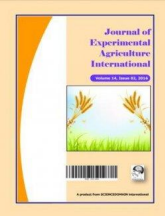

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Comparison of Insecticide Toxicity in Adults of the Fruit Flies *Anastrepha fraterculus* (Wied.) and *Anastrepha grandis* (Macquart) (Tephritidae)

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Authors' contributions

This work was carried out with the contribution of all authors. Authors LTG, SBS and FBB contributed to the maintenance of fruit fly colonies and development of the experiments. Author AR designed the study and wrote the first draft of the manuscript. Author MES helped the statistical analysis and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Fruit flies (Diptera: Tephritidae) of economic and quarantine significance are responsible for both quantitative and qualitative losses in horticulture. Most producers in Brazil use insecticides as a cover spray for the control of fruit flies. Here new insecticide molecules were evaluated under laboratory conditions as potential replacements for organophosphates to provide protection and prevent damage to horticultural crops. Five pairs of *Anastrepha fraterculus* (Wied.) and *Anastrepha grandis* (Wied.) were placed into Petri dishes and exposed to eight insecticides using a Potter spray tower. The number of insect deaths was monitored until 21 hours after spraying. In general, both *Anastrepha* species exhibited similar susceptibility to the insecticides. Different fruit fly mortalities were observed among the insecticide treatments beginning 30 minutes after exposure. Interactions were verified between the compounds and fruit fly species and between evaluation periods and treatments. Acetamiprid, deltamethrin, flypyradifurone (1.60 ppm), imidacloprid, phosmet, thiamethoxam and zeta-cypermethrin caused similar mortalities 21 hours after treatment for both fruits fly species.

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1. INTRODUCTION

The South American fruit fly, *Anastrepha fraterculus* (Wied.) and the South American cucurbit fruit fly, *Anastrepha grandis* (Macquart) are important pests of fruits and cucurbit crops, respectively, in Latin American countries [1,2,3]. *Anastrepha fraterculus* attacks 116 host plants in Brazil [4], while *A. grandis* exclusively infests wild and commercial cucurbit fruits, especially species and hybrids of *Cucurbita* [5].

The risk of transportation of infested host fruits is very high because *A. fraterculus* and *A. grandis* produce an average of 446 eggs [6] and 538 eggs per female [7], respectively. One *A. grandis* female may oviposit up to 96 eggs per puncture [8]. Quarantine restrictions are required for the exportation of suitable hosts of both fruit fly species (Tephritidae). In the case of *A. grandis,* exportation of cucurbits is allowed from crops managed in fruit fly-free areas or under a risk mitigation system.

Ground applications of insecticides in the form of a cover spray or as toxic bait are the primary methods for the control fruit flies in Brazil [9]. Organophosphates were used against fruit flies for five decades in Brazil, especially malathion, dimethoate, ethion, phention and trichlorfon. In earlier studies, *Anastrepha fraterculus* and *Ceratitis capitata* (Wied.) exhibited high susceptibility to organophosphates, pyrethroids [10], spinosad [9] and neonicotinoids [11] in the laboratory.

Malathion is the most widely used insecticide against fruit flies around the world [12,13,14,15, 16,17], under both cover spray or toxic bait systems.

Flupyradifurone is the first representative of the novel butenolide class of insecticides, which are active against various insect pests, with an excellent safety profile. Flupyradifurone acts reversibly as an agonist on insect nicotinic acetylcholine receptors but is structurally different from known agonists (e.g. neonicotinoids). This insecticide exhibits excellent field efficacy on several crops with different application methods, including foliar, soil, seed treatment and drip irrigation [18].

There are no reports of field fruit fly populations resistant or multi-resistant to insecticides in Brazil. However, Brazil recently banned several organophosphates and growers demand new active ingredients for use as adulticides for fruit flies. The aim of this study was to determine under laboratory conditions, the susceptibility of adults of *A. fraterculus* and *A. grandis* to some new insecticides, to provide fruit fly control options for growers.

2. MATERIALS AND METHODS

Adults of *A. fraterculus* and *A. grandis* were obtained from colonies that have been maintained at the Instituto Biológico, in Campinas, State of São Paulo (SP), Brazil, since 1993 and 2002, respectively. Larvae of *Anastrepha fraterculus* were reared in papaya fruits [19] and *A. grandis* reared in orange pumpkin (*Cucurbita maxima*). During larval development, the fruits were kept in plastic boxes, with a layer of vermiculite until pupation. The boxes were kept in a temperature controlled rearing room (25°C ± 3°C). Three days before emergence, pupae were sieved from the vermiculite. After emergence, the fruit flies were transferred to rearing cages (1.00 m x 0.50 m x 0.50 m) and received water and a mixture of sugar (49.1%), brewer´s yeast (24.5%), yeast extract (12.2%), wheat germ (12.2%) and Sustagen® (2.0%).

Table 1. Insecticides tested against fruit flies in laboratory

Chemical group	Chemical name	Trade name	Concn (ppm)
Neonicotinoids	acetamiprid	Mospilan WG	0.725
Pyrethroids	deltamethrin	Decis 25 EC	1.00
Butenolide	flupyradifurone	Sivanto Prime 200 SL	0.50
Butenolide	flupyradifurone	Sivanto Prime 200 SL	1.60
Neonicotinoids	imidacloprid	Evidence 700 WG	2.10
Organophosphates	malathion	Malathion 1000 EC	15.0
Organophosphates	phosmet	Imidan 500WP	7.50
Neonicotinoids	thiamethoxam	Actara 25 WG	0.50
Pyrethroids	zeta-cypermethrin	Mustang 350 EC	0.35

All tested chemicals are described in Table 1. Deltamethrin, imidacloprid, and acetamiprid are registered for use in some fruit and cucurbit crops, while malathion, pyradifurone, zeta-cipemethrin and phosmet are registered for fruit crops in Brazil [20].

Five females and five males of 8-9 day-old *A. fraterculus* and 10-20 day-old *A. grandis* were captured in glass tubes that were then closed with cotton. Prior to the spraying, the tubes were stored in the refrigerator at approx. -15°C for 4 minutes, and the flies were transferred immediately to glass Petri dishes (8.5 cm diameter).

An insecticide suspension (2 mL) was applied to the adult flies under a Potter spray tower at 60.0 kPa. After the treatment, the flies were maintained at room temperature (25 ± 3°C) and ambient humidity (50 ± 10%), deprived of water and food. Evaluations of survivorship were conducted at 30, 60, 90, 120, 150, 180, 240, and 360 minutes and 21 h after initial exposure. Irreversible knockdown followed by the death of the adults was the criterion to determine mortality [11].

Ten replicates were established for each treatment. Each Petri dish corresponded to one replication per treatment. We performed ANOVA (The SAS System for Windows, version 9.2) using ranked data [21]. Three-factor ANOVA was used to compare the mortality of fruit flies. The LT_{50} (lethal time) values for each compound were estimated using Probit analysis (Polo PC).

3. RESULTS AND DISCUSSION

The mortality caused by the insecticides did not differ significantly between fruit fly species (females + males) (F = 1.360; df = 0.58; P = 0.448) or sexes (F = 1.360, df = 3.74; P = 0.054). Different mortality levels were obtained among the insecticides (F = 9,360; df = 155.96; P < 0.001). Interactions were observed between the compounds and fruit fly species (F = 9.360; df = 2.26; P = 0.018) and between evaluation periods and treatments (F = 27.1080; df = 32.85; P < 0.001).

Deltamethrin, malathion, and zeta-cypermethrin were highly toxic to both species of *Anastrepha*, with 100% mortality within 30 minutes after treatment (Tables 2 and 3).

Table 2. Comparison of adult mortality (number of dead insects per dish, *n* = 5) of *Anastrepha fraterculus* (AF) and *Anastrepha grandis* (AG) in two periods after exposure to insecticides under cover spray in the laboratory

Treatment	30 min		21 h	
	AF	AG	AF	AG
	Females			
Acetamiprid 0.725 ppm	3.70bcA	0.10cB	5.00aA	5.00aA
Deltamethrin 1.00 ppm	5.00aA	5.00aA	5.00aA	5.00aA
Flupyradifurone 0.5 ppm	1.00cdA	1.00cA	2.80bA	2.80bA
Flupyradifurone 1.6 ppm	2.20cdA	0.20cB	4.10aA	5.00aA
Imidacloprid 2.1 ppm	3.70bA	1.40cA	5.00aA	5.00aA
Malathion 15.0 ppm	5.00aA	5.00aA	5.00aA	5.00aA
Phosmet 7.5 ppm	4.30bA	3.40bA	5.00aA	5.00aA
Thiamethoxam 2.10 ppm	1.60cdB	4.30abA	4.70aA	5.00aA
Zeta-cypermethrin 0.35 ppm	5.00aA	5.00aA	5.00aA	5.00aA
Control	0.00dA	0.00cA	0.10bA	0.00cA
	Males			
Acetamiprid 0.725 ppm	4.50aA	0.30cB	5.00aA	5.00aA
Deltamethrin 1.00 ppm	5.00aA	5.00aA	5.00aA	5.00aA
Flupyradifurone 0.5 ppm	0.90cdA	0.90bcA	2.60cA	2.60bA
Flupyradifurone 1.6 ppm	3.10bcA	0.20cB	4.10bcB	5.00aA
Imidacloprid 2.1 ppm	4.10abA	1.10bcB	5.00aA	5.00aA
Malathion 15.0 ppm	5.00aA	5.00aA	5.00aA	5.00aA
Phosmet 7.5 ppm	4.30abA	2.50bB	5.00aA	5.00aA
Thiamethoxam 2.10 ppm	1.80cdB	4.50aA	4.90abA	5.00aA
Zeta-cypermethrin 0.35 ppm	5.00aA	5.00aA	5.00aA	5.00aA
Control	0.00dA	0.00cA	0.30dA	0.00cA

Means within columns followed by the same lower case letter are not significantly different (ANOVA – Tukey´s test, P<0.05). Means within rows followed by the same upper case letter in each time are not significantly different (ANOVA – Tukey´s test, P<0.05)

In the case of *A. grandis*, high mortality (86%) was also obtained for thiamethoxam (Table 2). The mortalities of *A. grandis* males at 30 minutes were similar (≥ 90%) for deltamethrin, malathion, thiamethoxam and zeta-cypermethrin. Males of *A. fraterculus* were more susceptible than those of *A. grandis* to imidacloprid and phosmet, with mortalities above 80%, which is similar to the results from the mentioned pyrethroid and organophosphate insecticides (Table 2).

The fruit fly mortality increased between the evaluations for each insecticide treatment (F = 3.1080; df = 344.51; P < 0.001). The levels of fruit fly mortality were higher at 21 hours after exposure for most of the insecticides (Table 2). Except for flupyradifurone sprayed on males (1.6 ppm), no statistically significant differences in mortality were observed between the fruit fly species at 21 hours.

The number of dead females of *A. fraterculus* was higher than that of *A. grandis* at 30 minutes for acetamiprid and imidacloprid (Table 2).

Considering the lethal times of the insecticides, *A. fraterculus* was more susceptible than *A. grandis*, with lower LT_{50} values for imidacloprid (F and M), flupyradifurone 1.6 ppm (F and M), acetamiprid (M) and phosmet (F) (Table 3).

Table 3. Comparison of lethal times (LT_{50}) obtained for both sex of *Anastrepha fraterculus* (*Af*) and *Anastrepha grandis* (*Ag*) exposed to insecticides under cover spray in the laboratory

Treatment	Species	Sex	LT_{50} (min)	Slope ± SE	X^2	df
Acetamiprid	*Af*	Females	15.1 (5.95 – 24.5)	1.65 ± 0.29	0.71	7
0.725 ppm	*Ag*	Females	52.0 (1.69 – 77.1)	3.69 ± 1.27	2.36	6
	Af	Males	8.02 (1.06 – 16.6)	1.84 ± 0.46	0.06	7
	Ag	Males	52.3 (47.8 – 56.8)	6.91 ± 0.63	0.49	7
Deltamethrin	*Af*	Females	< 30.0*	-	-	-
1.00 ppm	*Ag*	Females	< 30.0*	-	-	-
	Af	Males	< 30.0*	-	-	-
	Ag	Males	< 30.0*	-	-	-
Flupyradifurone	*Af*	Females	516.5 (293.6 – 1850.7)	0.56 ± 0.14	0.71	7
0.5 ppm	*Ag*	Females	145.3 (94.4 – 219.1)	0.70 ± 0.14	3.54	7
	Af	Males	700.2 (348.3 – 5093.5)	0.48 ± 0.14	1.96	7
	Ag	Males	119.2 (78.9 – 168.3)	0.80 ± 0.14	2.90	7
Flupyradifurone	*Af*	Females	2.76 (0.00056 – 14.7)	0.45 ± 0.16	2.91	7
1.6 ppm	*Ag*	Females	77.0 (67.7 – 86.1)	3.31 ± 0.30	1.93	7
	Af	Males	6.55 (0.000001 – 22.6)	0.74 ± 0.35	0.21	3
	Ag	Males	69.7 (61.6 – 77.4)	3.86 ± 0.35	1.68	7
Imidacloprid	*Af*	Females	22.7 (14.8 – 28.3)	3.90 ± 0.76	0.34	7
2.1 ppm	*Ag*	Females	48.9 (29.5 – 65.5)	4.84 ± 1.15	13.9	7
	Af	Males	13.7 (5.02 – 21.7)	2.26 ± 0.46	0.072	7
	Ag	Males	53.8 (36.9 – 68.7)	5.17 ± 1.07	13.8	7
Malathion	*Af*	Females	< 30.0*	-	-	-
15.0 ppm	*Ag*	Females	< 30.0*	-	-	-
	Af	Males	< 30.0*	-	-	-
	Ag	Males	< 30.0*	-	-	-
Phosmet	*Af*	Females	3.70 (0.065 – 11.7)	1.28 ± 0.37	0.092	7
7.5 ppm	*Ag*	Females	24.0 (16.6 – 28.9)	4.70 ± 1.02	0.014	7
	Af	Males	≤ 30.0	-	-	-
	Ag	Males	29.7 (23.3 – 35.0)	4.01 ± 0.58	0.025	7
Thiamethoxam	*Af*	Females	57.6 (25.6 – 85.2)	1.95 ± 0.41	2.73	7
2.10 ppm	*Ag*	Females	6.31 (0.55 – 14.9)	1.53 ± 0.39	0.04	7
	Af	Males	37.4 (15.6 – 56.0)	1.95 ± 0.37	1.61	7
	Ag	Males	1.57 (0.00058 – 8.25)	0.98 ± 0.32	0.038	7
Zeta- cypermethrin	*Af*	Females	< 30.0*	-	-	-
0.35 ppm	*Ag*	Females	< 30.0*	-	-	-
	Af	Males	< 30.0*	-	-	-
	Ag	Males	< 30.0*	-	-	-

In the case of thiamethoxam, there was a tendency opposite to the other insecticides tested, with greater susceptibility (lower LT_{50}) in *A. grandis* compared to *A. fraterculus*, with significant differences between species for both sexes (Table 3).

No significant differences in LT_{50} values were detected between males and females of *A. fraterculus* and of *A. grandis* for any of the evaluated insecticides (Table 3).

Flupyradifurone at its highest concentration (1.6 ppm) presented significantly shorter lethal times (LT_{50}) (2.76 to 77.0 min) than at its lowest concentration (0.5 ppm) (119.2 to 700.2 min) for the two fruit fly species (Table 3).

Flupyradifurone, imidacloprid and acetamiprid (LT_{50}s ≥ 48.9 min) were not effective against *A. grandis* up to 60 minutes after exposure (Fig. 1).

Thiamethoxam is registered for many horticultural crops in Brazil, including zucchini, watermelon, melon and cucumber [20]. *Anastrepha grandis* is a quarantine pest for many countries, with a risk of spreading to fruit fly free areas and those under mitigation risk systems on the American continent [5]. In some regions of the state of São Paulo, *A. grandis* infestations cause significant yield losses in orange pumpkin (*Cucurbita maxima*) and Tetsukabuto hybrid (*C. maxima* x *C. moschata*). Due to the long duration of immature stages of *A. grandis* [7], economic losses may also occur during the post-harvest period and commercialization. Thus, the growers spray insecticides to kill adults to prevent oviposition in cucurbits.

An earlier study found that females and males of *A. fraterculus* were more susceptible than *C. capitata* to cover spraying of imidacloprid and thiamethoxam in laboratory [11]. The resistance or susceptibility of populations of *Bactrocera cucurbitae* Coquillett (Tephritidae) to insecticides in China is variable according to the crop [22], especially due to differences in selection pressure and across geographic regions. In the laboratory, zeta-cypermethrin killed adults of *Rhagoletis indifferens* (Tephritidae) more quickly than malathion and spinetoram, causing up to 100% mortality 2 hours after exposure [23].

Here, deltamethrin, malathion and zeta-cypermethrin caused complete mortality for adults of both species within a very short period (< 30 min) after application. Similar results were previously obtained for organophosphates and pyrethroids against *A. fraterculus* and *C. capitata* in the laboratory [10]. Deltamethrin and malathion are effective at preventing infestation of *B. cucurbitae* in bitter gourd in India [24]. Insecticides should kill fruit flies within 2 hours after exposure to prevent oviposition in the field [23].

The differences in efficacy and lethal time observed among the insecticide treatments are probably associated with the mode of action and chemical properties of each compound. The neonicotinoids acetamiprid, imidacloprid and thiamethoxam and the butenolide flupyradifurone are classified as systemic insecticides; however, the organophosphates phosmet, malathion, and the pyrethroids deltamethrin and zeta-cypermethrin are non-systemic with contact action [25], implying in some contrasts like solubility in water and cuticle penetration ability.

Deltamethrin, for instance, is extremely lipophilic and easily penetrates the cuticles of insects; and induces "long-lasting" inhibition of the sodium channel activation gate (Type II pyrethroid with α-cyano group) [26]. These factors may explain its high toxicity and short lethal time to the fruit flies as verified in the present study.

The lower efficacy and slower action of the neonicotinoid imidacloprid against fruit flies [*Bactrocera tryoni* (Froggatt)] in comparison with some organophosphate (dimethoate) and pyrethroid (alpha-cypermethrin) insecticides were also reported by other authors [27], corroborating the results obtained for *A. grandis*.

Direct spraying kills fruit flies more rapidly than topical and residual contact methods [23]. Consequently, calibrating pesticide spray equipment is essential to ensure the best foliar coverage and insect body contact. Although many insecticides do not immediately kill the flies, they may cause insects to drop to the lower canopy or onto the ground, where the adults are exposed to predation by ants and spiders [28, 29].

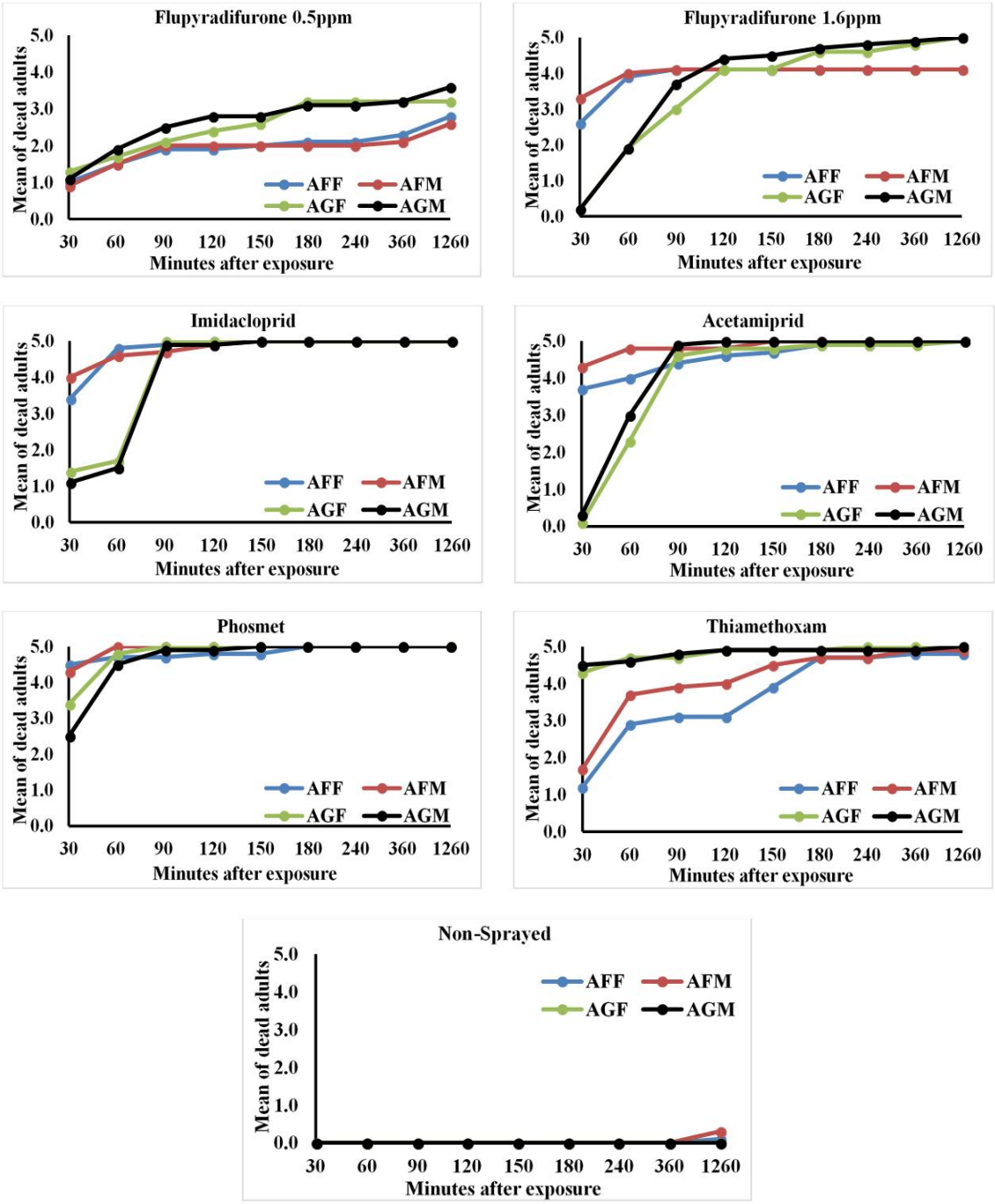

Fig. 1. Cumulative time-mortality curves for females (F) and males (M) of *Anastrepha fraterculus* (AF) and *Anastrepha grandis* (AG) exposed to insecticides that did not cause total mortality up to 30 minutes of exposure in the laboratory

4. CONCLUSION

Results from this study demonstrate the potential efficacy of insecticides from different chemical groups for killing adults of two *Anastrepha* species of economic and quarantine significance. The use of cover sprayed insecticide to manage resident or immigrant populations of *Anastrepha* may be effective when the spray equipment provides effective contact with the fly body.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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