, 15.0, 30.0, 63.7, 131.2 and 266.2 mg (a.i.) L$^{-1}$ for the residual assay, and 0, 0.05, 0.10, 0.218, 0.437 and 0.875 mg (a.i.) L$^{-1}$ for the ingestion assay. These concentrations were selected after previous experiments. Thiamethoxam was used in plants cultivated in pots maintained in an open-sided greenhouse and in the field at recommended concentrations [25, 50, 100 and 200 g (a.i.) ha$^{-1}$ (0.5, 1, 2 and 4 mg (a.i.) per plant]. Untreated plants were used as control treatments. Cotton plants were treated with 20 ml of acqueous insecticide solution drenched at the base of each plant 15 days after sprouting in the open-sided greenhouse and in the field experiments, using a 200-ml pistol with hand applicator (Paasche Airbrush®). One treatment was simultaneously carried out with imidacloprid at the rate of 50 g (a.i.) ha$^{-1}$ [1 mg (a.i.) per plant] for comparisons in open-sided greenhouse and in the field.

**Laboratory Experiment.** Nymphs of *P. nigrispinus* were obtained from a colony established from adults collected in a passion fruit orchard at the Agronomy Department Farm of the Universidade Federal Rural de Pernambuco, Recife (PE). They were fed on larvae and pupae of *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), according to Zanuncio *et al.* (1994), at 26 ± 1.5°C, 60-75% RH, and L12:D12 photoperiod. Humidity was maintained by inserting a 1.3 cm$^3$ vial, filled with water and stopped with moistened cotton, into the rearing container (ca. 500-ml white plastic). Nymphs were obtained after 17 generations in the laboratory with no previous contact with any insecticide.

Second and 5$^{th}$-instar nymphs of *P. nigrispinus* were exposed to the insecticides by residual and ingestion contact. Residual effect was obtained by dipping cotton leaves into each insecticide concentration for six seconds and left to dry around 1h at room conditions. Fifteen individuals of both 2$^{nd}$- or 5$^{th}$-instar nymphs of *P. nigrispinus* (24h to 48h-old) were placed in petri dishes (18 x 1.5 cm) with five nymphs per petri dish containing one treated cotton leaf. For ingestion treatment, 15 nymphs either 2$^{nd}$- or 5$^{th}$-instar were exposed to insecticides via treated drinking water. Insect exposure to the insecticide solution was obtained by using an anaesthetic tube closed with a piece of cotton and attached to the cover of the petri dish. These 1.5 cm$^3$-tubes were filled either with insecticide solution or water and provided to nymphs during five days. Nymphs of both treatments received no prey during

24h, but were supplied with two *T. molitor* pupae in the following days. Control treatment was constituted of cotton leaves dipped into distilled water or tubes filled with distilled water. Data were collected during five days with mortality summarized at the end of the observation period.

**Plants.** Cotton (*Gossypium hirsutum* L.) seeds of the genotype Precocious CNPA1 nectaried were provided by the Seed Department of the EMBRAPA-Algodão, Campina Grande, PB. Two seeds of cotton were planted in pots (25 cm diameter and 20 height) filled with a mixture of soil and humus (4+1 by weight) in the open-sided greenhouse, and plants were subsequently thinned to one seedling per pot after plant emergence. Additional fertilizers were watered into the pots at 15-day intervals [N supplied by Nitrato de Cálcio Hydro™ (84.5 g $L^{-1}$) and K by Kristalon marron Hydroä (100 g $L^{-1}$)].

**Potted Plant Experiment.** Sixteen plastic pots with a single cotton plant each were used per each insecticide concentration and control treatment. The cotton plants were watered from above until insecticide application, and afterwards at the plant base, but only lightly, so that the soil did not become too wet. Whitefly (*Bemisia tabaci* Genn. biotype B) immature stages were sampled prior to insecticide application on upper fully expanded leaf (15-d-old) and 11, 20, 28, 40, 54, 64 and 74 days after insecticide application. Ten leaves from 10 randomly selected plants were harvested and eggs, nymphs and pupae were counted under a stereoscopic microscope covering an area of 1 cm wide along both sides of the main vein. Adults were quantified 20, 30, 45 and 60 days after insecticide application in the underside leaf surface turning up the leaf delicately during the first hours in the morning when insects are commonly resting in groups. The impact of insecticides on *P. nigrispinus* nymphs was evaluated on $2^{nd}$-instar (24h to 48h-old) confined on treated and untreated cotton plants. Eighteen nymphs per insecticide concentration and untreated plants were confined in sleeve cage bags (30 by 25-cm organdy bags) in groups of three on cotton foliage ($4^{th}$ leaf from the apex). The first interval from insecticide application to caging nymphs was eight days, and the following time intervals were determined by the survival of the nymphs previously caged (ca., the $2^{nd}$, $3^{rd}$ and $4^{th}$ times of caging nymphs were conducted 25, 42 and 52 days) after insecticide application. After caged, nymphs, were deprived of prey and their mortalities were daily scored.

**Field Experiment.** A plot of 1200 $m^2$ was fertilized with 400 kg $hectare^{-1}$ of 4-14-18 (N-P-K) and seeded in 23 September 1999, at the Agronomy Department Farm of the Federal Rural University of Pernambuco, Recife, PE. The plots consisted of 50,000 plants per hectare spaced 0.25 m within row and 0.8 m between rows. Two plot sides were surrounded by cassava (ca. 6 months old) and green beans (flowering stage) and the other two sides were surrounded by grass. The experiment was carried out in a completely randomized block design in a continuous field with four replicates consisting of six lines, i.e. insecticide concentrations and control treatments, 10 m long (ca. 35 plants) with a two untreated cotton lines between blocks. Data on whitefly immatures were obtained from 10 randomly selected plants (costituting one replication within block) in the central rows for each block. The sampling procedure, in the field, was similar to the open-sided greenhouse method by evaluating collected leaves under scope. For aphid (*Aphis gossypii* Glover) infestation the presence of wingless or winged individuals was registered in the two upper fully expanded leaves of cotton plants in two central rows of each plot (ca. 40-50 pls). Infestations of immature whitefly *B. tabaci* biotype B were evaluated before and after 11, 20, 30, 38, 49 and 63 days of insecticide application. Likewise aphids were evaluated before and after 10, 20, 30, 38, 46 and 54 days of insecticide application. Impact of insecticides on $2^{nd}$-instar *P. nigrispinus* was determined after 9, 27 and 40 days of application by caging nymphs on plants as carried out in the open-sided greenhouse experiment.

**Statistical Analysis.** Probit regression lines were generated using the Probit option of SAEG (Gomes 1985), after correction for natural mortality according to Abbott (1925) for the laboratory data. Lethal concentrations (LC) were estimated in mg a.i. $L^{-1}$ and 90% fiducial limits (FL) that were determined for $LC_{50}$- and $LC_{90}$-values. The number of whitefly eggs, nymphs, pupae (ca. immature) and adults in the open-sided greenhouse were averaged per sampled cotton leaf and transformed by $\log_{10}(x + 1)$ before ANOVA to stabilize variances that were found to be heterogeneous through Lavene's test (SAS Institute 2000). Survivorship of *P. nigrispinus* nymphs in the open-sided and in the field was likewise transformed (Öx + 0.5) to meet ANOVA assumptions. Whitefly immature and adults, and survival of the predatory stinkbug nymphs in the open-sided greenhouse were submitted to repeated measures ANOVA using the PROC ANOVA of SAS (SAS Institute 2000) taking as major factors insecticide concentrations and time intervals post-applicaton. Data on predator nymph survival, the average number of plants infested by aphids and whitefly immature in the field were also submitted to repeated measures ANOVA on time, under a three-way factor design, including block as major factor to control possible differences in the data across replicates. Means subjected as significant over time and among treatments by ANOVA were compared using Tukey HSD test ($P < 0.05$).

## Results

**Laboratory Experiment.** Fifth-instar stinkbugs were 8.2 and 3.8 times more tolerant to thiamethoxam and imidacloprid than the $2^{nd}$-instar ones, based on $LC_{90}$ when exposed to insecticide residues on treated cotton leaves (Table 1). Toxicity by ingestion was similar for $2^{nd}$- and $5^{th}$-instar insects for both insecticides. Exposure by ingestion of drinking water was 217.6 and 1435.2 ($LC_{90}$) times more toxic to $2^{nd}$- and $5^{th}$-instar insects than residual exposure to thiamethoxam; and 223.4 and 346.8 times for imidacloprid, respectively. In general, $2^{nd}$-instar nymphs of *P. nigrispinus* were more susceptible to insecticides than $5^{th}$-instar and exposure by ingestion was more toxic than residual exposure for either $2^{nd}$- or $5^{th}$-instar nymphs.

**Survival of the Predatory Stinkbug Caged on Treated Plants.** The main effects of insecticide concentrations and time

Table 1. Toxicity[1] of thiamethoxam and imidacloprid to 2nd- and 5th-instar of *P. nigrispinus* according to different methods of exposure.

| Application method | n | Stage | Slope | $LC_{50}$ (FL) | $LC_{90}$ (FL) | $\chi^2$ (df) | P-values |
|---|---|---|---|---|---|---|---|
| | | | | Thiamethoxam | | | |
| Residual | 75 | 2nd | 3.42 ± 0.18 | 18.39 (12.4-26.4) | 43.52 (29.4-113.1) | 4.65 (6) | 0.61 |
| | 75 | 5th | 2.28 ± 0.28 | 98.84 (53.8-170.1) | 358.80 (197.4-791.0) | 2.13 (6) | 0.90 |
| Ingestion | 90 | 2nd | 2.24 ± 0.35 | 0.05 (0.03-0.08) | 0.20 (0.11-1.00) | 4.97 (6) | 0.68 |
| | 90 | 5th | 2.26 ± 0.35 | 0.06 (0.04-0.10) | 0.25 (0.14-0.86) | 9.59(8) | 0.32 |
| | | | | Imidacloprid | | | |
| Residual | 75 | 2nd | 3.26 ± 0.20 | 34.40 (20.7-45.3) | 84.89 (62.9-164.3) | 2.74 (6) | 0.83 |
| | 75 | 5th | 3.77 ± 0.13 | 147.66 (109.2-212-3) | 322.56 (221.0-963.1) | 2.83 (6) | 0.85 |
| Ingestion | 75 | 2nd | 2.32 ± 0.31 | 0.13 (0.09-0.18) | 0.38 (0.26-0.75) | 5.17 (13) | 0.97 |
| | 90 | 5th | 1.39 ± 0.51 | 0.44 (0.10-0.97) | 0.93 (0.75-2.69) | 4.54 (8) | 0.80 |

[1]Mortality scored for nymphs up to five days. Lethal concentrations, mg (a.i.) $L^{-1}$; FL, fiducial limits

intervals post-application on the predatory stinkbug nymphs were highly significant on plants in the field (concentrations: $F = 73.98$, $df = 5, 90$, $P < 0.0001$; time intervals: $F = 581.48$, $df = 2, 90$, $P < 0.0001$), as well as on potted plants in the open-sided greenhouse (concentrations, $F = 200.35$, $df = 5, 120$, $P < 0.0001$; time intervals, $F = 6.22$, $df = 3, 120$, $P = 0.0006$). In addition, interactions between the effects of insecticide concentrations and post-application time intervals were also significant for predator nymphs caged on plants in the field ($F = 17.26$, $df = 10, 90$, $P < 0.0001$), and on potted plants in the open-sided greenhouse ($F = 19.16$, $df = 15, 120$, $P < 0.0001$). Nymph survival on treated potted plants were significantly lower than those in untreated plants up to 52 days after insecticide application, except with 0.5 mg (a.i.) of

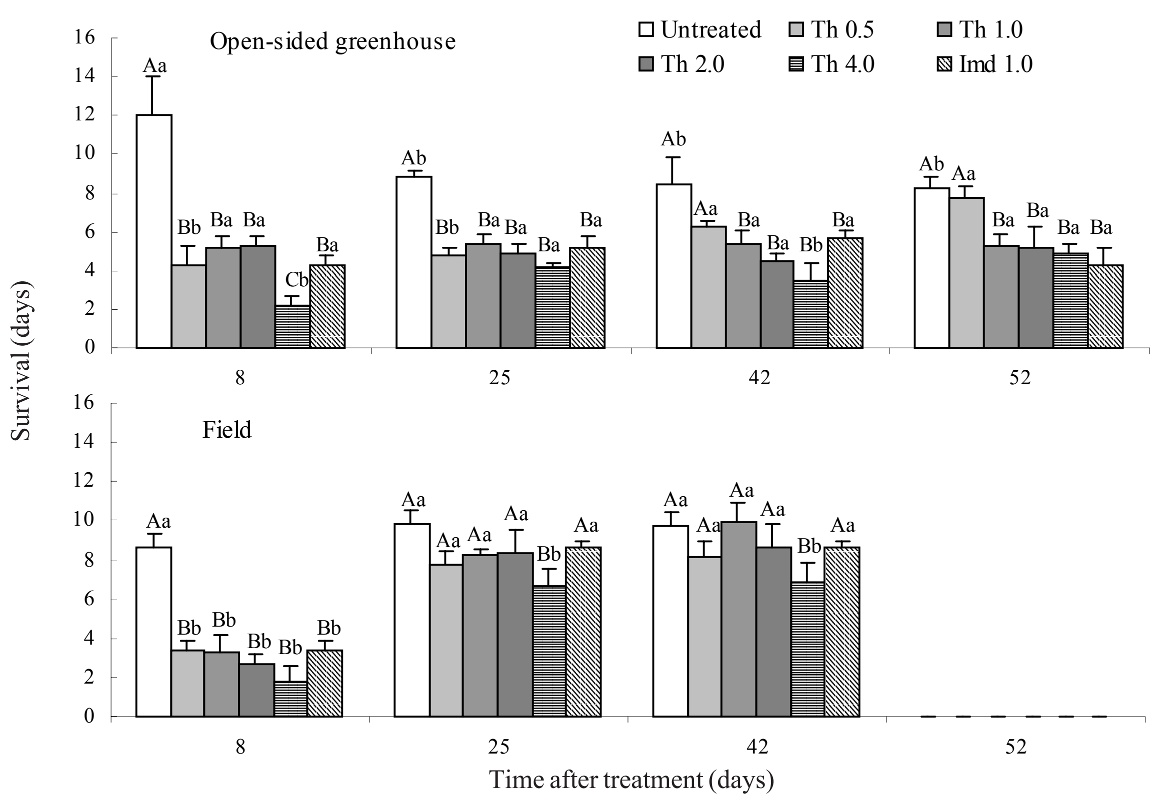


Figure 1. Survival (days) of *P. nigrispinus* 2nd-instar nymphs deprived of prey and caged on cotton plants treated with thiamethoxam (th) and imidacloprid (Imd) as a drench 15d after sprouting in open-sided greenhouse and in the field conditions and an untreated control. Bars (+ SE) within dates having an identical capital letter or within same insecticide concentration having an identical small letter over time are not significantly different at the 0.05 level by Tukey HSD test.

thiamethoxam per plant (Fig. 1). Survival of *P. nigrispinus* nymphs caged on treated plants with 0.5 mg (a.i.) did not differ from those in untreated plants after 42 days of application in potted plants. Overall shorter residual effect on predator nymph survival was observed in the field. Although all thiamethoxam and imidacloprid concentrations significantly reduced nymph survival when caged on nine days treated-plants, the difference was similar for untreated and treated plants with insecticide concentrations up to 2 mg (a.i.) at day 27 after application (Fig. 1). The highest thiamethoxam concentration [ca. 4 mg (a.i.) per plant], however, reduced predator nymph survival up to 40 days post-application in the field (Fig. 1).

**Whitefly Control.** Whitefly population was 7.6 ± 2.51 individuals per leaf (n = 60) prior to insecticide application on 14-day-old plants and in the open-sided greenhouse. These individuals were mainly eggs [98.6% (450/457)] and nymphs 1.4% (7/457).

The two-way ANOVA indicated a significant effect of insecticide concentrations on immature ($F = 931.38$, $df = 5, 378$, $P < 0.0001$) and adult whiteflies ($F = 107.74$, $df = 3, 216$, $P < 0.0001$) in plant cultivated in pots. A significant interaction between the effect of insecticide concentrations and time intervals after application was also found (immature: $F = 33.38$, $df = 30, 378$, $P < 0.0001$; adult: $F = 5.41$, $df = 15, 216$; $P < 0.0001$). Whitefly immature population was suppressed with all thiamethoxam concentrations applied in potted plants up to day 28. After this day only concentrations above 1 mg (a.i.) per plant affected whitefly immatures, what lasted up to day 74 (Fig. 2). The number of whitefly adults per plant treated with 0.5 mg (a.i.) was similar to that on untreated plants at day 74 (Fig. 2), what may result from higher population of nymphs associated to this thiamethoxam concentration.

Eggs, nymphs and pupae of whitefly were only observed in the field on 35-day-old plants (i.e., 20 days after insecticide application). Data analyses showed no variation among blocks ($P = 0.8733$), but significant effect of insecticide concentrations ($F = 11.45$, $df = 5, 123$, $P < 0.0001$), time intervals post-application ($F = 22.19$, $df = 6, 123$, $P < 0.0001$) and on their interaction ($F = 2.35$, $df = 30, 123$, $P < 0.0001$). All thiamethoxam concentrations prevented plants from being colonized by whitefly up to 45 days after application (Fig. 3). However, after 45 days only thiamethoxam at 2 and 4 mg (a.i.) per plant maintained immature population at levels lower than one individual per leaf. Additionally, these two higher thiamethoxam concentrations promoted residual control of whitefly up to 63 days, while 0.5 and 1 mg (a.i.) of thiamethoxam and 1 mg (a.i.) of imidacloprid per plant held immature population lower than one individual up to 38 days under natural infestation.

**Aphid Control.** Fifteen plants were selected per plot in the

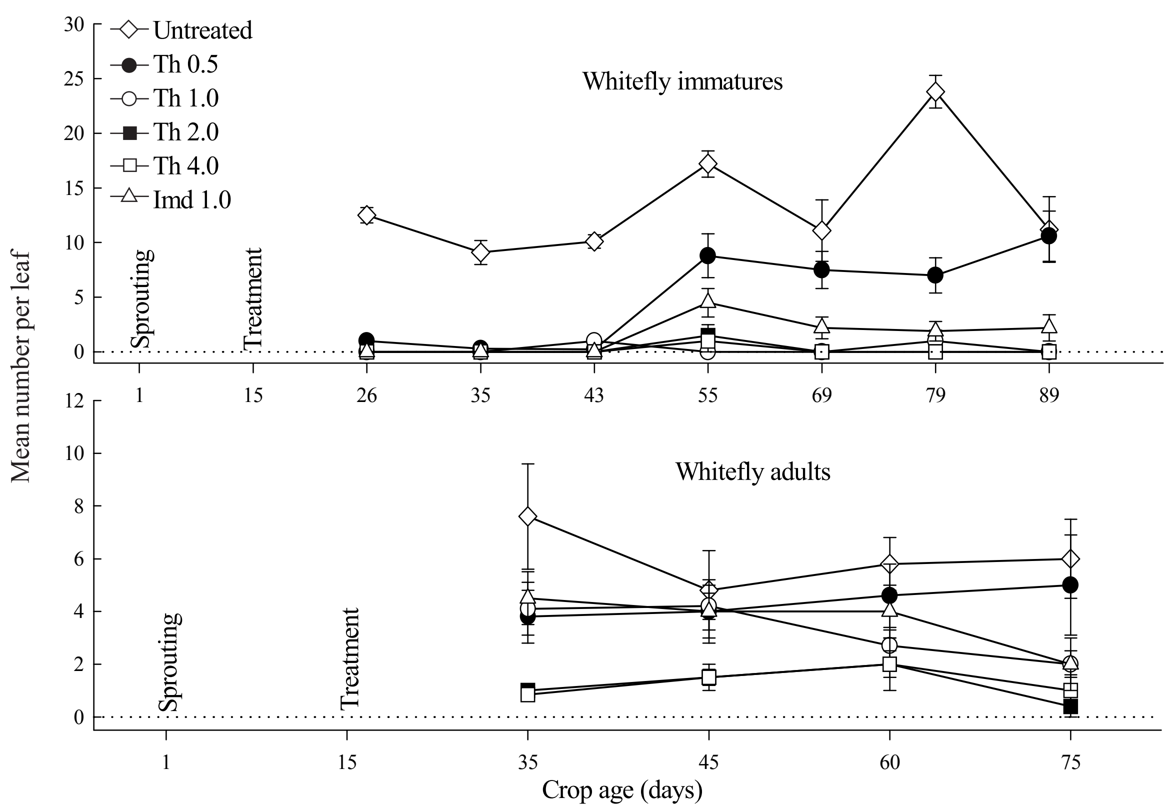


Figure 2. *B. tabaci* biotype B immatures and adults sampled from cotton plants treated with thiamethoxam (Th) at 0.5, 1, 2 and 4 mg [a.i.] per plant, and imidacloprid (Imd) at 1 mg [a.i.] per plant as a drench 15 d after sprouting in the open-sided greenhouse conditions and an untreated control.

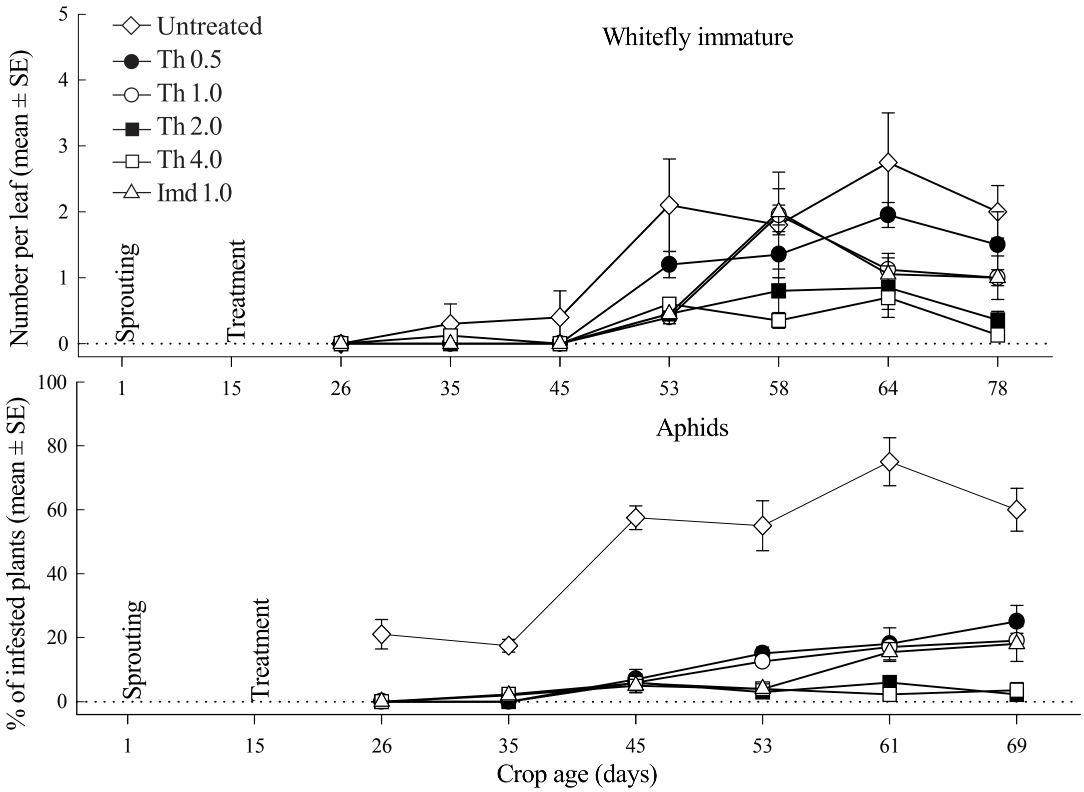


Figure 3. *B. tabaci* biotype B immatures and percentage of plants infested by aphids sampled from cotton plants treated with thiamethoxam (Th) at 0.5, 1, 2 and 4 mg [a.i.] per plant, and imidacloprid (Imd) at 1 mg [a.i.] per plant as a drench 15 d after sprouting in the field conditions and an untreated control.

field, before insecticide application, which were used to evaluate infestation of the cotton aphid. Plants infested ranged from 16.7 to 30.7% with no difference between treatments ($F = 0.22$, $P = 0.9225$, df = 4, 69). Aphid mummies were collected two days latter and maintained in the laboratory until parasitoid emergence or parasitism failure. All parasitoid emerged belonged to *Lysiphlebus* sp. and accounted for 97.7 to 100% of parasitism. No effect of insecticides on parasitoid emergence was found ($F = 1.39$, $P = 0.2646$, df = 4, 26) through drench treatment.

The block effect was not significant ($P = 0.0713$), but insecticide concentrations and evaluation intervals post-application varied significantly (concentrations, $F = 295.06$, $P < 0.0001$, df = 5, 105; time intervals, $F = 68.37$, $P < 0.0001$, df = 5, 105) as their interactions ($F = 11.82$, $P < 0.0001$, df = 25, 105) on determining aphid control. Insecticide application reduced aphid population to zero in the first evaluation with 20% higher infestation in the untreated plots and increased infestation up to 69 days of plant age (Fig. 3). Population of aphids in treated plots with thiamethoxam and imidacloprid did not increase up to 30 days post-application. Thiamethoxam at above 1 mg (a.i.) per plant, however, suppressed aphid population to a level lower than 10% of infested plants, what lasted up to day 53 (Fig. 3). Apparently, thiamethoxam at 0.5 mg (a.i.) was not present in the plants at sufficiently higher concentration to prevent aphid from feeding and colonizing plants after this period.

## Discussion

The laboratory study focused on possible routes of insecticide exposure to the predatory stinkbugs. They represent predator contamination by ingestion of systemic insecticide, either by feeding on plant sap or by drinking insecticide droplets on leaf surface, and residual contact by moving on plant canopy. Both insecticides were highly toxic to the predatory stinkbug nymphs. In addition, the contamination through direct or residual contact with the predator showed that thiamethoxam and imidacloprid affected the predatory behavior of *P. nigrispinus* and their survival in the field (Torres *et al.* 2002a). These findings agree with De Cock *et al.* (1996) who reported similar results for $5^{th}$-instar and adults of *P. maculiventris* through topical and ingestion exposure of imidacloprid. Additionally, these authors found higher toxicity of imidacloprid to *P. maculiventris* $5^{th}$-instar by ingestion than by residual contact what supports the results found with thiamethoxam on *P. nigrispinus* nymphs.

Nymphs of *P. nigrispinus* were highly sensitive to thiamethoxam and to imidacloprid through treated drinking water and plants as well to dry residue on leaf. The high toxicity of thiamethoxam and imidacloprid to the predator nymphs through treated water and plants is justified due to low rates of these insecticides required to control efficiently sucking pests. Therefore, the ingestion of active ingredients and their metabolites via plant sap or from reservoirs in the cotton leaves by heteropteran predators may impose problems to preserve these natural enemies in cotton treated with these insecticides. Systemic insecticides applied in soil may be less harmful to chewing predators and parasitoids than foliar spray because direct contact is less likely with these natural enemies (Ruberson *et al.* 1998, Torres *et al.* 2002a). Baldson *et al.* (1993) reported no effect of imidacloprid, a related thiamethoxam neonicotinoid insecticide, on emergence of *Anagrus takeyanus* Gordh, an egg parasitoid of the azalea lace bug *Stephanitis pyrioides* (Scott). Slight to moderate toxicity of thiamethoxam to parasitized whitefly pupae and cotton aphid mummies was reported by Ogata (1999) and Torres *et al.* (2003a), respectively. The placement selectivity of systemic insecticides through soil treatment, however, can be diminished when natural enemies supplement their diet by feeding on plant (e.g., pollen, nectar, sap) (Sclar *et al.* 1998, Smith & Krischik 1999, Torres *et al.* 2003b). Smith & Krischik (1999) reported reduced survival and mobility and delay on preoviposition period of *Coleomegilla maculata* (DeGeer), a facultative pollen feeder, confined with inflorescences from imidacloprid-treated plants 25 days post-aplication. Also, Sclar *et al.* (1998) showed that the predator *Orius tristicolor* (White) suffered higher mortality when confined on imidacloprid-treated corn seedlings than when confined on untreated plants.

Nymphs of *P. nigrispinus* caged on untreated potted plants and on plants in the field without prey lived 2-3 times longer than those caged on treated plants. *P. nigrispinus* 2$^{nd}$-instar deprived from prey and caged on cotton plants lived from 8.5 to 12 days (Oliveira *et al.* 2002). Therefore, thiamethoxam applied above 1 mg (a.i.) per plant in pots showed residual effect on survival of predatory stinkbug nymphs up to 52 days post-application. This effect was attenuated to nine days in the field, except for the treatment with 4 mg (a.i.) of thiamethoxam per plant that reduced survival of nymphs up to 40 days post-application. Long residual period of thiamethoxam on potted plants and under protected conditions suggest that further investigations should be addressed for other heteropteran predators (e.g. *Geocoris*, *Orius*), which are released in greenhouses for pest control because they have similar feeding behavior. To our knowledge, few studies have been carried out on side effects of thiamethoxam on other natural enemies.

In the field, *P. nigrispinus* can be preserved after 27 days from thiamethoxam application, which seems compatible considering the crop age desired for action of both control methods (i.e., systemic insecticide used at early season by drench, seed or soil treatments against sucking pests, and conservation of predatory stinkbug at middle and late season for control of defoliators). However, a single application in a concentration enough to successfully suppress pest populations may be harmful to nymphs of *P. nigrispinus.* Topical toxicity of thiamethoxam to *P. nigrispinus* through foliage sprayings or through pesticide-intoxicated prey (Torres *et al.* 2002a) can be diminished by drench treatment (Torres *et al.* 2003b). This fact, however, is clearly dependent on prey availability and its use need to be considered cautiously. The absence of prey, as manipulated in this study, was a determinant factor for toxicity of imidacloprid to *Orius insidiosus* (Say) exposed to insecticide-treated sorghum and corn seedlings (Al-Deeb *et al.* 2001).

Although presenting residual effects for *P. nigrispinus* nymphs, a single application of thiamethoxam and imidacloprid at rate of 1 mg (a.i.) per plant was enough to control immature whitefly population on cotton plants in potted plants and suppress whitefly and aphid colonization on plants in the field (Figs. 2 and 3). Although with shorter residual effect against whitefly colonization compared to higher doses, thiamethoxam at rate of 0.5 mg (a.i) per plant showed potential of use and should be better studied. Lower pest exposure to thiamethoxam and imidacloprid residues by single application and shorter residual contact in each cotton season should be considered within a cotton pest management program. This strategy reduces the selection pressure on whitefly and on other pests for resistance extending the insecticide lifespan, and also seems less harmful to the heteropteran predators due to shorter residual effects. Moreover, extended residual effects of the systemic insecticides, including neonicotinoids, have been claimed as a major cause for resistance selection on whitefly populations (Elbert & Nauen 2000, El Kady & Devine 2003).

## Acknowledgements

We extend our thanks to Dra. Maria R. Vilarinhos de Oliveira (Embrapa-Recursos Genéticos) for identifying the whitefly colony and Christian S.A. Silva-Torres and Walter S. Evangelista Jr for technical assistance. We also thank two anonymous reviewers for their valuable suggestions. This research was partially sponsored by SEAG (Syngenta Entomological Advisory Group).

## Literature Cited

**Abbott, W.S. 1925.** A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

**Al-Deeb, M.A., G.E. Wilde & K.Y. Zhu. 2001.** Effect of insecticides used in corn, sorghum, and alfalfa on the predator *Orius insidiosus* (Hemiptera: Anthocoridae). J. Econ. Entomol. 94: 1353-1360.

**Baldson, J.A., S.K. Bramam, A.F. Pendley & K.E. Espelie. 1993.** Potential for integration on chemical and natural enemy suppression of azalea lace bug (Heteroptera: Tingidae). J. Environ. Hortic. 11: 153-156.

**Cock, A. De, P. De Clercq, L. Tirry & D. Degheele. 1996.** Toxicity of diafenthiuron and imidacloprid to the predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae). Environ. Entomol. 25: 476-480.

**Elbert, A., B. Becker, J. Hartwig & C. Erdelen. 1991.** Imidacloprid – a new systemic insecticide. Pfla. Nachr. Bayer 44: 113-136.

**Elbert, A. & R. Nauen. 2000.** Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidade) to insecticides in southern

Spain with special reference to neonicotinoids. Pest Manage. Sci. 56: 60-64.

**Lemos, W.P., R.S. Medeiros, F.S. Ramalho & J.C. Zanuncio. 2001.** Effects of plant feeding on the development, survival and reproduction of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae). Int. J. Pest Manage. 47: 89-93.

**Maiensfisch, P., H. Huerlimann, A. Rindlisbacher, L. Gsell, H. Dettwiler, J. Haettenschwiler, E. Syeger & M. Walti. 2001.** The discovery of thiamethoxam: a second-generation neonicotinoid. Pest Manage. Sci. 57: 165-176.

**Mullins, J.W. 1993.** Imidacloprid: a new nitroguanidine insecticide. Am. Chem. Soc. 524: 183-198.

**Ogata, Y. 1999.** Integration of biological control and chemical control in case of Japan. IOBC Bulletin 22: 189-191.

**Oliveira, E.J.M., J.B. Torres, A.F. Carrano-Moreira & F.S. Ramalho. 2002.** Biologia de *Podisus nigrispinus* com escassez de lagartas de *Alabama argillacea* em condições de campo. Pesq. Agropec. Bras. 36: 7-14.

**Ruberson, J.R., H. Nemoto & Y. Hirose. 1998.** Pesticides and conservation of natural enemies, p.207-220. In P. Barbosa (ed.), Conservation of biological control. San Diego, Academic Press, 396p.

**SAS Institute. 2000.** SAS/STAT User's guide, Version 8 for Windows. Cary, SAS Institute Inc., 1383p.

**Smith, S.F. & V.A. Krischik. 1999.** Effects of systemic Imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). Environ. Entomol. 28:1189-1195.

**Torres, J.B., C.S.A. Silva-Torres & J.V. Oliveira. 2003a.** Toxicity of pymetrozine and thiamethoxam to *Aphelinus gossypii* and *Delphastus pusillus*. Pesq. Agropec. Bras. 38: 459-466.

**Torres, J.B., C.S.A. Silva-Torres, M.R. Oliveira & J. Ferreira. 2002a.** Compatibilidade de inseticidas e acaricidas com o percevejo predador *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) em algodoeiro. Neotrop. Entomol. 31: 311-317.

**Torres, J.B., C.S.A. Silva-Torres & R. Barros. 2003b.** Relative effects of the insecticide thiamethoxam on the predator *Podisus nigrispinus* and the tobacco whitefly *Bemisia tabaci* in nectaried and nectariless cotton. Pest Manage. Sci. 59: 315-323.

**Torres, J.B., W.S. Evangelista Jr., R. Barros & R.N.C. Guedes. 2002b.** Dispersal of *Podisus nigrispinus* (Het., Pentatomidae) nymphs preying on tomato leafminer: effect of predator release time, density and satiation level. J. Appl. Entomol. 126: 326-332.

**Zanuncio, J.C., J.B. Alves, T.V. Zanuncio & J.F. Garcia. 1994.** Hemipterous predators of eucalypt defoliator caterpillars. For. Ecol. Manage. 65: 65-73.

*Received 23/04/03. Accepted 25/10/03.*