Pesticide Toxicity to *Anastrepha suspensa* (Diptera: Tephritidae)[1]

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Abstract Twenty-four materials were assessed for toxicity to *Anastrepha suspensa* (Loew) in laboratory assays. Compounds which should be effective for managing *A. suspensa* were abamectin, azinphos-methyl, baythroid, bifenthrin, chlorpyrifos, emamectin, fipronil, imidacloprid, methamidophos, and spinosad. Oviposition was inhibited up to 168 h in surviving females by abamectin, baythroid, bifenthrin, fipronil, imidacloprid, methidathion, and spinosad. By varying chemistries, *A. suspensa* management should provide effective and responsible control with minimal environmental impact while providing opportunities for resistance management.

Key Words *Anastrepha suspensa* management, oral toxicity, fly-free zone

Anastrepha suspensa (Loew), the Caribbean fruit fly, was first discovered in Key West, FL, in 1931 but was believed to have been established in Key West for many years prior to its discovery. From 1931-1965, *A. suspensa* was assumed to be non-existent in Dade, Broward, and Palm Beach counties, FL, was in very low numbers in the Florida Keys, and was never reared from a field host. In 1965, more than 14,000 adult *A. suspensa* were trapped in Dade Co., and it was postulated that these *A. suspensa* were a Puerto Rican strain which, in contrast to the Florida Keys strain, infests many tropical and subtropical fruits, citrus included (Weems 1965).

On 4 June 1974, the Japanese Ministry of Agriculture, Forestry, and Fisheries intercepted 3 live pinhead-sized larvae in a decaying Florida white grapefruit from a 10,000 carton load. These were determined to be *Anastrepha* spp. (American Embassy 1974, Nishimura 1974, Rainwater 1974). Although the shipment was eventually released for sale, the initial reaction was to "dump the rejected fruits into the sea" because "Entry of fruit flies into Japanese territory will affect and ruin the domestic fruit industry of Japan" (Nishimura 1974). This one incident resulted in the expenditure of many millions of dollars by the Florida citrus industry for postharvest treatment and basic scientific studies of *A. suspensa* (Hallman et al. 1990, Sharp 1990, Sivinski and Calkins 1990, Sivinski and Heath 1988, Greany et al. 1983, 1985, 1987, 1991, McDonald et al. 1987). Although citrus appears to be the most important crop for *A. suspensa* economically, this fruit fly has at least 84 'field' hosts in Florida and has decisively prevented the Florida cultivation of peaches and many tropical fruits (Swanson and Baranowski 1972).

A current approach to avoid some of the export costs associated with *A. suspensa* is to certify "fly-free zones" (Simpson 1991, Nigg et al. 2004a). The fly-free zone program requires the removal of hosts and monitoring for the presence of *A. sus-*

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pensa with McPhail traps baited with a yeast-borax mixture (Burditt 1982, Nigg et al. 2004a). The detection of a fly may require the application of a mixture of 71.04 mL malathion and 284.2 mL of Nu-Lure™ (Miller Chemical & Fertilizers Corp., Hanover, PA) protein hydrolysate bait to an area as well as additional trapping to certify fruit for export (Nigg et al. 2004a). We previously investigated the toxicity of organophosphate pesticides to *A. suspensa* (Nigg et al. 1994). Organophosphate pesticides, including malathion, have proven unacceptable to the public for area-wide eradication of fruit flies in Florida (H. Nigg, pers. obs.). The purpose of this study was to investigate the toxicity of pesticide chemistries to provide possible management tools for the *A. suspensa* fly-free zone program.

Materials and Methods

The pesticide assays described herein generally follow the method of Nigg et al. (1994). Briefly, pupae were received from the Biological Control Rearing Facility, FL Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS 1990), Gainesville Florida Fly Rearing Facility as an overnight shipment, and were caged as approximately 5,000 pupae in a 30 × 30 cm aluminum cage. After 5-6 d, emergence began, and adults were allowed to emerge for 24 h. The pupae then were placed in a new cage and removed after 24 h for known-age adults. These adults were provided with food, water and oviposition squares until they were 12 d old (Nigg et al. 1994). Twenty-five female and 25 male 12-d-old flies were used for each replicate test. Each test (dose) was replicated three times, including three control cages presented with an agar patty without pesticide. Pesticides were obtained as technical materials from the manufacturer with a listed purity range of 89-100% (Table 1). Concentrations were not adjusted for purity. Flies were presented for 24 h with 100 ppm pesticide active ingredient in an agar patty. Agar patties containing pesticide were prepared by mixing 0.5 g agar, 2 g yeast hydrolysate enzymatic (MP Bioedicals, LLC, Aurora, OH) and 0.8 ml 2 M NaOH with 55 ml of glass-distilled, deionized water in a 250-mL glass beaker. The pH of this mixture was 7.0. This mixture was heated in a microwave until it boiled briefly. Ten grams of sucrose was added with stirring. This mixture was scaled up or down dependent on the needs of the experiment. When the mixture began to thicken, appropriate aliquots of 100 mg/ml pesticide (in 95% ethanol) solutions were added with stirring; 30 mL of this solution was transferred to 9.0-cm diam Petri dishes to solidify as gels. After gelling, glassine weighing paper was placed on the patty and the patty was placed on the upper screen of the cage, glassine paper up. Regular adult fly diet, granulated sugar cubes and a 1% agar patty were supplied after the 24 h pesticide exposure period. Mortality by sex and egg production were monitored every 24 h for 168 h after pesticide exposure. Dead flies were removed daily. Oviposition squares were constructed by coating double cheesecloth (5.0 × 5.0 cm) with a liquid paraffin wax, petroleum jelly (1:2 by wt) mixture dyed red by adding red candle wax to a deep red color and marking a black 1 cm grid on one side to be placed up away from the flies. The grid and color of the ovipositon square are for ease in seeing and counting white eggs. One oviposition square was placed on the top screen of each cage. Oviposition squares were removed and replaced daily at 0,800 h. Eggs were counted and discarded. At 1400 h the oviposition squares were removed and replaced with fresh ones. The eggs on these squares were counted. Egg production was monitored for 168 h from the start of each experiment. Egg production was calculated as eggs per female. Pesticides with a mortality

Table 1. *Anastrepha suspensa* mean % mortality after 24 h exposure to 100 ppm pesticide active ingredient

Common name	Trade name	Company	Chemical type	24 h		48 h	
				Males	Females	Males	Females
Abamectin	Agri-Mek*	Syngenta	Glycoside	92.0	100.0	100.0	100.0
Azinphos-methyl	Guthion*	Bayer Crop Science	Organophosphate	96.0	24.0	100.0	76.0
Bacillus thuringiensis spp. *kurstaki*	Dipel	Valent Biosciences	Bacterium	0.0	8.7	19.2	17.4
Bacillus thuringiensis spp. *kurstaki*	Thuricide	Certis	Bacterium	50.0	4.2	76.9	12.5
Bacillus thuringiensis spp. *kurstaki*	Foray	Ecogen Inc.	Bacterium	3.8	0.0	26.9	8.0
Baythroid	Baythroid*	Bayer Crop Science	Pyrethroid	53.6	43.5	100.0	91.3
Bifenthrin	Talstar	FMC	Pyrethroid	64.0	32.0	100.0	96.0
Carbaryl	Sevin XLR	Bayer Crop Science	Carbamate	18.8	3.1	37.5	9.4
Chlorfenapyr	Alert	BASF Corp.	Pyrrole	0.0	0.0	80.0	21.4
Chlorpyrifos	Lorsban*	Gowan	Organophosphate	100.0	64.0	100.0	100.0
Diflubenzuron	Micromite	Crompton	Difluorobenzamide	0.0	0.0	0.0	0.0
Emamectin benzoate	Proclaim*	Syngenta	Natural product	100.0	92.9	100.0	96.4
Fenoxycarb	Fenoxycarb	Syngenta	Carbamate	0.0	0.0	0.0	0.0
Fenpropathrin	Danitol	Valent	Pyrethroid	38.5	0.0	96.2	24.0
Fipronil	Fipronil*	Bayer Crop Science	Aryl heterocycle	100.0	76.0	100.0	80.0
Imidacloprid	Admire*	Bayer Crop Science	Chloronicotinyl	96.3	69.6	100.0	95.7
Methamidophos	Monitor*	Bayer Crop Science	Organophosphate	100.0	100.0	100.0	100.0
Methidathion	Supracide*	Gowan	Organophosphate	96.2	50.0	100.0	84.6
Metiram complex	Polyram	BASF Corp.	Dithiocarbamate	4.8	0.0	23.8	3.8

Table 1. Continued.

Common name	Trade name	Company	Chemical type	24 h		48 h	
				Males	Females	Males	Females
Neem Oil	Trilogy	Certis	Botanical	0.0	0.0	0.0	0.0
Pymetrozine	Fulfill	Syngenta	Pyridine, IGR	0.0	0.0	0.0	0.0
Pyridaben	Nexter	BASF Corp.	Pyridazinone	0.0	0.0	0.0	0.0
Pyriproxyfen	Knack	Sumitomo	Pyridine, IGR	0.0	0.0	0.0	0.0
Spinosad	GF-120*	Dow Agrosciences	Natural product	52.0	30.8	100.0	96.2

* 85% + mortality, n = 3

Table 1. Continued.

Common name	72 h		96 h		120 h		144 h		168 h	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Abamectin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Azinphos-methyl	100.0	92.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Bacillus thuringiensis spp. *kurstaki*	23.1	17.4	30.8	21.7	34.6	26.1	34.6	39.1	34.6	43.5
Bacillus thuringiensis spp. *kurstaki*	80.8	12.5	80.8	12.5	80.8	12.5	80.8	12.5	80.8	12.5
Bacillus thuringiensis spp. *kurstaki*	26.9	8.0	26.9	24.0	26.9	24.0	34.6	28.0	38.5	28.0
Baythroid	100.0	95.7	100.0	95.7	100.0	100.0	100.0	100.0	100.0	100.0
Bifenthrin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Carbaryl	37.5	9.4	37.5	9.4	37.5	9.4	37.5	12.5	37.5	18.8
Chlorfenapyr	100.0	35.7	100.0	46.4	100.0	46.4	100.0	53.6	100.0	60.7
Chlorpyrifos	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Diflubenzuron	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Emamectin benzoate	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fenoxycarb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fenpropathrin	96.2	36.0	96.2	44.0	96.2	56.0	100.0	68.0	100.0	76.0
Fipronil	100.0	88.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Imidacloprid	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Methamidophos	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Methidathion	100.0	88.5	100.0	88.5	100.0	88.5	100.0	88.5	100.0	88.5
Metiram complex	38.1	3.8	38.1	3.8	38.1	3.8	95.2	61.5	95.2	65.4

Table 1. Continued.

	72 h		96 h		120 h		144 h		168 h	
Common name	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Neem Oil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pymetrozine	0.0	0.0	4.0	0.0	8.0	0.0	8.0	3.7	8.0	3.7
Pyridaben	4.0	0.0	4.0	0.0	20.0	0.0	24.0	0.0	24.0	0.0
Pyriproxyfen	0.0	0.0	4.0	0.0	4.0	0.0	4.0	0.0	4.0	0.0
Spinosad	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* 85% + mortality, n = 3

Table 2. Mean percent reduction in oviposition per 12-d-old female *Anastrepha suspensa* after 24-h exposure to 100 ppm of pesticide active ingredient (experimental/control X 100 ± S.D.)

Compound	Eggs/Female						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Abamectin*	100	—	—	—	—	—	—
Azinphos-methyl*	0	44 ± 6	100	—	—	—	—
Baythroid*	100	—	—	—	—	—	—
Bacillus thuringiensis spp. *kurstaki*	98 ± 4	0	79 ± 13	0	32 ± 17	36 ± 14	0
Bacillus thuringiensis spp. *kurstaki*	71 ± 12	0	50 ± 16	52 ± 6	51 ± 5	61 ± 12	62 ± 7
Bacillus thuringiensis spp. *kurstaki*	92 ± 7	0	54 ± 2	0	0	0	41 ± 7
Bifenthrin*	100	100	—	—	—	—	—
Carbaryl	38 ± 9	42 ± 11	33 ± 5	51 ± 8	49 ± 8	0	0
Chlorfenapyr*	0	0	34 ± 18	0	0	0	97 ± 5
Chlorpyrifos*	100	—	—	—	—	—	—
Diflubenzuron	0	0	0	0	0	0	0
Emamectin*	0	100	—	—	—	—	—
Fenpropathrin*	58 ± 17	68 ± 11	77 ± 12	95 ± 7	58 ± 16	98 ± 7	85 ± 7
Fenoxycarb	0	0	0	0	0	0	0
Fipronil*	95 ± 7	100	100	—	—	—	—
Imidacloprid*	100	100	—	—	—	—	—
Methidathion*	0	100	100	100	0	0	0
Methamidophos*	—	—	—	—	—	—	—

Table 2. Continued.

Compound	Eggs/Female						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Metiram complex	29 ± 5	73 ± 14	52 ± 6	333 ± 1	89 ± 7	0	0
Neem Oil	0	0	0	0	0	0	0
Pymetrozine	0	77 ± 8	49 ± 3	19 ± 7	0	0	0
Pyridaben	0	0	0	0	0	0	0
Pyriproxyfen	45 ± 8	21 ± 11	52 ± 12	76 ± 3	75 ± 2	83 ± 11	81 ± 10
Spinosad*	100	100	—	—	—	—	—

* 85% + mortality or reduction in oviposition, – = 100% mortality, n = 3

of ≥85% or reduced oviposition of ≥85% at 100 ppm were tested further at 50, 40, 30, 20, and 10 ppm or lower to obtain an LC_{50} (lethal concentration resulting in 50% mortality in 24 h unless otherwise stated) for comparative studies. LC_{50} determinations were replicated three times. LC_{50}s were calculated with PROCNLIN (SAS 1989) to fit a nonlinear regression with a forced 100% survival at time zero with 95% confidence intervals. Control data were not used in calculations as there were less than 10% deaths (two flies or less) in any control cage. The female/male ratio was calculated by dividing the female LC_{50} by the male LC_{50}.

Results and Discussion

Anastrepha suspensa prefers to feed upside down as patties were presented here and, although contact is possible, consumption appears to be the primary exposure route from an agar patty (Nigg et al. 2004b). *Bacillus thuringiensis* Berliner, carbaryl, diflubenzuron, fenoxycarb, metiram, neem, pyretrozine, pyridaben and pyriproxyfen were essentially nontoxic to *A. suspensa* (Table 1). Compounds that produced 100% mortality in 72 h after a 24 h exposure were abamectin, bifenthrin, chlorpyrifos, emamectin, imidacloprid, methamidophos, and spinosad (Table 1). At 168 h, fipronil, baythroid, and azinphos-methyl produced 100% mortality for males and females (Table 1). Some compounds displayed delayed toxicity: azinphos-methyl, bifenthrin, emamectin, fipronil, imidacloprid, methidathion, and spinosad. When toxicity is delayed it would be advantageous in a fruit fly management program for oviposition to be inhibited in surviving females.

This is the first report of the reduction of oviposition by abamectin, azinphos-methyl, baythroid, bifenthrin, chlorpyrifos, emamectin, fenpropathrin, fipronil, imidacloprid, methidathion, and spinosad (Table 2). In some cases, females survived for 24-96 h and then died, but did not oviposit after exposure to 100 ppm of abamectin, azinphos-methyl, bifenthrin, chlorpyrifos, emamectin, fipronil, imidacloprid, and spinosad for 24 h (Table 2). Methidathion inhibited oviposition at 48, 72, and 96 h after which oviposition resumed (Table 2).

In our previous study of organophosphate pesticide toxicity to *A. suspensa* (Nigg et al. 1994), methamidophos exhibited an LC_{50} of 3.4 ppm for males and 4.1 ppm for females, ♀/♂ ratio 1.21. In the present study, methamidophos yielded an LC_{50} of 4.7 for males and 5.9 for females, ♀/♂ ratio 1.26 (Table 3). The previous LC_{50} for chlorpyrifos for males was 17.7 and for females 23.2, ♀/♂ ratio 1.32. The present chlorpyrifos LC_{50} was 12.8 males and 22.2 females, ♀/♂ ratio 1.73 (Table 3). These data suggest little change in the susceptibility of the laboratory colony to pesticides. Our data are in agreement with that of Hennessey and King (1996) for male response to abamectin; however, King and Hennessey (1996) found the 24 h LC_{50} for spinosad as 4.6 females, 3.4 males, whereas we determined 8.8 females, 3.7 males in our study (Table 3).

Differential toxicity to one sex or another might provide a management advantage by eliminating one of the sexes and breaking the life cycle. With the organophosphates, there was little or no differential toxicity to either sex (Nigg et al. 1994; Tables 1 and 3). In the present study, other chemistries demonstrated differential toxicity to males: abamectin (♀/♂ LC_{50} ratio 5.29), baythroid (17.00), fenpropathin (7.35), fipronil (8.00), and emamectin (4.30) (Table 3). Chlorfenapyr was 100% toxic to males at 100 ppm, but was toxic at only 60.7% to females over 168 h (Table 1). Methidathion was also differentially toxic to males at 100 ppm (Table 1). Although females were, in

Table 3. Calculated 24 or 48 h LC_{50}s with female/male ratios

	Males			Females			
Compound	Time	LC_{50} (ppm)	95% CI	Time	LC_{50} (ppm)	95% CI	♀/♂%
Abamectin	24	3.1	(1.6-4.6)	24	16.4	(13.7-19.1)	5.29
Azinphosmethyl	24	5.4	(3.7-7.2)	24	9.5	(7.6-11.4)	1.76
Baythroid	48	0.5	(0.3-0.7)	48	8.5	(6.7-10.3)	17.00
Befenthrin	24	7.0	(6.1-8.0)	24	12.6	(11.1-13.1)	1.80
Chlorpyrifos	24	12.8	(9.4-16.2)	24	22.2	(19.7-24.7)	1.73
Emamectin	24	5.6	(3.7-7.5)	24	24.1	(17.0-31.2)	4.30
Fenpropathrin	24	12.5	(10.5-14.6)	24	91.9	(85.6-98.2)	7.35
Fipronil	48	0.1	(0.6-0.14)	48	0.8	(0.5-0.10)	8.00
Imidachloprid	24	0.7	(0.4-1.0)	24	1.6	(1.2-2.1)	2.29
Methamidophos	24	4.7	(3.6-5.8)	24	5.9	(5.1-6.6)	1.26
Methidathion	48	13.1	(10.7-15.5)	48	23.5	(18.2-28.7)	1.79
Spinosad	48	3.7	(3.1-4.3)	48	8.8	(8.6-9.0)	2.38

general, more difficult to kill (Nigg et al. 1994; Tables 1 and 3), the higher toxicity to males by these compounds should be studied for management of *A. suspensa*.

This is the first study that compares the toxicity of different chemistries to *A. suspensa* and the first *A. suspensa* study to include oviposition inhibition as a standard toxicity endpoint. In our previous study, we allowed flies access to treatments for 48 h. We suggest that future toxicity research on insects with a crop include a 72-h mortality monitoring period, oviposition monitoring and, to minimize hydrolysis of susceptible pesticides, adjustment of the food source pH to 7.0.

It appears from these data that *A. suspensa* could be managed with pesticides other than malathion, perhaps obviating malathion's beneficial insect impact (Ehler and Endicott 1984). Spinosad is currently being substituted for malathion for the *A. suspensa* fly-free zone program (King and Hennessey 1996, Burns et al. 2001). These data provide indications for alternate materials for *A. suspensa* dependent on environmental impact and human health concerns.

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