TOXICITY OF NEONICOTINOIDS TO *CERATITIS CAPITATA* AND *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE)

Adalton Raga[1]*, *Mário Eidi Sato*

Centro Experimental do Instituto Biológico
Secretaria da Agricultura e Abastecimento
P.O. Box 70, 13001-970, Campinas, São Paulo, Brazil

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Abstarct: The effects of imidacloprid and thiamethoxam against *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) adults were evaluated under broadcast spray and toxic bait systems in the laboratory. In general, when *C. capitata* and *A. fraterculus* were sprayed with insecticides, the time required for killing them was inversely proportional to the neonicotinoid concentrations. By cover spray, females and males of *A. fraterculus* were more susceptible to imidacloprid and thiamethoxam than *C. capitata,* presenting significantly lower LT_{50}. In the case of imidacloprid bait, no differences at LT_{50} were detected between females or males of both fruit flies or between sexes of respective fruit fly species. In general, *C. capitata* and *A. fraterculus* adults died in all the toxic baits up to 7 days after application on citrus leaves in the field. The data emphasize the viability of the use of neonicotinoids for the control of fruit flies.

Key words: medfly, South American fruit fly, imidacloprid, thiamethoxam, toxic bait

INTRODUCTION

The medfly – *Ceratitis capitata* (Wiedemann) and South American fruit fly – *Anastrepha fraterculus* (Wiedemann) were considered the most important fruit fly species (Diptera: Tephritidae) in Brazil in terms of economic importance, due to their large distribution, dominance and number of known hosts. Besides damage to the yield, the incidence of both species provides specific restrictions to export trade fruit to several countries around the world.

A wide range of fruits and vegetables are infested by *C. capitata* and *A. fraterculus* in Brazil (Zucchi 2000, 2001), including almost all commercial fruits. In the state of São Paulo, both tephritid species have been captured in McPhail traps almost all year long, although with different population peaks.

In Brazilian, malathion and others organophosphates have been used in the field against fruit flies over the past 40 years (Sampaio *et al.* 1966; Orlando and Sampaio 1973; Reis Filho 1994; Salles 1995). Nowadays, five organophosphates, two pyrethroids and spinosad were registered in Brazil for controlling fruit flies (Agrofit 2010). Although the applications are effective in initial fruit fly infestations, the successive sprays of organophosphates and pyrethroids have caused secondary insect and mite pest outbreaks in many Brazilian orchards, like orange (personal information) and pome fruits (Kovaleski *et al.* 2000). Furthemore, organophosphate applications are restricted (Vargas *et al*. 2002; Barry and Polavarapu 2004) in many countries. The restrictions make it difficult to manage tephritid populations in fruit crops.

Hsu *et al.* (2004) suggest that Oriental fruit fly *Bactrocera dorsalis* (Hendel) can develop resistance to various insecticides, including malathion. Cross-resistance between chemicals may also be found for this species. Koren *et al.* (1984) concluded that medfly females can develop slight resistance to malathion after a nine generation selection process.

Although registered insecticides in Brazil are still effective against *C. capitata* and *A. fraterculus* adults (Raga and Sato 2006), chemicals of low and moderate impact are demanded by growers to support the Integrated Plant Management (IPM) programs (Raga and Sato 2005; Thomas and Mangan 2005; Braham *et al.* 2007) and the integrated fruit production. In other countries, aerial sprays are also used by eradication programs for suppressing medfly populations before sterile adult releases in other countries (Jones and Casagrande 2000).

Neonicotinoid insecticides are the only major new class of insecticides developed in the past three decades and these insecticides have higher selectivity factors for insects versus mammals than the organophosphates, methylcarbamates, and organochlorines (Tomizawa and Casida 2005; Preetha *et al.* 2010). Neonicotinoid insecticides act upon nicotinic acetylcholine receptors (nAChR).

Several authors reported the control of tephritids by exposing adults to neonicotinoids (Hu *et al.* 1998; Prokopy *et al.* 2000; Stelinski *et al.* 2001; Barry and Polavarapu

*Corresponding address:
adalton@biologico.sp.gov.br

2004; Liburd *et al.* 2004; Scoz *et al.* 2004), but compounds differ in their ability to incapacitate and kill flies (Barry and Polavarapu 2005). The present study was undertaken to compare the performance of neonicotinoids which were used to kill fruit flies under laboratory conditions.

MATERIALS AND METHODS

For these studies *C. capitata* and *A. fraterculus* adults were exposed to imidacloprid and thiamethoxam under two systems: cover spray and toxic bait. Besides these tests, an assay was conducted, exposing the flies of both species to neonicotinoid residues (baits) of different ages of treated citrus leaves.

Colonies

The flies were obtained from colonies of both species that were maintained at the Laboratory of Economic Entomology, Instituto Biológico, Campinas, State of São Paulo, since 1993. Medfly was reared using artificial media described by Raga *et al.* (1996). South American fruit fly larvae were reared in papaya fruit. After emergence, adults were fed on a mixture of yeast extract and sugar at a 1:3 ratio.

Cover spray assay

Five females and five males of 2–4-day-old *C. capitata* and 2–7-day-old *A. fraterculus* were individually placed in ten plastic Petri dishes (8.5 cm diameter). Each dish corresponded to one replication. Approximately 2.0 ml of insecticide suspension was applied with Potter spray tower at 60.0 kPa. The methodology was based on Raga and Sato (2006). Imidacloprid was tested at 60.0; 90.0; 120.0 and 150.0 mg of active substance (a.s.)/liter of distilled water. For thimethoxam, the rates were equivalent to 75.0; 150.0; 225.0 and 300.0 mg of a.s./liter of distilled water. Both neonicotinoids were compared with deltamethrin at 12.5 mg of a.s./liter of water, and the control. Evaluations of survivorship were conducted at intervals of ten minutes, from 20 to 140 minutes after initial exposure.

Approximately 2 h before the beginning of the experiments, flies were deprived of food and water. During the test, only baits were available to the flies. Tests were carried out in the laboratory under 25±1°C, 70±10% relative humidity (RH) and 14 h photophase.

Toxic bait assay

The same insecticides and respective concentrations tested for cover spray were tested under toxic baits. The method of fly exposure to insecticide baits was based on Raga and Sato (2006). Five females and five males of 2–4-day-old *C. capitata* and 2–3-day-old *A. fraterculus* were each placed in small cages (1400 cc). The commercial corn protein Bio Anastrepha™ (Bio Controle Métodos de Controle de Pragas Ltda, São Paulo, SP) at 5% was used to prepare the baits, and also allowed to the control adults. About 18h before the beginning of the experiments, flies were deprived of food and water. Tests were carried out at 25±1°C, 70±10% RH and 14 h photophase. The adult survival was registered at 15, 30, 45, 60, 75, 90, 120, 150, 180, 240 and 300 min after initial exposure.

Residual bait assay

Treatments consisted of imidacloprid (60.0 and 150.0 mg of a.s./liter) and thiamethoxam (75.0 and 300 mg of a.s./liter). Both neonicotinoids were compared with deltamethrin (12.5 mg of a.s./l) and the control. Diluted baits were manually sprayed on citrus trees until the upper surface of foliage was completely covered. Protein with insecticide was used for the control. Baits were allowed to dry at an ambient temperature before testing. The leaves were collected at intervals of 0, 1, 2, 4 and 7 days after application (DAA). Five females and five males of 2–6 d *C. capitata* and 3–8 d *A. fraterculus* were placed (separately for each species) into a plastic cage (1,400 cc) containing one leaf. Cages were put on the ground. Mortality was assessed at 30, 60 and 90 minutes after exposure. New adults were used for each test, with different ages of insecticide residue. During the period of the experiment (one week), the average temperature in the field was 23.4°C and the rainfall was 47 mm, with at least 24 h without rain after treatment.

Statistical analysis

A completely randomized design was used for selection of flies and treatments. For each test we used ten replications, except for residual tests (five replications). Data were analyzed by ANOVA, and means separated using Tukeyś test ($p = 0.05$). Irreversible knockdown followed by death of the adults were the criteria which were used to determine the mortality. The LT_{50} values for each compound were estimated using Probit analysis (Polo PC) (LeOra Software 1987).

RESULTS AND DISCUSSION

Cover spray assay

In general the time required for killing *C. capitata* and *A. fraterculus* was inversely proportional to the tested neonicotinoid concentrations, when the insecticides were sprayed on the adults (Table l). Imidacloprid presented the lowest LT_{50} values for the South American fruit fly: 10.6 min for males and 13.0 for females, exposed respectively to 120.0 mg and 150.0 mg/l. Females of *A. fraterculus* were more susceptible to imidacloprid and thiamethoxam than *C. capitata* females in all tested concentrations.

Except for the highest concentrations of neonicotinoids, medfly was more tolerant to imidacloprid and thiamethoxam than *A. fraterculus*. Comparing both neonicotinoids at 150 mg/l, imidacloprid presented significantly lower LT_{50} than thioamethoxam, based on the non-overlapping of 95% confidential limit values of LT_{50}, for females and males of *C. capitata*.

No differences between species and sexes were detected when the adult flies were exposed to deltamethrin. Deltamethrin at 12.5 mg/l caused 50% mortality for both sexes of *C. capitata* in a shorter period than imidacloprid and thiamethoxam at any concentration.

The South American fruit fly was found to be more tolerant to deltamethrin, ethion, trichlorfon, fenthion and fenpropathrin than medfly by Raga and Sato (2006). Those authors obtained low values of LT_{50} for *A. fraterculus*

Table 1. Comparison of lethal times (LT_{50}) obtained for females (F) and males (M) of *A. fraterculus* (Af) and *C. capitata* (Cc) exposed to neonicotinoids in cover spray assay

Treatment active substance	Species	Sex	LT_{50} (min)	Slope ±SE	X^2	df
Imidacloprid (60.0 mg/l)	Af	F	22.1 (14.2–27.9)	1.93±0.13	1.49	5
	Cc	F	40.3 (36.2–44.2)	3.65±0.14	9.76	5
	Af	M	15.0 (7.07–20.8)	1.95±0.17	1.66	4
	Cc	M	35.0 (30.8–38.7)	3.40±0.12	3.58	6
Imidacloprid (90.0 mg/l)	Af	F	17.0 (8.78–23.2)	1.81±0.14	5.23	5
	Cc	F	37.5 (33.8–40.9)	3.90±0.13	4.39	6
	Af	M	13.0 (3.23–19.8)	1.89±0.27	1.82	2
	Cc	M	29.3 (25.0–32.9)	3.37±0.18	4.62	4
Imidacloprid (120.0 mg/l)	Af	F	15.0 (6.88–20.8)	1.98±0.18	2.14	4
	Cc	F	33.1 (28.7–36.9)	3.24±0.14	4.95	5
	Af	M	10.6 (2.35–16.8)	1.89±0.24	0.028	3
	Cc	M	26.0 (21.9–29.3)	3.66±0.19	1.90	4
Imidacloprid (150.0 mg/l)	Af	F	13.0 (5.03 -18.4)	2.30±0.25	0.24	3
	Cc	F	25.7 (20.9–29.6)	3.09±0.18	2.51	4
	Af	M	15.2 (6.62–20.7)	2.14±0.24	0.48	3
	Cc	M	22.9 (17.0–27.3)	2.65±0.18	1.49	4
Thiamethoxam (75.0 mg/l)	Af	F	28.8 (24.1–32.7)	3.06±0.14	1.32	5
	Cc	F	55.3 (46.1–62.4)	2.74±0.25	0.91	4
	Af	M	27.6 (23.4–31.0)	3.54±0.23	1.17	3
	Cc	M	62.0 (49.2–68.7)	4.43±0.48	0.85	3
Thiamethoxam (150.0 mg/l)	Af	F	23.8 (19.1–27.5)	3.31±0.24	1.16	3
	Cc	F	48.1 (40.9–55.0)	2.55±0.21	3.79	4
	Af	M	21.0 (15.6–24.8)	3.16±0.24	3.95	3
	Cc	M	55.8 (48.8–62.0)	3.37±0.22	0.15	4
Thiamethoxam (225.0 mg/l)	Af	F	19.6 (14.1–23.5)	3.15±0.25	0.76	3
	Cc	F	37.3 (27.4–44.2)	2.22±0.18	0.87	4
	Af	M	13.5 (7.07–17.9)	3.09±0.30	0.058	3
	Cc	M	33.4 (22.7–40.4)	3.30±0.25	0.66	4
Thiamethoxam (300.0 mg/l)	Af	F	16.3 (4.05–21.1)	2.35±0.43	0.45	2
	Cc	F	33.6 (26.3–38.8)	3.13±0.22	0.096	4
	Af	M	17.0 (10.0–20.7)	3.28±0.37	0.15	3
	Cc	M	25.0 (15.7–31.6)	2.67±0.20	0.43	4
Deltamethrin (12.5 mg/l)	Af	F	16.1 (10.6–18.8)	5.73±0.71	0.36	2
	Cc	F	13.1 (5.62–17.6)	3.25±0.37	2.94	3
	Af	M	12.8 (3.79–16.9)	4.66±0.77	0.099	2
	Cc	M	15.5 (9.56–19.2)	3.84±0.36	0.84	3

females (3.3 and 4.7 min) and males (5.1 and 4.7 min) exposed to fenpropathrin and malathion, respectively.

In terms of susceptibility, except for medfly exposed to imidacloprid at 90.0 mg/l, no differences between LT_{50}s were detected between females and males of respective species in each tested concentration of neonicotinoids (Table 1). Except for trichlorphon, Raga and Sato (2006) did not detect any differences between *A. fraterculus* females and males exposed to six organophosphates and two pyrethroids under Potter spray tower.

Toxic bait assay

In the case of imidacloprid, no differences at LT_{50} were detected between fruit fly species (females or males) or between sexes of respective species. On the other hand, medfly females were more susceptible to thiamethoxam baits than *A. fraterculus* females (Table 2).

In terms of susceptibility, no differences were detected between females and males of each species exposed to thiamethoxam baits (Table 2). The highest value

Table 2. Comparison of lethal times (LT_{50}) obtained for females (F) and males (M) of *A. fraterculus* (Af) and *C. capitata* (Cc) exposed to neonicotinoids in toxic bait assay

Treatment active substance	Species	Sex	LT_{50} (min)	Slope ±SE	X^2	df
Imidacloprid (60.0 mg/l)	Af	F	80.5 (71.4–88.7)	3.98±0.28	1.68	3
	Cc	F	64.6 (55.1–72.8)	2.96±0.14	0.41	5
	Af	M	65.6 (55.6–74.3)	2.83±0.18	3.66	4
	Cc	M	62.3 (55.1–68.7)	3.89±0.16	1.30	5
Imidacloprid (90.0 mg/l)	Af	F	63.5 (56.7–69.6)	4.12±0.20	1.26	4
	Cc	F	66.0 (59.1–72.2)	4.08±0.20	1.69	4
	Af	M	59.4 (49.6–67.2)	3.04±0.19	1.69	4
	Cc	M	55.8 (48.8–61.6)	4.26±0.22	1.17	4
Imidacloprid (120.0 mg/l)	Af	F	56.4 (48.2–63.0)	3.66±0.20	3.22	4
	Cc	F	59.8 (53.7–65.0)	4.77±0.23	0.60	4
	Af	M	52.0 (43.4–58.4)	3.87±0.28	5.11	3
	Cc	M	48.1 (38.0–55.6)	3.26±0.21	0.35	4
Imidacloprid (150.0 mg/l)	Af	F	47.6 (38.0–54.3)	3.76±0.29	2.26	3
	Cc	F	53.8 (47.2–59.1)	4.76±0.30	0.99	3
	Af	M	44.9 (32.4–52.9)	3.11±0.28	1.81	3
	Cc	M	49.3 (44.1–54.2)	4.11±0.24	2.95	3
Thiamethoxam (75.0 mg/l)	Af	F	73.1 (62.1–82.0)	3.46±0.20	2.43	4
	Cc	F	29.7 (18.5–38.4)	1.87±0.12	1.51	5
	Af	M	63.2 (54.2–70.9)	3.17±0.19	1.82	4
	Cc	M	30.7 (20.6–39.0)	1.97±0.10	3.34	6
Thiamethoxam (150.0 mg/l)	Af	F	63.2 (55.0–70.5)	3.42±0.18	1.48	4
	Cc	F	17.7 (6.27–27.2)	1.64±0.13	0.29	5
	Af	M	58.2 (50.7–64.6)	3.83±0.17	0.71	5
	Cc	M	24.5 (14.2–32.9)	1.88±0.11	2.09	6
Thiamethoxam (225.0 mg/l)	Af	F	55.7 (48.0–62.0)	3.88±0.21	2.94	4
	Cc	F	13.2 (6.73–19.3)	1.49±0.10	1.07	4
	Af	M	49.2 (42.1–55.6)	2.92±0.13	2.92	5
	Cc	M	15.3 (3.68–24.2)	1.98±0.24	0.029	3
Thiamethoxam (300.0 mg/l)	Af	F	39.6 (32.5–45.4)	3.06±0.17	2.16	4
	Cc	F	9.66 (0.74–17.7)	1.03±0.15	1.05	3
	Af	M	40.7 (34.5–45.8)	3.58±0.23	2.18	3
	Cc	M	10.4 (2.76–17.3)	1.25±0.14	0.39	3
Deltamethrin (12.5 mg/l)	Af	F	68.2 (60.3–75.4)	3.54±0.19	0.38	4
	Cc	F	75.2 (64.8–83.8)	3.61±0.20	3.98	4
	Af	M	66.6 (60.2–72.6)	4.31±0.21	3.74	4
	Cc	M	70.5 (64.2–75.6)	6.42±0.50	0.32	2

(80.5 min) for a neonicotinoid product was obtained for *A. fraterculus* females treated with imidacloprid bait at 60.0 mg/l. The lowest LT_{50} were observed for thiamethoxam at 300.0 mg/l for *C. capitata* females (9.66 min) and *C. capitata* males (10.4 min). In the case of deltamethrin bait at 12.5 mg/l, the LT_{50}s were similar for both species and sexes, with values ranging from 66.6 to 75.2 min.

The lethal times observed for *A. fraterculus* in toxic bait assay were higher than in the cover spray test for all concentrations of both neonicotinoids. However, in the case of *C. capitata*, for thiamethoxam at 150.0 and 225.0, the LT_{50} values for toxic bait were lower than for cover spray assay.

For deltamethrin, the LT_{50}s for toxic bait were higher than for the cover spray for both sexes and species. The highest contrasts between the methods were observed for this pyrethroid, for which the values obtained for the toxic bait were up to 5.7 times higher than for the cover spray.

According to Raga and Sato (2006), when medfly females were treated with fenpropathrin and trichlorfon baits, the lowest LT_{50} values were lower than 5.0 min. The same authors reported that medfly females were more susceptible than males when fed on chlorpyrifos and dimethoate baits.

The thiamethoxam LT_{50}s estimated for males of *C. capitata* exposed to toxic bait were inferior to those for spray application (Table 2). According to Yee and Alston (2006), imidacloprid and thiacloprid were more toxic to *Rhagoletis indifferens* Curran adults (Diptera: Tephritidae)

when the insecticides were ingested than when they were topically applied.

The lethal times for thiamethoxam at all concentrations were shorter than for deltamethrin in *A. fraterculus* and *C. capitata* (Table 2). The lethal times for neonicotinoids, observed in the present study, were also shorter than those reported for spinosad in *C. capitata* and *A. fraterculus* (Raga and Sato 2005). The spinosad at 80 mg/l tested as bait against medfly presented LT_{50}s around 106 min (97.1–117 min). Higher LT_{50} values of spinosad in comparison to those of neonicotinoid compounds is probably related to the mode of action of spinosad. This insecticide normally kills the insects by causing the cessation of feeding and paralysis (Salgado *et al.* 1998).

Barry and Polavarapu (2005) observed 80% knockdown in *Ragoletis mendax* Curran, one hour after exposures of adults (5 min exposure in baits containing the insecticide at 40 mg/l) to the neonicotinoid imidacloprid. The knockdown for spinosad (40 mg/l) was only 5% in the same period.

Residual bait assay

All toxic baits caused significant mortality in *C. capitata* (Fig. 1) and *A. fraterculus* (Fig. 2) adults up to 7 DAA, when the insects were exposed to the bait residues for 60 or more minutes. At 90 minutes of adult exposure to bait residual on leaves collected at 4 DAA, thiamethoxam (300 mg of a.s./liter) was not significantly different from deltamethrin in the efficacy against both fruit fly species. In the case of medfly, the mortality provided by thiamethoxam (300 mg of a.s./liter) at 90 minutes, was similar to that by deltamethrin up to 4 DAA. The highest concentrations of neonicotinoids showed similar mortalities as deltamethrin in the South American fruit fly (Fig. 2). Residues of spinosad, malathion, fenthion and deltamethrin, in toxic baits applied on citrus leaves, caused high mortalities of medfly adults up till two days after treatment (Raga and Sato 2005).

Bait spray will continue to be an integral part of the management of many tephritid pests (Barry *et al.* 2006). Cover sprays are not as specific as bait sprays and remove many of the nontarget organisms, especially beneficials

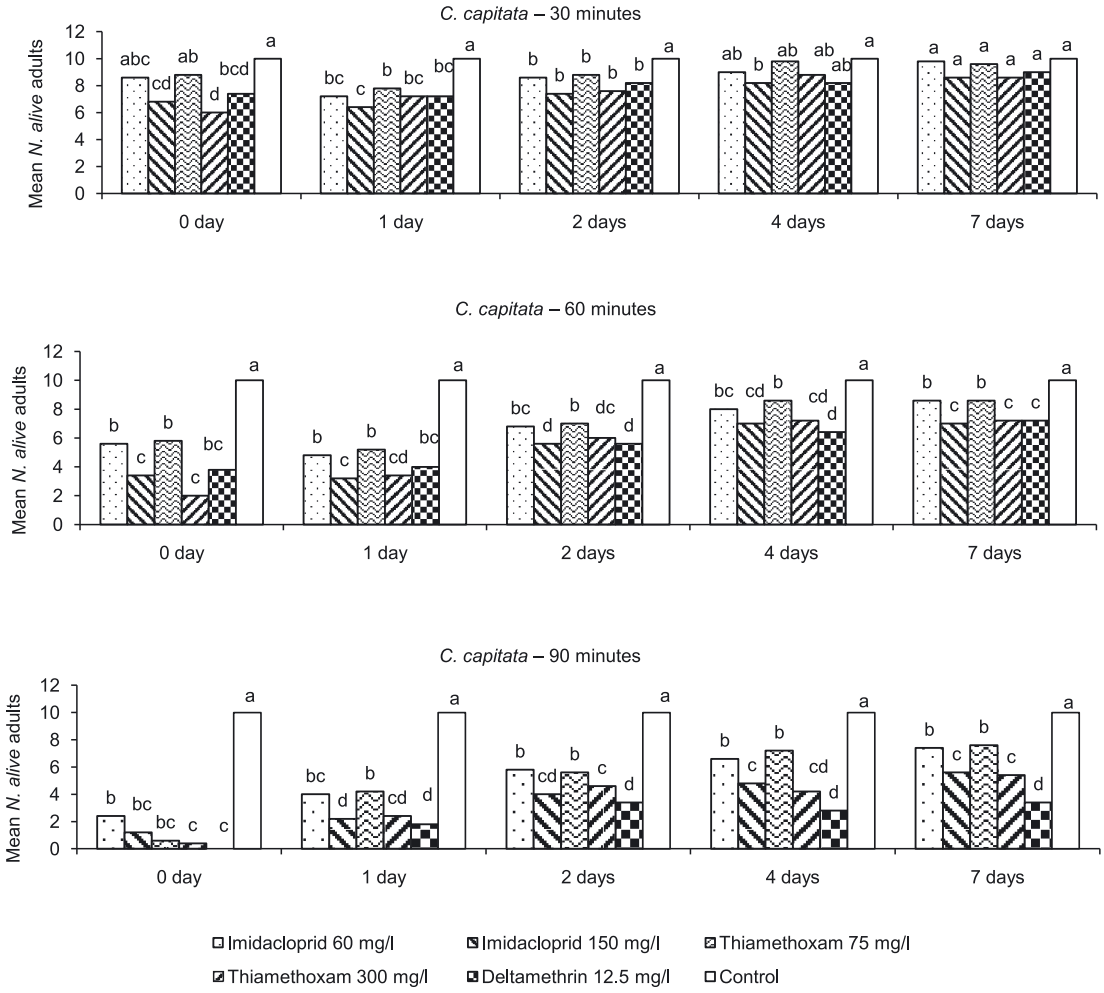

Fig. 1. Mean number of alive adults of *C. capitata* after exposure to bait residues in citrus leaves collected at different times in the field. In the initial period (0 day), the leaves with baits were exposed approximately 30 minutes after spraying. Columns in the same date with the same letter are not significantly different by Tukey's test at 5% level

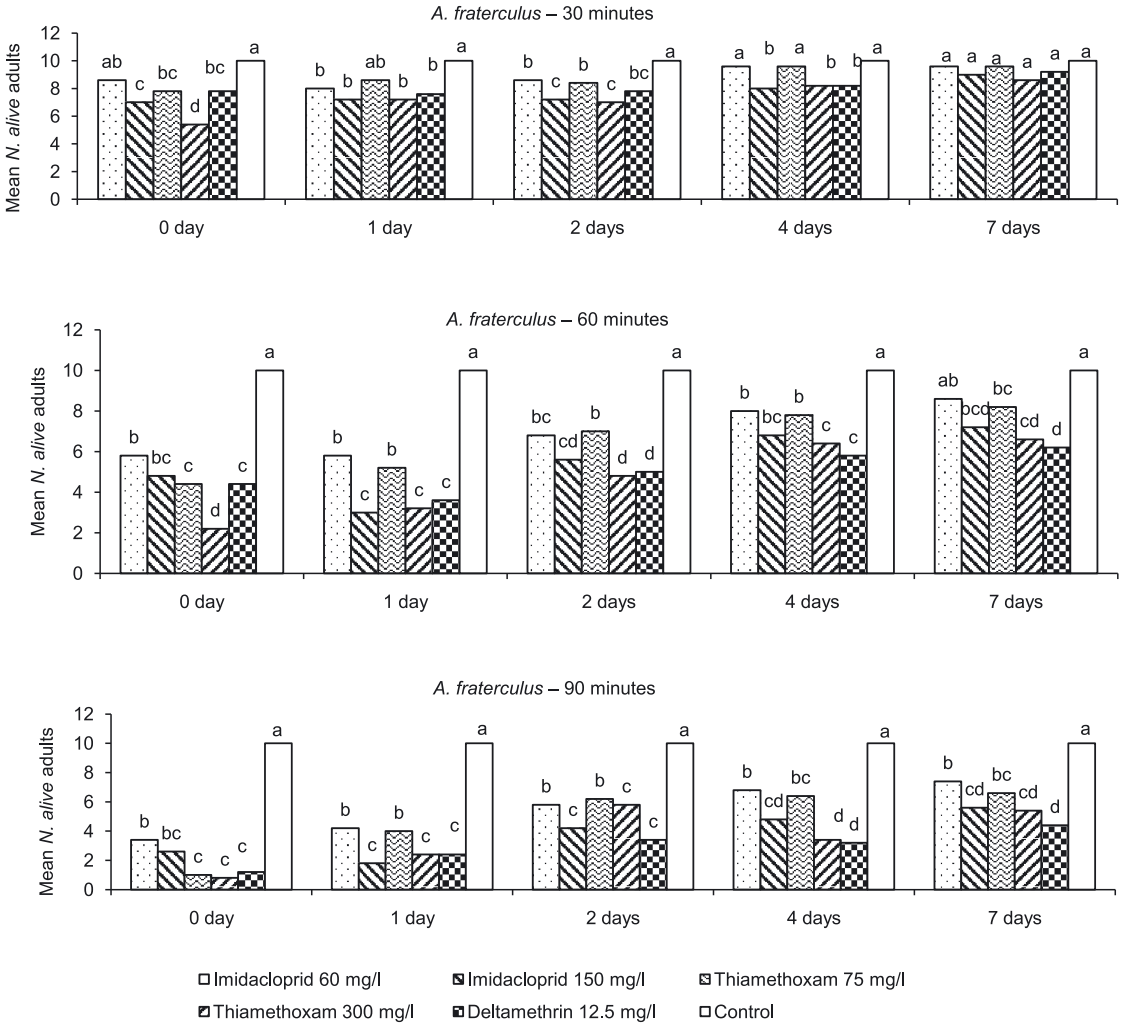

Fig. 2. Mean number of alive adults of *A. fraterculus* after exposure to bait residues in citrus leaves collected at different times in the field. In the initial period (0 day), the leaves with baits were exposed approximately 30 minutes after spraying. Columns in the same date with the same letter are not significantly different by Tukey's test at 5% level

(Calkins and Malavasi 1995). Bait sprays containing feeding stimulants, have several advantages to conventional sprays because the mortality of fruit flies is primary from oral toxicity (Barry and Polavarapu 2005). Bait spray efficacy is also dependent of the attractiveness and stability of proteins.

In some cases, as for controlling vectors, fruit growers have applied neonicotinoids under cover spray. In that situation imidacloprid and thiamethoxam can reduce fruit fly adult populations inside the treated orchards.

In our study, we use different ways to measure the effects of neonicotinoids against fruit flies. Future field experiments are needed to determine if the neonicotinoids is a viable alternative to substitute organophosphate for controlling tephritids.

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