Toxicity of Imidacloprid-Treated Spheres to Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae) and Its Parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in the Laboratory

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ABSTRACT No-choice cage tests were used to study the toxicity of imidacloprid-treated spheres to Caribbean fruit fly, *Anastrepha suspensa* (Loew), and its associated parasitoid, *Diachasmimorpha longicaudata* (Ashmead), in the laboratory. Three imidacloprid sphere treatments (2, 4, and 8% active ingredient [AI] Provado 1.6 F) and an untreated control sphere (no toxicant) were evaluated against *A. suspensa*. Throughout the observation period (2–72 h), all concentrations of imidacloprid-treated spheres killed significantly more *A. suspensa* compared with control spheres. After 4 h of exposure to imidacloprid-treated spheres, significantly more *A. suspensa* were killed on spheres treated with 8% compared with 2% (AI). At 48 and 72 h, there were no significant differences in the mean number of *A. suspensa* killed at 2, 4, and 8% (AI), potentially indicating that a period of 24 h was sufficient for flies to ingest a lethal dose of the pesticide. Overall, significantly more *A. suspensa* males were killed after 72 h of exposure to imidacloprid-treated spheres compared with females. For *D. longicaudata*, only two imidacloprid sphere treatments, 2 and 4% (AI), and an untreated sphere (control) were evaluated for mortality in cage tests. There were no significant differences in mortality of *D. longicaudata* between the 2 and 4% (AI) imidacloprid-treated spheres. Both rates killed significantly more *D. longicaudata* compared with the control. However, after 24, 48, and 72 h of exposure to imidacloprid-treated spheres, significantly more *D. longicaudata* were killed in cages containing 4% compared with 2% (AI) and untreated control spheres. The study demonstrates the potential use of imidacloprid-treated spheres for control of *A. suspensa* in areas where it may be difficult to apply broad-spectrum insecticides.

KEY WORDS Caribbean fruit fly, *Diachasmimorpha longicaudata*, imidacloprid-treated spheres

INCREASINGLY, APPLICATION OF PESTICIDES by air and occasionally by ground for fruit fly control is meeting resistance from environmentalists and the general public. Access to critically sensitive areas such as hospitals, bodies of water, and school zones is becoming increasingly problematic for fruit fly eradication programs that emphasize broad-spectrum insecticides (Clark et al. 1996). The situation is further complicated because biological (Baranowski et al. 1993) and cultural methods of control do not yield immediate results necessary for successful eradication programs (AliNiazee and Croft 1999). To prevent fruit flies from harboring in these areas and reinfesting surrounding areas, alternative strategies for managing these critically sensitive areas must be developed.

Several types of traps, as well as trap-lure combinations and baits specifically designed to improve monitoring capabilities through increased trap captures while minimizing environmental impacts and decreasing chemical residues on fruit crops, have been tested for control of Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Greany et al. 1978, Burditt 1982). Thomas et al. (2001) evaluated trap-lure combinations against populations of *A. suspensa* and Mexican fruit fly, *Anastrepha ludens* (Loew), as substitutes for the glass McPhail trap and found that open-bottom, plastic traps baited with a two-component synthetic lure (ammonium acetate and putrescine) caught as many or more fruit flies than McPhail traps baited with torula yeast. In their study, fruit fly captures varied among seasons and locations, but more females and fewer nontarget insects were captured with synthetic lures.

Several opiine brachonid parasitoid species, including *Diachasmimorpha longicaudata* (Ashmead) and *Doryctobracon areolatus* (Szepligeti), were introduced into the United States for biological control of *A. suspensa* (Lawrence et al. 1978, Baranowski et al.

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1993). Baranowski et al. (1993) reported a 40% decline in trap catches of *A. suspensa* 5 yr postrelease of *D. longicaudata.* Later, Sivinski et al. (1999) showed that *A. suspensa* larvae and their brachonid parasitoids, including *D. longicaudata,* were evenly distributed within the canopy of host trees, suggesting that height above ground or distance from canopy edge of trees did not significantly affect oviposition preferences for *A. suspensa.* The development of effective pest management strategies for *A. suspensa* without negatively impacting parasitoid populations is essential to maintaining long-term control of *A. suspensa* in a production system.

Recently, the strategy of using imidacloprid-treated spheres for management of key fruit fly pests, *Rhagoletis pomonella* (Walsh) and *Rhagoletis mendax* Curran, have been given much attention in northeastern United States (Liburd et al. 1999, Prokopy et al. 2000, Stelinski and Liburd 2001, Hamill et al. 2003). This strategy may also work well for other fruit fly species, including *A. suspensa,* a key pest of tropical fruit in Florida and the Caribbean region (Norrbom and Kim 1988). Although efforts are underway to develop bait stations (Sivinski, personal communication), there are no studies in the literature that have investigated the potential of using imidacloprid-treated spheres for control of *A. suspensa.* In addition, there are no reports (for any fruit fly species) on how imidacloprid-treated spheres may impact fruit fly parasitoid densities. Nevertheless, before any large-scale trials are developed, laboratory assays must be performed to determine the response of *A. suspensa* and its natural enemies to imidacloprid-treated sphere tactics.

The objective of this study was to conduct laboratory assays to investigate the toxicity of *A. suspensa* to imidacloprid-treated spheres and to explore the potential nontarget effects of using imidacloprid-treated spheres on *D. longicaudata,* a key parasitoid of *A. suspensa.*

Materials and Methods

Experiments to evaluate the toxicity of imidacloprid-treated spheres to *A. suspensa* and *D. longicaudata* were conducted at APHIS-Plant Protection and Quarantine, Center for Plant Health Science and Technology (CPHST) Laboratory in Gainesville, FL, and the Fruit and Vegetable Integrated Pest Management Laboratory, University of Florida, Gainesville, FL. The unit used was a starch/sugar 9-cm-diameter sphere composed of gelatinized corn flour, corn syrup, sugar, cayenne pepper, and sorbic acid (Liburd et al. 1999, Stelinski and Liburd 2001). The sugar/starch sphere was coated with a mixture of 70% yellowish orange florescent paint ([4CI-3 Behr Flat Yellow Cluster], Home Depot, Gainesville, FL), 20% sucrose solution (wt:vol), and varying amounts of toxicant (imidacloprid) and water, depending on the treatment.

Experimental design was a randomized complete block with five replicates per treatment. Each cage contained 25 male and 25 female fruit flies or parasitoids, totaling 250 individuals per treatment. Four treatments were evaluated: 1) spheres brush painted with a mixture of 2% ([AI]) imidacloprid (Provado 1.6 F, Bayer Cropscience, Kansas City, MO) and 8% water; 2) same as 1 except 4% imidacloprid and 6% water; 3) same as 1 except 8% imidacloprid and 2% water, and 4) control 10% water, no toxicant. Each sphere was placed on an inverted 236.8-ml plastic cup (Solo Cup Co., Urbana, IL), pedestal equidistant from the top, sides, and bottom of a 30 by 30-cm cubed Plexiglas cage. Before the start of the experiment, each sphere was misted with water to simulate "morning dew" and again at 24 and 48 h.

Source of Insects. *A. suspensa* pupae were obtained from colonies maintained at the Florida Department of Agriculture and Consumer Service's Division of Plant Industry in Gainesville, FL (Burns 1995). Adults were maintained on a diet of sugar (sucrose), protein (yeast hydrolysate), and water. Flies were protein-sugar starved 24 h before commencement of assays to increase their responsiveness. Only water was provided in two souffles cups (59.2 ml). Each cup with water had a dental wick protruding through its lid to allow flies within treatment cages easy access to water. The only other moisture source came from misting the spheres. Sexually mature 7–10-d-old flies were tested.

D. longicaudata were obtained from Division of Plant Industry (Biocontrol Rearing Facility) and were tested when 5–8 d old. Before placing *D. longicaudata* into test cages, they were fed honey, presented in a gelatinous form (i.e., agar/water/honey block) in an open petri dish on the bottom of the cage. Water was supplied as described above for *A. suspensa* by using two souffles cups with dental wicks placed inside the cages. *D. longicaudata* were subjected to two and 4% imidacloprid treatments. Temperature and humidity best suited for responsiveness were maintained where possible; 25.6–30°C and 60–78% RH. A photoperiod of 12:12 (L:D) h was provided with florescent lamps with the aid of a timer.

Sampling. *A. suspensa* and *D. longicaudata* visits to the spheres to attempt feeding were recorded at the following intervals: 2, 4, 24, 48, and 72 h. Data were recorded according to sex by counting the number of males and females that were killed after contact (feeding or alighting) with the sphere.

Statistical Analysis. Male and female data were initially pooled to examine the overall effects of imidacloprid-treated spheres on *A. suspensa* and *D. longicaudata.* Finally, data were separated according to sex to determine the toxicity of imidacloprid-treated spheres to males and females independently. Data from all experiments were square root transformed ($x + 0.5$) to stabilize variances and subjected to a repeated measures analysis of variance (ANOVA) followed by mean separation by using the least significant difference (LSD) test (SAS Institute 2001). The results were considered statistically significant when $P < 0.05$. Data are presented as untransformed means and standard errors.

Table 1. Mean ± SEM percentage of *A. suspensa* killed on spheres treated with different concentrations of imidacloprid

% Imidacloprid	Hours posttreatment				
	2	4	24	48	72
0	0.1 ± 0.1b	0.1 ± 0.1c	0.1 ± 0.1c	0.4 ± 0.1b	7.7 ± 1.3b
2	5.7 ± 0.9a	7.6 ± 1.2b	17.4 ± 1.5b	53.4 ± 2.2a	87.6 ± 1.0a
4	6.6 ± 1.2a	8.2 ± 1.0ab	21.4 ± 1.0a	56.2 ± 3.0a	88.1 ± 2.1a
8	7.3 ± 1.0a	10.2 ± 0.8a	24.8 ± 1.1a	58.2 ± 3.4a	86.5 ± 3.3a

Means within column followed by the same letter are not significantly different ($P < 0.05$, LSD test; SAS Institute Inc. 2001). Analyses were performed on square root-transformed data, but means shown are backtransformed data. For 2 h, $F = 71.2$; df = 3, 12; $P < 0.01$; for 4 h, $F = 155.3$; df = 3,12; $P < 0.01$; for 24 h, $F = 357.8$; df = 3,12; $P < 0.01$; for 48 h, $F = 313.3$; df = 3,12; $P < 0.01$; and for 72 h, $F = 516.9$; df = 3, 12; $P < 0.01$. For all treatments, $n = 250$.

Results

Laboratory Assays for *A. suspensa*. Throughout the observation period (2–72 h) all concentrations (2, 4, and 8% [AI]) of imidacloprid-treated spheres killed significantly more *A. suspensa* compared with control spheres (Table 1). After 2 h, there was no significant difference in the mean number of *A. suspensa* killed at 2, 4, and 8% (AI). After 4 h of exposure to treated spheres, significantly more *A. suspensa* were killed at 8% compared with 2% (AI). There was no significant difference in the number of *A. suspensa* killed by the 2 and 4% imidacloprid-treated spheres.

Results from the 24-h observation period differed from those at 4 h. A significantly greater number of *A. suspensa* were killed at 4 and 8% (AI) compared with flies exposed to 2% (AI) imidacloprid-treated spheres. At 48 and 72 h, there were no significant differences in the mean number of killed *A. suspensa* when exposed to imidacloprid-treated spheres at 2, 4, and 8% (AI). All treatments (2, 4, and 8% [AI]) killed ≈12 times as many *A. suspensa* compared with untreated imidacloprid spheres. All treatments with imidacloprid killed significantly more *A. suspensa* compared with the control over time ($F = 590.8$; df = 1, 38; $P < 0.0001$). There were no significant differences among imidacloprid sphere treatments over time ($F = 0.02$; df = 2, 29; $P = 0.98$).

Susceptibility of Male and Female *A. suspensa* to Imidacloprid-Treated Spheres. Overall, *A. suspensa* males were on average 1.2 times more susceptible to imidacloprid-treated spheres compared with females. Treatments 2 and 4% (AI) killed significantly more males than females (Table 2). There were no significant differences between males and females for 0 and 8% (AI). Significantly more males ($F = 160$; df = 3,12; $P < 0.0001$) and females ($F = 124.5$; df = 3, 12; $P < 0.0001$) were killed with imidacloprid treatments compared with the control (Table 2).

Susceptibility of *D. longicaudata* to Imidacloprid-Treated Spheres. There was no significant difference in mortality of *D. longicaudata* exposed to 2 and 4% (AI) imidacloprid-treated spheres at 2 or 4 h (Table 3). However, 2% (AI) killed significantly more *D. longicaudata* compared with the control after 24 h. After 24-, 48-, and 72-h exposures to imidacloprid-treated spheres, significantly more *D. longicaudata* were killed with 4% compared with 2% (AI) and the control (Table 3).

There were no significant differences among imidacloprid sphere treatments for *D. longicaudata* over time ($F = 1.4$; df = 1, 87; $P = 0.25$). However, both imidacloprid sphere treatments (2 and 4% [AI]) killed significantly more *D. longicaudata* compared with the control [($F = 47.4$; df = 1, 82; $P < 0.001$) and ($F = 101.5$; df = 1, 87; $P < 0.001$) for 2 and 4%, respectively].

Discussion

These results demonstrate that imidacloprid-treated spheres were effective in killing *A. suspensa* at all rates of imidacloprid (2, 4, or 8%) evaluated. The mortality of *A. suspensa* to imidacloprid-treated spheres is not surprising considering that flies were not given an option to alight or forage on other nontreated surfaces and because these spheres have been reported to be highly toxic to other tephritids (Liburd et al. 1999). Nevertheless, if it is possible to induce fly mortality as early as 2 h after setting on a treated sphere (in the field), residual effectiveness may not be critical, although certainly desirable, from a replacement standpoint (Hamill et al. 2003). However, it is unlikely that all the flies in the field would be exposed to a toxic dose of the pesticide during a 2-h period; therefore, it would be important for spheres to remain effective for a fairly long period after they are deployed in the field.

After 4-h exposure to imidacloprid-treated spheres, significantly greater numbers of flies were killed by 8%

Table 2. Mean ± SEM percentage male and female *A. suspensa* killed on spheres treated with different concentrations of imidacloprid

% Imidacloprid	Male	Female
0	10.4 ± 5.3b	4.6 ± 2.8b
2	95.0 ± 1.6a*	79.0 ± 2.2a
4	95.0 ± 1.5a*	78.2 ± 2.3a
8	96.7 ± 2.7a	82.2 ± 4.3a

Means within column followed by the same letter are not significantly different ($P < 0.05$, LSD test; SAS Institute Inc. 2001). Analysis was performed on square root-transformed data, but means shown are backtransformed data.

* The number of killed males in imidacloprid treatments at 2 and 4% is significantly higher than females. For 0% (AI), $F = 1.0$; df = 1,4; $P = 0.34$; for 2% (AI), $F = 34.5$; df = 1,4; $P < 0.01$; for 4% (AI), $F = 34.1$; df = 1,4; $P < 0.01$; and for 8% (AI), $F = 2.8$; df = 1, 4, $P = 0.1$. For all treatments, $n = 125$.

Table 3. Mean ± SEM percentage *D. longicaudata* killed on spheres treated with different concentrations of imidacloprid

% Imidacloprid	Hours posttreatment				
	2	4	24	48	72
0	0.0 ± 0.0b	0.2 ± 0.1b	2.2 ± 0.4c	5.4 ± 0.8c	11.1 ± 1.5c
2	1.2 ± 0.5ab	1.8 ± 0.7ab	7.4 ± 1.3ab	34.7 ± 2.0b	57.6 ± 2.5b
4	4.4 ± 1.8a	6.2 ± 2.3a	20.0 ± 4.2a	48.4 ± 5.9a	77.0 ± 6.2a

Means within column followed by the same letter are not significantly different ($P < 0.05$, LSD test; SAS Institute Inc. 2001). Analyses were performed on square root-transformed data, but means shown are backtransformed data. For 2 h, $F = 9.00$; df = 2,8; $P < 0.01$; for 4 h, $F = 7.97$; df = 2,8; $P = 0.013$; for 24 h, $F = 17.43$; df = 2,8; $P < 0.01$; for 48 h, $F = 68.29$; df = 2,8; $P < 0.01$; and for 72 h, $F = 105.40$; df = 2,8; $P < 0.01$. For all treatments, $n = 250$.

compared with 2% (AI), suggesting that higher concentrations of insecticide may be required over time to ensure greater efficacy. Stelinski et al. (2001) reported that the effectiveness of imidacloprid-treated spheres against *R. pomonella* flies decreased significantly after 12 wk of field exposure when treated with 2% (AI) imidacloprid, but mortality was not affected after 12 wk on spheres treated with 4 and 8% (AI) imidacloprid. In our study, the importance of higher insecticide concentration over time became more apparent after 24 h, when greater numbers of *A. suspensa* succumbed to the 4 and 8% compared with 2% (AI) treatments. Apparently, a 24-h exposure period was sufficient, because results were similar at the 48- and 72-h observations. However, these studies should be tested either in field cages or a commercial citrus grove to determine whether the results observed in the laboratory can be duplicated in the field.

The yellowish orange florescent sphere used in our study is believed to be visually attractive to both males and females (Greany et al. 1978). It is not clear why male *A. suspensa* were 1.2 times more susceptible than females to imidacloprid, a neonicotinoid insecticide, that has both contact and systemic mode of action (Liburd et al. 2003). It is possible that males landed on spheres more often than females to feed, or perhaps with the intent to mate, and as a result were more likely to come into contact with a toxic dose of imidacloprid. Imidacloprid has been shown to have lethal and sublethal effects on other tephritids (Hu and Prokopy 1998).

The benefits of using imidacloprid-treated spheres include a reduction in pesticide residues on crops, as well as reduced environmental and worker hazards (Hamill et al. 2003). In addition, unlike sticky spheres, insects killed from feeding on pesticide-treated spheres do not accumulate on spheres and reduce their effectiveness.

Before using imidacloprid-treated spheres in an agricultural or environmentally sensitive ecosystem, a measure of their effects on beneficials (parasitoids and predators) must be assessed. Our study measured the effects of imidacloprid-treated spheres on an opiine parasitoid used throughout the world in tephritid fruit fly biological control programs (Sivinski et al. 2000). Although higher mortality of *D. longicaudata* was recorded with 4% compared with 2% (AI) after 24 h it is believed that the seasonal population dynamics of fruit fly parasitoids inhabiting various *A. suspensa* niches (for feeding) are so limited that populations would not be significantly reduced (Sivinski et al. 1998). Vargas et al. (2001, 2002) reported that spinosad or phloxine B bait sprays had little or no effect on *Fopius arisanus* (Sonan), the major parasitoids of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Hawaii. Nevertheless, further research using field plots would be necessary to determine susceptibility of *D. longicaudata* to imidacloprid-treated spheres under natural conditions.

Fruit fly control in some sensitive areas in the U.S. and internationally preclude the use of aerially applied bait-sprays, or the application of sterile fruit flies due to topography (Burns et al. 2001). For instance, the use of bait sprays is a concern in potentially sensitive areas (e.g., due to water, hospitals) in Texas and California where Mexican fruit fly is of economic importance. Florida has also experienced a problem with the occurrence of oriental fruit fly, *Bactrocera dorsalis* Hendel, and guava fruit fly, *Bactrocera correcta* (Bezzi).

Presently, the insecticide imidacloprid is registered for soil and foliar treatment in field crops, vegetables and selected fruits. The sugar/starch sphere evaluated in this study has been reported to be susceptible to rodent feeding (Stelinski and Liburd 2001). A new plastic version of spheres with sucrose cap is targeting *Rhagoletis* spp. (Hamill et al. 2003). The data derived from the current study and future field studies will hopefully lead to commercialization of insecticide-treated spheres in citrus as well as other crops currently threatened by *A. suspensa.* However, field tests will be necessary because of the ambiguities inherent in laboratory cage studies. The potential danger of using imidacloprid-treated spheres to nontarget organisms, including people and birds, also remains to be examined.

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