# Dilution of Fluon Before Trap Surface Treatment Has No Effect on Longhorned Beetle (Coleoptera: Cerambycidae) Captures

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## Abstract

Several studies have observed that trap captures of longhorned beetles (Coleoptera: Cerambycidae) can be increased by treating the surface of intercept traps with a lubricant. In addition to being expensive, these treatments can alter the spectral properties of intercept traps when applied neat. These surface treatments, particularly Fluon, are commonly used diluted as a low friction coating to prevent insects from climbing out of rearing containers. The purpose of this study was to examine the effect of diluting Fluon on the spectral properties of treated corrugated plastic traps and the capture of longhorned beetles including Monochamus scutellatus (Say), Monochamus mutator (LeConte), and Monochamus notatus (Drury). Intercept panel traps were baited with attractant semiochemicals and treated with either undiluted (i.e., 100%) Fluon, a 1:1 mixture of Fluon and water (50%), a 1:9 mixture of Fluon and water (10%), or untreated. There were no obvious differences in the relative reflectance of untreated black Coroplast plastic or black Coroplast plastic treated with 50 or 10% Fluon. Traps treated with 100% Fluon had similar patterns of peak reflectance to the other treatments but overall had higher relative reflectance. In general, no effect of diluting the Fluon was observed for male or female M. scutellatus or M. mutator, but an effect of treating traps with Fluon was observed. Similar results were observed for the combined captures of Clytus ruricola Olivier, Cyrtophorus verrucosus Olivier, Megacyllene caryae (Gahan), Xylotrechus colonus (F.), Neoclytus acuminatus (F.), Neoclytus mucronatus (F.), and Phymatodes testaceus (L.). No treatment effect was observed for *M. notatus*.

Key words: Cerambycidae, Monochamus, trap, survey and detection, Fluon

As a result of increasing global demands for forest products combined with a decreasing land base to meet these demands and increasing numbers of introductions of nonnative species, the need for effective pest management programs in forest systems is high. Operational survey (e.g., Cooperative Agricultural Pest Survey) and monitoring (e.g., Early Detection Rapid Response) programs are critical components of management programs for forest insects and rely on flight intercept traps baited with attractants (e.g., host volatiles and pheromones). Intercept trap systems capable of detecting low-density populations of target species are essential because: 1) as populations of the target invasive species increase, the available management options decrease and their associated costs increase; 2) the distribution of the target species needs to be accurately defined; and 3) the success of management efforts cannot be evaluated without good monitoring tools (Sharov and Liebhold 1998, Myers et al. 2000, Liebhold and Tobin 2008). Recently, there have been several high profile introductions of large woodborers (*Anoplophora glabripennis* (Motschulsky), *Agrilus planipennis* Fairmaire, *Sirex noctilio* (F.)), and woodborers are now recognized as one of the most serious threats to forest health globally (Aukema et al. 2011). Consequently, large woodborers, particularly longhorned beetles, are frequent targets of many of these survey and monitoring programs.

Considerable research effort has been focused on the optimization of trapping systems for large woodborers. These studies have either quantitatively or qualitatively enhanced the attractant(s) used to bait the trap or improved the performance of the trap. Numerous attractants for longhorned beetles have been identified including kairomones [e.g., host volatiles (Suckling et al. 2001, Sweeney et al. 2004), bark beetle pheromones (Billings and Cameron 1984; Allison et al. 2001, 2003, 2013)] and pheromones (Allison et al. 2004, 2012; Sweeney et al. 2010; Mitchell et al. 2013; Wickham et al. 2015; Ray et al. 2015). Factors known to affect the performance of intercept traps for longhorned beetles include the trap type (McIntosh et al. 2001, Allison et al. 2014), collection reservoir (Miller and Duerr 2008), the addition of a large collar to the bottom of the trap to prevent insects from striking the trap and falling outside the collection cup (Morewood et al. 2002, de Groot and Nott 2003, Allison et al. 2014), and treating the trap surface with a lubricant (de Groot and Nott 2003; Sweeney et al. 2004; Graham et al. 2010; Allison et al. 2011, 2014; Graham and Poland 2012).

In addition to trap surfaces, the walls of containers used to house laboratory colonies of insects are commonly treated with Fluon (Northern Specialty Chemicals, Dudley, MA) to prevent escape. Unfortunately, treatment of trap surfaces changes the spectral properties of traps which may reduce the impact of treatment. For example, Francese et al. (2013) examined the reflectance spectra of purple and green multiple-funnel traps and reported that although treating the trap surface with Fluon did not alter the pattern of reflectance across wavelengths (i.e., the wavelength of peak reflectance did not change) it did increase reflectance. Trap color (wavelength) and brightness (reflectance) may be important in optimizing woodborer attraction. Francese et al. (2010) found that green traps (wavelength = 530-540 nm) painted in the 23-66% reflectance range were optimal for attracting emerald ash borer, A. planipennis (Coleoptera: Buprestidae), and that traps painted with 49% reflectance resulted in significantly higher trap catches than traps painted with 9% reflectance paint. Additionally, formulations of polytetrafluoroethylene (Teflon and Fluon) are expensive. Allison et al. (2011) calculated that the cost to treat an eight-unit multiple-funnel trap with Fluon was \$3.88 USD. Fortunately, there is some evidence that dilution of Fluon before treatment may reduce both cost and impact on reflectance (and ultimately attraction and capture of woodborers). Chen and Wei (2007) evaluated the effect of dilution of Fluon on its ability to prevent escape of fire ants from laboratory containers and observed that depending on the container type that Fluon could be diluted with distilled water to between 1/16 and 1/ 256 Fluon to distilled water with no increase in escapes. The objective of this study was to determine if dilution of Fluon as a trap surface treatment reduced its effect on the capture of longhorned beetles or changed the reflectance of the trap.

#### **Materials and Methods**

An experiment was conducted to test the effect of Fluon on the reflectance of treated trap material. Two field trapping experiments were then conducted to examine the effect of diluting the trap surface treatment Fluon with distilled water on the capture of longhorned beetles in Canada and Michigan.

#### Experiment 1: Reflectance Test

Samples of black, Coroplast corrugated plastic (4 mm thick, Coroplast, Vanceburg, KY), the material used for construction of intercept panel traps, were cut into 7.5 cm<sup>2</sup> for application of Fluon and measurement of reflectance spectra. Fluon was applied by cloth as undiluted or diluted with DDI water to 50% or 10% (v/v) concentrations. Reflectance spectra of samples were generated relative to a white standard using a Labsphere RSA-HP-84 integrating sphere (Labsphere, North Sutton, New Hampshire) equipped with a Tungsten Halogen bulb attached to a Hewlett-Packard HP 8452A Diode Array spectrophotometer (Agilent, Inc., Santa Clara, CA). Each line (Fig. 1) represents the mean of a single measurement taken from a haphazardly chosen location on each square with four replicates each for each treatment (i.e., 16 squares total).

#### Experiment 2: Canada Trapping

Insects were collected from traps weekly from 23 July to 29 August 2012. The species M. scutellatus, M. mutator, and M. notatus were targeted by baiting all of the traps with semiochemicals (ultra-high release [UHR] ethanol and  $\alpha$ -pinene, ipsenol, ipsdienol, and monochamol) known to be attractive to Monochamus spp. (Allison et al. 2001, 2003, 2004, 2013). Panel traps were deployed in a linear array of 10 replicate blocks of four traps per block. Each replicate block had a single trap treated with the following: 1) undiluted Fluon (100%); 2) Fluon diluted with equal parts distilled water (50%); 3) Fluon diluted with 9 parts distilled water to 1 part Fluon (10%); and 4) untreated (0%). All traps were equipped with a wet collection cup containing 150-200 ml of propylene glycol. Fluon and the distilled water were mixed in the appropriate ratios in plastic spray bottles and then applied outside under a covered shed to the entire trap surface until wet and allowed to air-dry prior to trap deployment. The experiment was conducted in a clear-cut north of Aubrey Falls, Ontario cut in the spring of 2012. The cut stand and adjacent stands were predominately (ca. 70%) jack pine with some red pine. Traps were suspended individually from metal conduit pipe with a bend at the top such that the collection cup of each trap was 0.5-1.0 m above the ground. There was a minimum of 25 m between traps within and between blocks. Species of Monochamus were identified using standard keys, and individuals were sexed using antennal and abdominal morphology (Yanega 1996, Lingafelter 2007). All traps were baited with UHR  $\alpha$ -pinene (chemical purity  $\geq$ 95%, enantiomeric purity 95% (-); release rate ca. 2 g/d at 20°C) and UHR ethanol (release rate ca. 0.5 g/d at 23°C) pouch lures, bubble cap lures loaded with  $(\pm)$ -ipsdienol (=racemic ipsdienol, 50:50 mix of plus and minus enantiomers; release rate ca. 0.1-0.2 mg/d at 25°C) and (±)-ipsenol (=racemic ipsenol, 50:50 mix of plus and minus enantiomers; release rate ca. 0.1-0.2 mg/d at 25°C), and monochamol high release pouches (99.3% pure; release rate ca. 0.4 mg/d at 20°C). All lures were purchased from ConTech Enterprises, Inc. (Victoria, B.C.), and purity and release rate data were supplied by the manufacturer. Total catches per trap of male and female M. scutellatus, M. mutator, and M. notatus were analyzed using a blocked multiresponse permutation procedure (MRBP; McCune et al. 2002). The catches from each collection period were summed by treatment

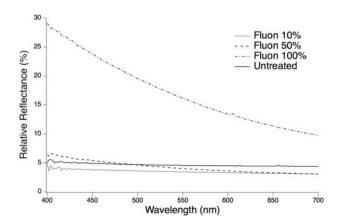


Fig. 1. Reflectance spectra of black, Coroplast plastic corrugated (4 mm thick, Coroplast, Vanceburge, KY) treated with Fluon applied by cloth as undiluted or diluted with DDI water to 50% or 10% (v/v) concentrations or untreated.

for males and females of each species. All analyses were conducted with PC-ORD 6.0 (MjM Software Design, Gleneden Beach, OR) by using Euclidean distances to construct the distance matrix with blocks aligned before analysis, and the multiplicity effect was controlled using step-up FDR (Benjamini and Hochberg 1995, Garcia 2004).

#### **Experiment 3: United States Trapping**

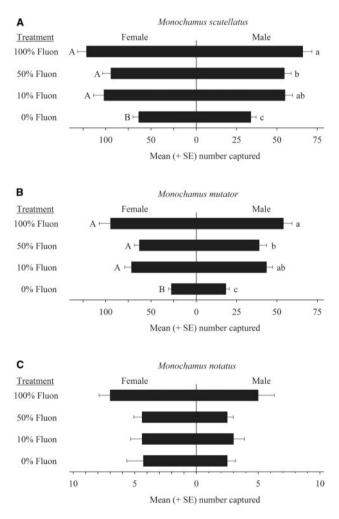
The second trapping experiment was conducted at the Michigan State University Tree Research Center in Lansing, MI. Traps were deployed from 13 May to 26 June. The treatments were identical to Experiment 1 (0, 10, 50, and 100% Fluon) with three blocks of each treatment. Trap contents were collected approximately every 4d, and treatments were re-randomized within blocks during each collection for a total of 27 replicates. Trap design and placement were the same as in Experiment 2 except the study was conducted in a mixed hardwood forest; therefore, different attractants were used. Lures consisted of 1 ml of citral solution (a 1:1 mixture of neral and geranial, Sigma-Aldrich Co., St. Louis, MO) diluted to 5% in ethanol and placed in clear polyethylene sachets ("Zipper" press-seal bags Catalog number 01-816-1 A; 5.1 7.6 cm, 0.05-mm wall thickness, Fisher, Pittsburg, PA) and the racemic pheromone blend of 3Rhydroxyhexan-2-one (Synergy Semiochemicals Corp., Burnaby, BC) released from bubble caps. Citral has been identified as a known pheromone component of Megacyllene caryae (Gahan) (Lacey et al. 2008), and 3R-hydroxyhexan-2-one is a pheromone component of several hardwood feeding species and has been used as a generic lure (Hanks et al. 2012). Total catches per trap of the most abundant species (see results) were analyzed using a blocked MRBP (McCune et al. 2002). All species for which >5 individuals were captured were summed by treatment. All analyses were conducted with PC-ORD 6.0 (MjM Software Design, Gleneden Beach, OR) by using Euclidean distances to construct the distance matrix with blocks aligned before analysis, and the multiplicity effect was controlled using step-up FDR (Benjamini and Hochberg 1995, Garcia 2004).

#### Results

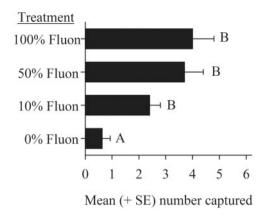
The reflectance spectra of black corrugated Coroplast plastic treated with Fluon applied by cloth as undiluted or diluted with DDI water to 50 or 10% (v/v) concentrations or untreated, were recorded for the visible spectrum (ca. 400–700 nm). There were no obvious differences in the reflectance spectra of the black Coroplast plastic squares untreated or treated with 50% and 10% diluted Fluon. Black Coroplast plastic squares treated with undiluted Fluon had higher relative reflectance across the entire visible spectra and this effect increased as wavelength decreased (Fig. 1). Despite this difference, the pattern of peak reflectance was similar for all treated and untreated samples of the black Coroplast plastic squares.

In Experiment 2, a total 10,348 *Monochamus* beetles was captured: 3,809 female and 2,094 male *M. scutellatus*, 2,566 female and 1,548 male *M. mutator*, and 201 female and 130 male *M. notatus*. There was a significant treatment effect on captures for male and female *M. scutellatus* (male: T = -5.9, P < 0.001; female: T = -3.5, P < 0.01) and *M. mutator* (male: T = -7.7, P < 0.0001; female: T = -8.3, P < 0.0001). There was no treatment effect on the captures of male (T = -0.72, P = 0.21) and female (T = -0.72, P = 0.21) *M. notatus*. In general, there was no effect of dilution on trap captures for male and female *M. scutellatus* and *M. mutator*. Traps treated with 100%, 50%, and 10% Fluon all captured more male and female *M. scutellatus* and *M. mutator* than untreated traps. Male *M. scutellatus* and male *M. mutator* were both captured in higher numbers in traps treated with undiluted Fluon (100%) than traps treated with the 1:1 mixture of Fluon and water (50%; Fig. 2). Otherwise for these three *Monochamus* spp. there were no differences among the traps treated with the three concentrations of Fluon.

In Experiment 3, 23 species of longhorned beetles were captured for a total of 362 beetles. The most abundant species were *Clytus ruricola* Olivier (57), *Cyrtophorus verrucosus* Olivier (56), *Megacyllene caryae* (53), *Xylotrechus colonus* (F.) (47), *Neoclytus acuminatus* (F.) (39), *Neoclytus mucronatus* (F.) (38), and *Phymatodes testaceus* (L.) (29). The remaining 16 species were singletons or fewer than five individuals were captured and were omitted from the analysis. The sum of these seven species was analyzed, and there was a significant treatment effect on this variable (the sum of these species; T = -10.98, P < 0.00001); traps treated with Fluon caught significantly more beetles than untreated traps regardless of dilution level (Fig. 3).



**Fig. 2.** Seasonal mean total captures (+SE) of *M. scutellatus* (**A**), *M. mutator* (**B**), and *M. notatus* (**C**) in panel traps baited with UHR ethanol, UHR  $\alpha$ -pinene, ipsenol, ipsdienol, and monochamol in Ontario, Canada. Traps were equipped with wet cups and treated with neat Fluon (100%), Fluon diluted with equal parts water (50%), Fluon diluted with nine parts water to one part Fluon (10%), or untreated (0%). There were 10 replicates per treatment. Means (+SE) followed by the same letter (uppercase: female, lowercase: male) within species are not significantly different at *P*=0.05.



**Fig. 3.** Mean total per collection of the combined captures  $(\pm SE)$  of the most abundant species (*C. ruricola, Cy. verrucosus, M. caryae, X. colonus, N. acuminatus, N. mucronatus,* and *P. testaceus*) of longhorned beetles captured in panel traps baited with Citral plus 3R-hydroxyhexanone in Michigan, United States. Traps were treated with neat Fluon (100%), Fluon diluted with equal parts water (50%), Fluon diluted with nine parts water to one part Fluon (10%), or untreated (0%). There were three blocks per treatment replicated over nine collection periods for 27 total replicates. Means (+SE) followed by the same letter are not significantly different at P = 0.05.

#### Discussion

Commercially available flight intercept traps were developed principally for ambrosia and bark beetles (Lindgren 1983) and do not work well for large woodborers (e.g., McIntosh et al. 2001, de Groot and Nott 2003). Additionally, the most effective trap design can vary among species (Holland 2006) and a recent study by Dodds et al. (2015) demonstrated that for large woodborers the intercept trap design that optimizes abundance is not necessarily the trap design that optimizes receively in survey, monitoring and mass trapping programs, and research trials targeting large woodborers (Allison et al. 2004, Animal and Plant Health Inspection Service [APHIS] 2006, Rabaglia et al. 2008). The advantages of flight intercept traps are that they are relatively inexpensive and easy to deploy.

The primary explanations for the poor performance of intercept traps for large woodborers include attraction to and subsequent failure to capture or capture of attracted individuals and subsequent escape. The proportion of attracted individuals attracted but not captured by flight intercept traps has not been quantified for large woodborers. Cooperband and Cardé (2006) quantified the capture efficiency of various trapping systems for the mosquitoes Culex quinquefasciatus Say and Culex tarsalis Coquillett and observed that the proportions of females captured per flights (approaches) ranged from 0.08-0.71. With respect to the capture and subsequent escape of individuals, several studies have demonstrated that flight intercept traps equipped with wet collection cups capture more large woodborers than traps equipped with dry collection cups (e.g., Miller and Duerr 2008, Graham and Poland 2012, Allison et al. 2014). These results strongly suggest that captured individuals are more likely to escape from dry than wet cups. Nakamura et al. (1999) reported a 30% daily loss of the large woodborer M. alternatus from flight intercept traps equipped with a dry cup.

The application of a lubricant to multiple-funnel and panel trap surfaces and the interior of dry collection cups has been demonstrated to increase trap captures and reduce escapes of large woodborers, primarily Cerambycidae and Buprestidae. The majority of these studies report the application of polytetrafluoroethylene (Fluon, Teflon, or both) to multiple-funnel and panel traps (Graham et al. 2010; Allison et al. 2011, 2014; Francese et al. 2011, 2013; Graham and Poland 2012; Lyons et al. 2012), but some report the effects of alternative lubricants (Czokajlo et al. 2001; de Groot and Nott 2003; Sweeney et al. 2004; Graham et al. 2010; Francese et al. 2011, 2013). The most likely explanation for the reported increased trap captures is that the lubricant prevents individuals from being able to land on the trap and subsequently depart without being captured or climb up the walls and escape from the dry collection cup.

Comparatively more effort has been invested in documenting the effects of trap surface treatments on different taxa of large woodborers than the effects of deployment protocol (e.g., time since application, trap coverage, concentration effects). Graham and Poland (2012) demonstrated that there was no difference in the numbers of Cerambycidae captured in traps freshly conditioned or conditioned one or two years previously. Laboratory studies of insect husbandry have demonstrated that escapes from rearing containers can be reduced by treatment of container surfaces with Fluon and that this effect is not diminished by dilution (Chen and Wei 2007). This study documented significant effects of treatment of the trap surface with Fluon for male and female M. scutellatus, M. mutator, and several species of hardwood feeding longhorned beetles. In general, traps treated with undiluted Fluon, Fluon diluted with equal (50%) or nine (10%) parts water to one part Fluon had similar trap captures. Conversely, while Black Coroplast plastic squares treated with equal (50%) or nine (10%) parts water to one part Fluon and untreated traps had similar reflectance spectra, Black Coroplast plastic squares treated with undiluted Fluon had higher relative reflectance across the entire visible spectra and this effect increased as wavelength decreased. These results suggest that the expense of treatment and impact on the spectral properties of trap surfaces can be minimized by diluting Fluon prior to application with no reduction in trap performance for large woodborers.

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