House Fly (Diptera: Muscidae) Resting Preference on Various Cords and Potential of Fipronil- or Indoxacarb- Impregnation on Cords for Fly Control¹

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Abstract Fly preference for resting on various cords was evaluated, and preferred cords were impregnated with insecticides for control of house flies, Musca domestica L. (Diptera: Muscidae). Numbers of house flies attracted to and resting on natural (manila, cotton, cotton wick, wool, and leather) and synthetic (nylon, polypropylene, and parachute cord) cords were counted. Synthetic cords attracted significantly fewer flies than natural cords, and the plant-based cords were more attractive than the animal-based cords. Manila cord had significantly more flies resting on it than any other cord. The most attractive cords were treated with 0.1% fipronil or 0.6% indoxacarb for laboratory testing. Insecticide-treated wool cord had the lowest LT_{50} 's (fipronil: $LT_{50} = 12.9$ h; indoxacarb $LT_{50} = 32.6$ h), and the impregnated cotton cord had the highest LT_{50} 's. The LT_{50} 's for the synthetic cords were significantly lower than those for the cotton and manila cords. Field evaluations in cages were conducted with wool cords impregnated with 0.1% fipronil or 1.2% indoxacarb. Both treatments significantly reduced fly counts at both 24 h and 48 h after placement, both initially and at 2-wk after treatment. Fipronil and indoxacarb both appear to be effective insecticides for house fly control, especially when they are applied to wool cords. With new active ingredients, insecticide-impregnated cords could be valuable tools in present-day urban, agriculture, and military fly management programs.

Key Words Musca domestica, fly cords, fipronil, indoxacarb

The house fly, *Musca domestica* L., is widely considered the most common nuisance pest. Its nuisance pest status can quickly change to a public health risk if fly populations occur near inhabited areas where pathogen-rich oviposition sites are found. Areas stressed due to natural disasters, humanitarian crises, or combat are often plagued by excessive fly populations (Rosales and Prendergast 2000, Burrus 2005, Thornton et al. 2005). The most effective way to control house flies and reduce the risk of disease transmission is by eliminating their pathogen-rich oviposition sites. However, this method may be impractical, especially in areas affected by natural disasters and combat, where the oviposition sites are too numerous or difficult to reach.

When fly populations need to be controlled, insecticides can provide rapid kill of house flies and fly density decreases can be achieved in 1 - 2 days. However, house flies have shown great propensity to develop insecticide resistance (Georghiou and Lagunes-Tejeda 1991, Liu and Yue 2000, Scott et al. 2000, White et al. 2007). At the same time, the number of registered insecticides available for house fly control in the United

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States has decreased (Kaufman et al. 2001). New application methods and insecticides are clearly needed to allow effective house fly control.

Insecticide-impregnated cords have been used with great success to control flies and are considered less likely to select for resistance than traditional residual sprays (Keiding 1976). Their first use was in the late 1940s (Baker et al. 1947) and by the mid-1950s, insecticide-impregnated cotton cords were commercially available and widely used (Fehn 1958, Smith 1958). The commercial fly cords contained 13.79% parathion and 3.54% diazinon (Smith 1958). Cords impregnated with high concentrations (up to 25% active ingredient) of other organophosphate and organochlorine insecticides were also tested yielding great success (Kilpatrick and Schoof 1959, Keiding 1976, Rabari and Patel 1976). Fly cords are no longer used today partially due to the popularity of insecticidal baits and space sprays, and because the Environmental Protection Agency, acting under federal legislation, eliminated the use of many active ingredients used on the cords. However, newer insecticides, like indoxacarb and fipronil, are now available and have potential for controlling urban pests, including house flies (Scott and Wenn 1997, N'Guessan et al. 2007).

The objective of this study was to determine whether cords impregnated with newer insecticides would be an effective tool for house fly control. Specifically, we wanted to: (1) determine the attractiveness of various natural and synthetic cords to house flies, (2) determine the effectiveness of fipronil and indoxacarb on the most attractive cord materials, and (3) evaluate the effectiveness of the best cord/treatment combination in field cages.

Materials and Methods

Insects. The Horse Teaching Unit (HTU) strain of house flies, *M. domestica* L., reared at the University of Florida in Gainesville for less than 10 generations was used in all experiments. Larvae were reared on a diet medium, modified from Hogsette (1992), containing 3 L wheat bran, 15 ml methyl paraben, 1.5 L water, and approx. 200 g (250 ml) dairy calf feed (Calf Manna® pellets, Manna Pro Corp., St. Louis, MO). All developmental stages were held at $26 \pm 1^{\circ}$ C and 55% RH. Adult flies emerged within screened rearing cages and were provided granulated sugar, powdered milk, and water *ad libitum*.

For all assays, adult house flies (3 - 5 d old) were aspirated from the screened rearing cages using a handheld vacuum with a modified crevice tool attachment. Flies used for the laboratory assays were placed into a 5°C environment for 5 min to subdue activity. Flies were then placed on a chilled aluminum tray, sexed, and counted. House flies used for field cage assays were not anesthetized, but were aspirated from the screened rearing cages and released directly into field cages.

Laboratory arenas. Arenas $(31 \times 25 \times 21 \text{ cm})$ were constructed using PVC pipe (1.27 cm [0.5 in]) and were enclosed with a transparent plastic bag $(3,721 \text{ cm}^2 [61 \times 61 \text{ cm}], 1 \text{ mil polypropylene}$, Uline, Waukegan, IL). Rubber bands were stretched between the top pipes and evenly distributed along the length of the arena to establish individual cord positions; 4 rubber bands were used in the cord attractiveness bioassay and 5 were used in the impregnated-cord bioassay. Cords were attached to the rubber bands using paper clips.

Cord attractiveness. Eight cord types were evaluated: nylon (Braided, MultiPurpose Braid 75 lb. load limit, Wellington Cordage LLC, Madison, GA), polypropylene (Braided, MultiPurpose Rope – 56 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton

(Braided, MultiPurpose Sash Cord – 28 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton wick (Sterilized roll, #200209, Richmond Dental Company, Charlotte, NC), manila (Twisted, Natural Rope – 108 lb. load limit, Wellington Cordage LLC, Madison, GA), wool (Twisted, Natural Cord, Wooded Hamlet Designs, Greencastle, PA), leather (Tan Iaces, #6192, Rothco, Ronkonkoma, NY), and parachute cord (550 test, white, purchased locally from M & C Army Surplus Store, Gainesville, FL).

An arena was organized with 2 cords randomly placed and hung from each rubber band. Fifty female flies were released into the arena and 10% sugar water was provided *ad libitum*. Number of flies resting on cords was counted every 10 min for 2 h. Arenas were lightly shaken between each count to displace flies from their resting positions. Four replications were performed in the laboratory ($28 \pm 1^{\circ}$ C) under continuous light on separate days, and using different flies.

Impregnated cords. Cotton, manila, wool, polypropylene, and nylon cords were selected from the cord attractiveness experiments to be evaluated in the impregnated-cord experiments. Cords (15.2 cm length, 0.6 cm diam) were impregnated with a 0.1% fipronil or a 0.6% indoxacarb solution. The 0.1% fipronil solution was prepared by combining 2.7 ml of the formulated insecticide (Termidor SC, 9.1% a.i., BASF, Research Triangle Park, NC) with 250 ml of tap water. The 0.6% indoxacarb solution was prepared by combining 5 g of formulated insecticide (DPX MP062, 30WG, DuPont, Wilmington, DE) with 250 ml of tap water. Cords were impregnated by dipping for ~2 sec in the insecticide solution and then were allowed to dry in a fume hood before being placed in the experimental arenas.

Separate experiments were conducted to evaluate the delivery of each insecticide by the different cord types. Each impregnated-cord experiment consisted of 6 arenas. Twenty-five arenas were organized into a 5×5 Latin square design with 5 cords per arena. Five additional arenas were set up to determine control mortality. The control arena had no treated cords and all cords within it maintained the same cord positions throughout all experiments (position 1 = cotton; 2 = wool; 3 = manila; 4 = polypropylene; 5 = nylon). The Latin square experiment was repeated once for a total of 2 trials.

Groups of 50 female flies were placed within each arena and provided a 10% sugar water solution *ad libitum*. Mortality was recorded from 6 - 72 h until \geq 80% mortality was observed for each toxicant. Due to the differences in the mode of action of the insecticides, mortality for flies exposed to fipronil-impregnated cords was defined as unresponsive to touch; flies exposed to indoxacarb-impregnated cords were considered dead if they were unable to stand. Each experiment was conducted in the laboratory (28 ± 1°C) under continuous light.

Field cage bioassay. Cages $(1.8 \times 3.7 \times 1.8 \text{ m})$ were constructed from PVC pipe (2.54 cm [1 in] diam) and enclosed with mesh screening (Outdoor Cage, #1412A, 18 × 14 mesh, Bioquip, Rancho Dominguez, CA). Black plastic sheeting (6 mil) was used to line the floor. A sampling stage, constructed of 2 vertical cinder blocks and an inverted storage bin (Palletote #1721, 37 L, Rubbermaid, Winchester, VA), was placed in the center of the cage. On top of the sampling stage there were 2 1-L chick waterers, one filled with 10% sugar water and the other with tap water, and a 60-ml plastic cup filled with 8 g of previously used larval house fly medium, a fly attractant, that was covered with a paper towel.

Treatments consisted of 2 long (0.9 m) and 8 short (0.6 m) lengths of 0.1% fiproniland 1.2% indoxacarb-impregnated wool cords. The 0.1% fipronil solution was prepared by combining 7.7 ml of the formulated insecticide (Termidor SC) with 700 ml of tap water, and the 1.2% indoxacarb solution was prepared by combining 28 g of formulated insecticide (DPX MP062) with 700 ml of tap water (indoxacarb dose was doubled in field cages compared with the laboratory arenas to assure faster mortality). Each cord was treated in the same manner as the laboratory experiments, except the cords were dipped and soaked for 1 min prior to drying.

Depending on fly availability, $27.5 - 35 \text{ ml} (9.8 \pm 1.8 \text{ flies/ml})$ of flies was released into each cage. After a 1-h acclimation period, pretreatment fly counts were taken. Before fly counts were taken, the operator walked 3 laps around the interior of the cage to disturb flies from their resting positions and to recover any dead flies from the cage floor. Four consecutive fly counts were then taken from the outside of the cage 1 min after the operator exited the cage. All flies that landed on the sampling stage, chick waterers, and plastic cup attractant were counted. Treatments were then hung vertically from the mesh ceiling using paper clips. Posttreatment fly counts were taken at 24 and 48 h using the same method described above for pretreatment counts. After the initial 48 h evaluation, cords were hung outdoors for 2 wk, at which point the field cage experiment was repeated as described above. Three replicates were performed at each treatment age (0 and 2 wk).

Statistical analysis. Mortality data were corrected using (Abbott's Eq. 1925), and all proportion data were arcsine square root-transformed before analysis. Statistical analyses were performed using JMP (SAS Institute 2005), except probit analysis estimates were performed using SAS (SAS Institute 2001). Fly resting behavior was submitted to analysis of variance blocking on cage and cord position, and contrasts were performed between natural versus synthetic cords and the animal-versus plantbased cords. The 24-h fipronil mortality and 48-h indoxacarb mortality was submitted to analysis of variance blocking on cage, cord position, and trial. Means for resting behavior and fly mortality were separated using LS Means Student-t. Median lethal times (LT₅₀'s) were estimated by probit-analysis (Finney 1971). Potency ratios, using the cotton cord as the standard, were performed (Robertson and Preisler 1991). Slopes, LT₅₀ values, and potency ratios were considered significantly different if the 95% confidence intervals did not overlap. For the field cage experiments, percent fly count reductions were calculated in relation to fly counts in control cages at 24 and 48 h after placement of treated cords. Fly count reductions and mortality data (number of dead flies recovered from cage floor) were then analyzed for each treatment age (0 and 2 wk). Means were separated using the Student-Newman-Keuls test ($\alpha = 0.05$).

Results

Cord attractiveness. All flies fully recovered from chilling after approx. 45 min, at which point the flies dispersed throughout the arena and selected resting surfaces. Cord position (F = 2.6071; df = 7; P = 0.0123) and cage (F = 3.2336; df = 3; P = 0.0224) significantly affected fly resting behavior, and their effects were accounted for in the analysis of variance. Flies were significantly attracted to certain cords over others (F = 14.883; df = 7; P < 0.0001) with more flies arresting on manila than any other cord (Fig. 1). Synthetic cords had significantly fewer flies resting on them than natural cords (t = 7.16; df = 382; P = < 0.0001), and the plant-based cords were more attractive than the animal-based cords (t = 3.41; df = 238; P = < 0.0007).

Impregnated cord bioassay. In the insecticide impregnated cord laboratory bioassay, trial, cage, and cord position did not significantly affect fly mortality for 0.1% fipronil (trial: F = 3.0661; df = 1; P = 0.0885, cage: F = 0.6353; df = 4; P = 0.6406, cord position: F = 0.6353; df = 4; P = 0.6638) or 0.6% indoxacarb (trial: F = 2.0182; df = 1;

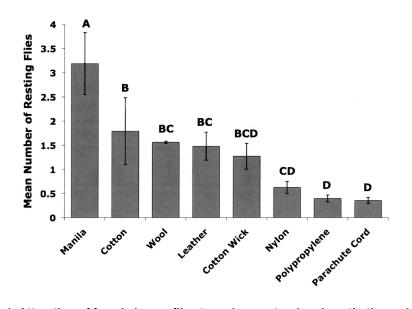


Fig. 1. Attraction of female house flies to various natural and synthetic cords in laboratory arenas.

P = 0.1640, cage: F = 0.2429; df = 4; P = 0.9121, cord position: F = 0.9314; df = 4; P = 04,567). Both fipronil- and indoxacarb-impregnated cords caused significant mortality (Fig. 2A and 2B); whereas, there was no mortality in the control cages.

House flies suffered significantly higher mortality (93%) when exposed to the 0.1% fipronil-impregnated wool cord than any other fipronil-impregnated cord. Cotton and manila fipronil-impregnated natural cords yielded percent mortality <15%. Likewise, 0.6% indoxacarb-impregnated wool cord caused significantly higher mortality (85%) than any other cord except for the cotton cord. No significant differences in mortality were seen among the indoxacarb-impregnated synthetic cords or between the indoxacarb-impregnated cotton and manila cords.

In general, 0.1% fipronil-impregnated cords had lower LT₅₀'s than 0.6% indoxacarbimpregnated cords (Table 1). Cotton cords were usually less effective than the other cords in delivering a lethal insecticide dose to house flies. Among the fipronilimpregnated cords, wool cord had the lowest LT₅₀ (13 h) and cotton cord had the highest (40 h). The LT₅₀'s for the synthetic cords were significantly lower than those for the cotton and manila cords.

All indoxacarb-impregnated cords had LT₅₀'s >32 h. The indoxacarb-impregnated wool cord had lower LT₅₀ (33 h) value than all other indoxacarb-impregnated cords except for the manila cord, which showed no significant difference in LT₅₀. No differences in LT₅₀'s were observed between the manila and nylon cords. In general, impregnated wool cord was 1.6 - 3 times more active than cotton cord, the standard material for fly cords in the 1950s.

Field cage bioassay. In the field cage experiments, both 0.1% fipronil and 1.2% indoxacarb caused similar fly count reductions after cords were hung in the cage for 48 h (Fig. 3). Fipronil caused faster reduction in the fly population as indicated by the

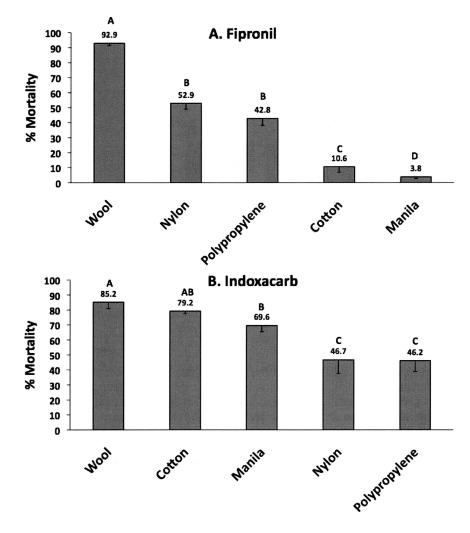


Fig. 2. Mortality of female house flies in the laboratory exposed to various natural and synthetic cords treated with (A) 0.1% fipronil evaluated after 24 h or (B) 0.6% indoxacarb evaluated after 48 h.

24 h counts. Resting fly counts did not significantly change throughout the experiment. Two wk after the cords had been hung and exposed to the outdoor weather conditions, fly counts were reduced in treatments similar to when the cords were freshly placed.

Discussion

Insecticide-impregnated fly cords are based on a fundamental component in a fly's behavior – flies prefer to rest on objects such as twigs, wires, cord, and line (Scudder 1949). Because insecticide-impregnated cords only represent a small proportion of

Table 1. Effic	acy of various cord	ls impregn	ated with 0.1% fi	Table 1. Efficacy of various cords impregnated with 0.1% fipronil or 0.6% indoxacarb on female house flies.	arb on femal	le house flies	
Treatment	Cord	ž	Slope ± SE	LT ₅₀ (95% CL) (h)	χ^{2}	P value	Potency Ratio (95% CL)
Fipronil	Cotton	2750	9.52 ± 0.36	39.7 (39.2 - 40.2)	9.060	0.1067	1.00
	Manila	1000	12.98 ± 0.83	35.0 (34.5 - 35.6)	1.213	0.5445	1.13 (1.12 - 1.15)
	Wool	1250	5.32 ± 0.29	12.9 (12.3 - 13.4)	0.650	0.4200	3.09 (3.01 - 3.16)
	Polypropylene	2500	4.65 ± 0.29	26.2 (25.6 - 27.0)	3.643	0.7249	1.51 (1.45 - 1.57)
	Nylon	1500	3.68 ± 0.25	23.0 (21.2 - 24.6)	1.455	0.6927	1.72 (1.66 - 1.79)
Indoxacarb	Cotton	2248	4.04 ± 0.13	52.2 (50.3 - 54.3)	1.3494	0.5093	1.00
	Manila	1659	5.10 ± 0.74	36.2 (32.3 - 38.7)	3.4030	0.3336	1.44 (1.35 - 1.54)
	Wool	4250	6.44 ± 0.16	32.6 (32.0 - 33.1)	8.6295	0.2804	1.60 (1.54 - 1.67)
	Polypropylene	2250	3.11 ± 0.20	52.2 (49.7 - 54.5)	0.6717	0.7147	1.00 (0.91 - 1.10)
	Nylon	2000	6.57 ± 0.54	39.2 (37.2 - 40.8)	2.9321	0.2308	1.33 (1.27 - 1.39)
* Total number of flies tested; (lies tested; (Probit [SAS Ir	(Probit [SAS Institute 2001]).					

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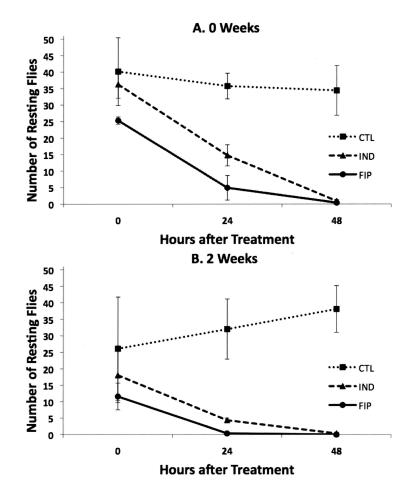


Fig. 3. Numbers of resting house flies counted in field cages before and after placement of 0.1% fipronil and 1.2% indoxacarb impregnated cords. (A) initial counts and (B) counts for cords aged 2 wk.

available resting surfaces available to flies, factors which enhance a fly's attraction to the cords are beneficial to the effectiveness of the treatment. All other factors being equal, surfaces which are more attractive to flies should cause quicker mortality due to increased exposure to the insecticide. Arevad (1965) found flies favored dark, rough surfaces over light, smooth surfaces. Fay and Lindquist (1954) reported sisal cord to be more attractive than jute or wool cords of the same size, but less attractive than a similar sized cotton cord.

In our attractiveness experiment, the cords varied by fiber type (animal, plant, or synthetic), color, and texture. All of the natural cords were more attractive than the synthetic cords. Although cotton was relatively attractive in our experiments, it had very poor efficacy when treated with either fipronil or indoxacarb compared with the other cords. Fipronil- and indoxacarb-impregnated wool cords had the greatest efficacy in

our experiments despite flies resting on it 50% less than the manila cord, as observed in the cord attractiveness experiments. This is contrary to the previous assumption that quicker mortality would result from increased exposure to a more attractive insecticide-impregnated cord. The assumption of greater exposure with greater attractiveness does not account for the insecticide-substrate interaction. Highly organic materials readily bind to pesticides and make them less available for acquisition by insects (Dell et al. 1994, Gardner et al. 2000). This fact may account for the high LT_{50} values seen in the cotton and manila cords. The exact reason wool outperformed the other cords in our experiments was not fully investigated, but it is likely due to the insecticide-substrate interaction.

In the laboratory, the indoxacarb-impregnated cords generally provided a much slower kill than the fipronil-treated cords; however, in field cages differences were not as apparent perhaps because the indoxacarb dose was doubled in the field cage experiments. However, indoxacarb is a proinsecticide that needs to be bioactivated within the insect and causes slower mortality than a contact insecticide, such as fipronil. Therefore, flies knocked down by indoxacarb in the laboratory are shielded from desiccation and predation, which may have prolonged fly survival in the laboratory experiments. Both insecticides showed efficacy in the field experiments even after being aged 2 wk. These results indicate that these newer insecticides are relatively stable under outdoor weather conditions typical of Florida.

Fipronil and indoxacarb impregnated into wool cord provided house fly mortality equivalent to cotton cords impregnated with older chlorinated hydrocarbon or organophosphate insecticides. Use of insecticide-impregnated cords would allow residual treatment of areas where traditional residual insecticides would be removed or degraded. With these new active ingredients, insecticide-impregnated cords could become valuable tools for use in present-day urban, agriculture, and military fly management programs.

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