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IGRs FOR CAT FLEA (SIPHONAPTERA: PULICIDAE) CONTROL REVISITED

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Abstract The intrinsic activity of combinations of methoprene and chlorfluazuron and methoprene and fipronil was determined against larval cat fleas, *Ctenochephalides felis* (Bouché). Methoprene synergized the activity of the IGR chlorfluazuron and the adulticide fipronil. The LC_{50}s of chlorfluazuron, fipronil, and methoprene were 0.187 (0.136-0.236), 0.983 (0.236-1.520), and 0.39 (0.212-0.574) ppm, respectively. Combinations of chlorfluazuron 1: methoprene 1 and chlorfluazuron 5: methoprene 1 were synergistic providing LC_{50}s of 0.028 (0.017-0.042) and 0.23 (0.112-0.337) ppm, respectively. All other combinations of these two IGRs were antagonistic. The combinations of fipronil 1: methoprene 1 and fipronil 5: methoprene 1 were synergistic providing LC_{50}s of 0.292 (0.221-0.360) and 0.927 (0.824-1.011) ppm, respectfully. The addition of methoprene to chlorfluazuron or fipronil allows for the reduction of amounts of both compounds needed to kill fleas and in the future may also retard the development of insecticide resistance.

Key words Methoprene, chlorfluazuron, fipronil, synergism.

INTRODUCTION

Insect growth regulators (IGRs) have been used to control cat fleas, *Ctenocephalides felis* (Bouché), on the host and in the environment for nearly three decades (Beugnet and Franc, 2012; Blagburn and Dryden, 2009; Rust, 2005). Most of the research has focused on three IGRs, lufenuron, methoprene and pyriproxyfen. Initially, the focus was on the use of IGRs to control fleas in the environment with applications of organophosphates and pyrethoids and methoprene or pyriproxyfen, combination treatments being routinely conducted by pest management professional (PMPs) (Rust and Dryden, 1997, Hinkle et al., 1997). In 1995 the emphasis switched to applications directly to the pet, and less attention has been given to IGRs and their application in the urban environment to control fleas.

Methoprene and pyriproxyfen have been combined with adulticides such as dinotefuran, fipronil, imidacloprid and permethrin and directly applied to the pelage of the pet. The development of these combination products has become very popular and their efficacy has been widely reported (Varloud and Hodgkins, 2015; Baker et al., 2014; Boushira et al., 2012; Ross et al., 2012; Beugnet et al., 2011; Beugnet et al., 2012; Dryden et al., 2008; Dryden et al., 2011 a, b, c). The objectives of this paper are to re-examine the potential role that IGRs may play in *C. felis* control both on-animal therapies and in the environment. The intrinsic activity of combinations of IGRs and methoprene and the adulticide fipronil is presented. Potential areas for further research are highlighted.

MATERIALS AND METHODS

Cat Flea Maintenance

A laboratory strain of cat flea (UCR) has been maintained without any exposure to insecticides for nearly 40 years (Rust and Hemsarth, 2016a). Cat flea eggs were collected from a tray underneath the cats and the debris and eggs were passed through metal sieves. The eggs were retained on the 60-mesh screen. The eggs were placed on larval rearing media consisting of 2950 g 30-mesh play sand, 375 g finely ground dog chow, 75 g hemoglobin freeze-dried (MP Biomedicals. LLC, Solon, OH), and 50 grams Brewer's yeast. The larvae were held in a desiccator chamber maintained at 75% RH (saturated NaCl solution, Winston and Bates, 1960) and 26.7º ± 1º C. On day 12 -13 after egg collection, the larval medium was passed through a 16-mesh sieve to remove the cocoons. Adults emerged 16-18 d after the egg collection.

Insecticides and IGRs

The IGRs chlorfluazuron (tech. 98.4 %, Chem Services, West Chester, PA), fipronil (tech. 97.8%, Pestanal ® Sigma-Aldrich, St. Louis, Mo) and methoprene (99.2%, Chem Service, West Chester, PA). Acetone was selected as the solvent.

Eleven serial dilutions of chlorfluazuron (chlor) and methoprene (meth) were tested ranging from 0.039 to 1.25 ppm and 0.0625 to 2.5 ppm, respectively. Serial dilutions of the fipronil (fip) and methprene combinations were tested ranging from 0.125 to 2.0 ppm. Dilutions of the chlorfluazuron and methoprene were tested ranging from 0.004 to1.0 ppm.

Larval Media Studies

Aliquots of 0.2 ml of each test solution were applied to 2 grams of larval rearing media in plastic vials (76 by 20 mm diameter Sarstedt, Newton, NC) and allowed to dry for at least 2 hours under a fume hood. The relative humidity of the work bench was increased by two humidifiers (TaoTronics TT-AH002, Fremont, CA) providing 75-80% RH, preventing the desiccation of larvae, and reducing the static electricity. Larvae were selected from the rearing media on day 4 or 5 after the egg collection for testing with a fine paint brush lightly moistened with water. Ten larvae were placed in glass petri dishes (5 cm diameter by 1.7 cm). The treated larval medium was transferred from the plastic vial to the glass petri dishes with the larvae. A piece of parafilm (ca. 1 cm by 3 cm) was stretched around about 2/3 of the bottom of the petri dish to help secure the lid and prevent adult fleas from escaping. The dishes were then placed into a desiccator maintained with 75% RH and held at 26.7º ± 1º C. After 21 days, the dishes were placed in the freezer for 2 hours and the number of adult fleas was counted.

The number of dead larvae and adults that failed to emerge from cocoons was counted. Adult fleas were counted as emerged or fully developed adults in cocoons if the legs were free from the body.

DATA ANALYSIS

The adult development data were analyzed by probit analysis with POLO program (Robertson et al., 2007). The program performs a likelihood ratio test of the equality of the slopes and intercepts of the probit lines for each IGR.

To determine the effects of the combination treatments the data were analyzed with the Chou-Talalay Method (Chou and Talalay, 1984; Chou, 2006, 2010). The median-effect plots allow the potency value or median dose and dose effect curve of each pesticide and their combination to be determined. These are used to calculate the Combination Index (CI) which provides a quantitative value and a basis for determining if combinations are synergistic, additive or antagonistic for each dose. Chou (2006) categorized CI values as follows: <0.1- very strong synergism, 0.1-0.3 – strong synergism, 0.3-0.7 – synergism, 0.7-0.85 – moderate synergism, 0.85-0.90 – slight synergism, 0.9-1.10 – nearly additive, 1.10-1.12 – slight antagonism, 1.20 -1.45 – moderate antagonism, 1.45 - 3.3 antagonism, 3.3 -10 – strong antagonism, >10 very strong antagonism. The Dose Response Index (DRI) measures the potential reduction of the dose of each IGR in the combination compared with the IGR alone.

RESULTS

The intrinsic activity of the two IGRs and fipronil (LC_{50}s) were chlorfluazuron (chlor) > methoprene (meth) > fipronil (fip) (Table 1). The LC_{50} and LC_{95} of chlorfluazuron and methoprene were 0.19 and 0.78 ppm and 0.39 and 5.07 ppm, respectively. Fipronil was the least active compound against larvae, the LC_{50} and LC_{95} being 0.98 and 22.28 ppm, respectively.

The combination of chlor 1: meth 1 and chlor 5: meth 1 provided LC_{50}s of 0.03 and 0.23 ppm, respectively. The combination of chlor 1: meth 1 was highly synergistic with Combination Indices (CI) ranging from 0.11 to 0.31 (Table 2), whereas the combination of chlor 5: meth 1 was slightly synergistic with CI values ranging from 0.72 to 0.98. The DRI's for chlor 1: meth 1 ranged from 6.04 to 68.24 suggesting that the concentrations of each IGR could be dramatically reduced and still provide the same activity of each IGR alone. The DRI's for chlor 5: meth1 were less, but the DRI's for methoprene ranged from 11.41 to 41.56 indicating that concentrations of methoprene could be significantly reduced.

Table 1. The intrinsic biological activity of IGRs against larval cat fleas, *C. felis*. Data in ppm.

Treatment	n	Slope ± (SEM)	LC_{50} (95% C.I.)	LC_{95} (95% C.I.)
Chlorfluazuron (Chlor)	720	2.65 ± 0.24	0.19 (0.137-0.236)	0.78 (0.600-1.146)
Methoprene (Meth)	610	1.48 ± 0.21	0.39 (0.212-0.574)	5.07 (2.531-16.526)
Chlor 5: Meth 1	520	4.10 ± 0.55	0.23 (0.112-0.337)	0.58 (0.403-0.974)
Chlor 1: Meth 1	450	1.59 ± 0.18	0.03 (0.017-0.042)	0.30 (0.172-0.824)
Fipronil (Fip)	940	1.21 ± 0.22	0.98 (0.236-1.520)	22.28 (7.782-3464.980)
Fip 5: Meth 1	600	5.72 ± 0.63	0.93 (0.824-1.011)	1.80 (1.592-2.183)
Fip 1: Meth1	690	2.32 ± 0.20	0.29 (0.221-0.360)	1.50 (1.173-2.126)

Table 2. Combination Index at each Effective Dose (ED) for the combinations of chlorfluazuron (Chlor) and methoprene (Meth).

Combination	ED_{50}	ED_{75}	ED_{90}	ED_{95}
Chlor 5: Meth 1	0.72	0.79	0.89	0.98
Chlor 1 : Meth 1	0.31	0.19	0.13	0.11

The combination of fip 1: meth 1 and fip 5: meth 1 provided LC_{50}s of 0.29 and 0.93 ppm, respectively. The CI's of the fip 1: meth 1 ranged from 0.26 to 2.62 and were slightly synergistic. Similarly, the CI's of fip 5: meth 1 ranged from 0.13 to 2.47. The DRI's for fipronil and methoprene combinations ranged from 0.55 to 21.89 and indicated that some reductions in the amounts of methoprene may be possible and still achieve the activity of fipronil alone.

Table 3. Dose-reduction Index at each Effective Dose (ED) for the combinations of chlorfluazuron (Chlor) and methoprene (Meth) against *C. felis* larvae.

Combination	ED_{50}		ED_{75}		ED_{90}		ED_{95}	
	Chlor	Meth	Chlor	Meth	Chlor	Meth	Chlor	Meth
Chlor 1: Meth 1	6.04	7.15	7.52	16.59	9.35	38.5	10.83	68.24
Chlor 5: Meth 1	1.56	11.41	1.34	19.74	1.16	34.14	1.05	41.56

Table 4. Combination Index at each Effective Dose (ED) for the combinations of fipronil (Fip) and methoprene (Meth).

Combination	ED_{50}	ED_{75}	ED_{90}	ED_{95}
Fip 5: Meth 1	2.47	0.81	0.27	0.13
Fip 1 : Meth 1	2.62	1.09	0.46	0.26

Table 5. Dose-reduction Index at each Effective Dose (ED) for the combinations of fipronil (Fip) and methoprene (Meth) against *C. felis* larvae.

Combination	ED_{50}		ED_{75}		ED_{90}		ED_{95}	
	Fip	Meth	Fip	Meth	Fip	Meth	Fip	Meth
Fip 1: Meth 1	1.22	0.55	3.99	1.23	10.53	2.72	21.89	4.67
Fip 5: Meth 1	0.58	1.32	1.96	3.35	6.59	8.51	15.04	16.04

DISCUSSION

IGRs have played an important role in IPM programs for cat flea control for nearly 3 decades. Initially they were important as environmental treatments to control immature stages of fleas (Osbrink et al., 1986; Rust and Dryden, 1997). With the advent of the spot-on therapies with fipronil and imidacloprid in the mid- 1990's, the IGRs also became an effective treatment on animal and the treatment of the environment applications became a secondary recommendation.

Chlorfluazuron is as active as methoprene and pyriproxyfen against larval cat fleas (Rust and Hemsarth, 2016b). Chlorfluazuron has been registered to control Dipteran and Lepidopteran pests in agricultural settings (Rust and Hemsarth, 2016a). Physiological resistance to methoprene and pyriproxyfen have been reported in Diptera and Lepidoptera (Rust and Hemsarth, 2016a). Chlorfluazuron might serve as a potential IGR if resistance were to develop to either methoprene or pyriproxyfen in fleas.

Methoprene is capable of synergizing the activity of the IGR chlorfluazuron. Recent research has shown that methoprene is synergized by pyriproxyfen and even at 20:1 ratios the combination is about 3-fold more active than methoprene alone (Rust and Hemsarth, 2016b). This is the first published data that methoprene is also capable of synergizing another IGR. The DRI clearly indicate that concentrations of chlorfluazuron and methoprene could be reduced for each IGR as a result of the synergy.

Young et al. (2004) suggested that methoprene may even synergize the activity of fipronil against immature stages of fleas when applied to the pelage of dogs. Our data provides evidence to support that observation. The addition of methoprene clearly increased the activity of the combination. Additional research with the combination against adult fleas is warranted to determine if any other effects might occur.

Our understanding of the mode of actions of the IGRs has changed since the first on-animal IGR/adulticide combination therapies registered some 20 years ago. In addition to altering insect development, studies have demonstrated IGR effects on behavior and synergistic activity towards other

active ingredients. Additional studies dealing with the interactions of IGRs and other active ingredients used to control cat fleas are warranted.

CONCLUSIONS

Methoprene synergized both chlorfluazuron and fipronil against larval *C. felis*. The DRI of the chlorfluazuron and methoprene suggest that the concentrations of both IGRs could be reduced at least 3-fiold and still provide the activity of either IGR alone. The DRIs of the fipronil and methoprene suggest that only modest reductions are probably possible.

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