Effects of pyriproxyfen on the reproduction of the housefly, *Musca domestica*, and the German cockroach, *Blattella germanica*

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Abstract: The activity of pyriproxyfen as an insect sterilant was studied. Total number of eggs/female and hatchability or total number of progeny of the housefly and the German cockroach decreased with the topical application of pyriproxyfen in a dose-dependent manner. The sexual transmission of sterility by pyriproxyfen-treated males was also observed, although the dosage required to cause sterility to his partner was relatively higher. Ten min exposure to pyriproxyfen formulated in rapeseed oil with dosages of 100 $\mu\text{g}/\text{cm}^2$ on aluminium surface for the housefly or 60 min exposure to more than 400 $\mu\text{g}/\text{cm}^2$ for the German cockroach enabled males to transfer sterility to females by sexual intercourse.

INTRODUCTION

Juvenile hormone (JH) is thought to play a principal role in controlling the insect oögenesis and vitellogenesis. This can be proved by the evidence that the ovarian maturation of allatectomized insect is activated by treatment of JH. Synthesized juvenile hormone mimics (JHMs) have also been known to play a similar role to natural JH (Adams, 1974; Sakurai, 1977). In contrast with these findings, the sterility caused by exogenous JHM has been reported in several papers (Masner *et al.*, 1968; Das and Gupta, 1974; Thomas, 1984; Langrey *et al.*, 1990).

We previously reported on the larvicidal effectiveness of a newly synthesized JHM, pyriproxyfen (Sumilarv®) against the housefly, mosquitoes and cockroaches (Hatakoshi

et al., 1987; Kawada *et al.*, 1987, 1988, 1989; Suzuki *et al.*, 1989). In the present paper, the activity of pyriproxyfen as an insect sterilant is reported, and the possibility of sterility transmission by male to female is discussed.

MATERIALS AND METHODS

Test compounds. Pyriproxyfen: 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether (purity 97.2%).

Test insects. The CSMA and Akagi strains of the housefly, *Musca domestica*, and the German cockroach, *Blattella germanica* were used.

The CSMA strain shows normal susceptibility to insecticides. The Akagi strain is highly resistant to pyrethroids and fairly resistant to organophosphorus compounds (Kawada *et al.*, 1987). The insects were reared for successive generations in the laboratory at $25\pm1^\circ\text{C}$ and 60% (RH) with a

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16L-8D photoperiod regime.

Evaluation methods. All tests were conducted under the same conditions as described above.

1) Inhibition test of reproduction by topical treatment to housefly adults: Newly emerged CSMA adults were separated by sex within 24 hr after emergence. Three days after emergence, a 0.5 μ l acetone solution of the test compound was topically applied to the dorsal thorax of the insect. Twenty-five females treated and the equivalent number of males untreated, or *vice versa*, were released into a cage (20 $\times$ 20 $\times$ 20 cm) and reared with food (skimmed-milk, 1 part and granulated sugar, 1 part; w/w) and water. Eggs were collected in gauze soaked with a solution of skimmed-milk, and the number of eggs per female was recorded. The collection of eggs was done 5 times during 23 days after emergence. The eggs were sampled on a slide glass and kept in a closed plastic cup with a moistened filter paper for 24 hr to observe hatchability. Inhibition of reproduction was calculated as follows:

$$\text{IR}(\%) = 100 - (N \times H) / (N_c \times H_c) \times 100.$$

IR, inhibition of reproduction; N , total number of eggs; N_c , total number of eggs in control; H , hatchability; H_c , hatchability in control.

Three-days-old virgin females treated with 10 μ g of pyriproxyfen and untreated females were reared in cages apart from males. Ten females were sampled at 5, 8, 17 days after emergence and dissected under a microscope to observe ovaries and the number of eggs in a stage higher than 7 (Adams, 1974) were counted.

2) Inhibition test of reproduction by topical treatment to German cockroach adults: The final instar nymphs of the German cockroach were separated by sex and were reared in containers. Seven days after emergence, a 0.5 μ l acetone solution of the test compound was topically applied to the sternal mesothorax of the insect. Ten females treated and the equivalent number of males untreated, or *vice versa*, were released into a plastic cup (650 cm³) and reared with food, water and harborage. Oothecae deposited were collected weekly and the number of progeny per female was recorded. Inhibition of reproduction was calculated as

follows:

$$\text{IR}(\%) = 100 - (N/N_c) \times 100.$$

IR, inhibition of reproduction; N , total number of progeny per female; N_c , total number of progeny per female in control.

3) Inhibition test of reproduction by contact of housefly males to the pyriproxyfen-treated surface: Fifty ml acetone solution of pyriproxyfen was mixed with 1 ml rapeseed oil. The solution was treated on the surface of aluminium foil (10 cm in diameter) at the rate of 12.7 μ l/cm² and the acetone was evaporated. Newly emerged Akagi adults were separated by sex within 24 hr after emergence. Three days after emergence, 10 males or females were exposed to the aluminium surface for 10 min. Fifty males treated and the equivalent number of females untreated, or *vice versa*, were released into a cage (20 $\times$ 20 $\times$ 20 cm) and reared with food (skimmed-milk, 1 part and granulated sugar, 1 part; w/w) and water. Eggs were collected in gauze soaked with a solution of skimmed-milk and the weight of eggs per female was recorded. Fifty eggs were inoculated in 15 g of medium (wheat bran, 7 parts; powdered animal food, 1 part; and deionized water, 14 parts) and reared to pupation. After 2 weeks, the number of adults was counted. The emergence rates were corrected by Abbott's formula for those of control. Inhibition of reproduction was calculated as follows:

$$\text{IR}(\%) = 100 - (W \times E) / (W_c \times E_c) \times 100.$$

IR, inhibition of reproduction; W , total weight of eggs; W_c , total weight of eggs in control; E , emergence rate; E_c , emergence rate in control.

4) Inhibition test of reproduction by contact of German cockroach males to the pyriproxyfen-treated surface: Ten ml acetone solution of pyriproxyfen was mixed with 1 ml rapeseed oil. The solution was treated on the surface of aluminium foil in the same manner as described above. The final instar nymphs of the German cockroach were separated by sex and were reared in containers. Seven days after emergence, 10 males were exposed to the aluminium surface for 60 min. Ten males treated and the equivalent number of females untreated were released into a plastic cup (650 cm³) and reared with food, water and harborage. Oothecae de-

posited were collected weekly and the number of progeny per female was recorded. Inhibition of reproduction was calculated in the same manner described in 2).

RESULTS

1. Effect of pyriproxyfen on the reproduction of housefly and German cockroach adults

The effects of topically applied pyriproxyfen on the reproduction of CSMA housefly adults are shown in Table 1. Total number of eggs/female and hatchability decreased with the increase of dosage. Total number of eggs produced by a female was *ca.* 17 times less in females treated with 10 or 20 μg of pyriproxyfen than that produced by untreated females. The reduction was lower in females treated with 2 μg . Hatchability was reduced to 5.8% in eggs produced by females treated with 20 μg . Hatchability was kept to less than 50% in females treated with 5 μg or more. Inhibition of reproduction, which is the product of % inhibition of oviposition and inhibition of egg hatch, for treated females became more than 98% with 10 or 20 μg treatment. IR_{90} (dosage required to cause 90% inhibition of reproduction) was 5 to 6 μg /female. Reductions in the number of eggs and hatchability were also observed in untreated females mated with treated males, although the effect was less than that for treated female. IR_{90} in this case was estimated to be around 20 μg /male or more. No significant differences in the number of eggs in stages higher than 7 were observed (Table 2).

Table 3 shows the effects of topically applied pyriproxyfen on the reproduction of the German cockroach adults. Observation was done on first oviposition (up to 6 weeks after emergence). Inhibition of reproduction was significantly higher in female or both sex treatment as compared with that in male treatment. Complete inhibition was obtained in 10 μg treatment of pyriproxyfen to both sexes. The inhibition activity was almost 20 times or more for female or both sex treatment than that for male treatment. The decrease in the number of progeny per ootheca or malformation of oothecae caused by infertility was observed in females treated.

2. Transmission of sterility by males contacted to the pyriproxyfen-treated surface

Figure 1 shows the changes in the amount of eggs, emergence rate and the inhibition of reproduction in the Akagi housefly females mated with males exposed to the pyriproxyfen-treated aluminium surface (100 $\mu\text{g}/\text{cm}^2$ as active ingredient). Evaluation was made for each week after treatment to males. The amount of eggs produced by untreated females mated with treated males was almost similar to that of control (corrected amount of eggs is more than 100) in the first week, but decreased to less than 50 during the next 2 weeks. Females treated and mated with untreated males produced fewer eggs than untreated females mated with treated males. Changes in the corrected rate of emergence of eggs drew similar reduction curves to those of the amount of eggs, *i.e.*, the reduction in the rate of emergence was slight in the first

Table 1 Reproduction of housefly* adults topically treated with pyriproxyfen.

Dosage ($\mu\text{g}/\text{insect}$)	Treatment to female**				Treatment to male			
	T	H	IO	IR	T	H	IO	IR
20	468	5.8	94.1	99.6	2,171	35.4	72.3	89.1
10	471	21.5	94.0	98.5	8,008	54.3	0	38.4
5	2,378	34.5	69.8	87.6	10,747	72.1	0	0
2	5,447	51.1	30.8	58.1	—	—	—	—
1	—	62.2	—	—	—	—	—	—
0.5	—	68.7	—	—	—	—	—	—
Control	7,872	84.3	0	0	7,838	90.1	0	0

* CSMA strain. ** T, total No. of eggs/female; H, % hatchability of eggs; IO, % inhibition of oviposition; IR, % inhibition of reproduction.

Table 2 Changes in the number of eggs (>stage 7) after emergence of pyriproxyfen-treated and untreated CSMA housefly female.

Days after emergence	Treated***		Untreated	
	No. of females with eggs in stage >7*	No. of eggs in stage >7/female**	No. of females with eggs in stage >7	No. of eggs in stage >7/female
1	0	0	0	0
3	0	0	0	0
5	5	97.20 (69.56-124.8)	7	112.4 (97.37-127.5)
8	8	116.6 (102.1-131.2)	8	113.0 (93.09-111.9)
17	10	89.10 (70.26-107.9)	10	101.8 (87.51-116.1)

* Ten females were sampled for each observation. ** Figures in parentheses indicate 95% confidence limits. *** Pyriproxyfen was topically treated to virgin females 3 days after emergence.

Table 3 Effect of pyriproxyfen on the reproduction of the German cockroach adult.

Dosage ($\mu\text{g}/\text{insect}$)	Treatment to female*		Treatment to male		Treatment to male/female	
	NP	IR	NP	IR	NP	IR
200	—	—	0.67	97.7	—	—
100	—	—	7.6	73.8	—	—
50	—	—	23.9	17.6	—	—
10	2.5	92.0	—	—	0	100
2	—	—	—	—	11.3	51.9
Control	31.4	0	29.0	0	23.5	0

* NP, No. of progeny/female; IR, % corrected inhibition of reproduction.

Fig. 1 Changes in the amount of eggs, emergence rate and the inhibition of reproduction in the Akagi housefly females contacted to the pyriproxyfen-treated aluminium surface ($100 \mu\text{g}/\text{cm}^2$ as active ingredient), or mated with males contacted to the treated surface.

♀, female contact; ♂, male contact.

Table 4 Reproduction of untreated German cockroach females after mating with males contacted to the pyriproxyfen-treated surface.

Dosage ($\mu\text{g}/\text{m}^2$)	First oviposition*		Second oviposition	
	NP	IR	NP	IR
400	8.3	79.1	16.0	57.3
200	24.9	37.4	28.9	22.8
100	38.2	3.9	39.2	0
Control	39.8	0	37.4	0

* NP, No. of progeny/female; IR, % corrected inhibition of reproduction.

week in untreated females mated with treated males and became substantial in the next 2 weeks. The inhibition of reproduction, which is the product of % inhibition of emergence of progeny and inhibition of egg production, was retained at more than 70% with treated females throughout the observation period. In the case of untreated females mated with treated males, the inhibition of reproduction was lower in the first week and increased to the same level as that of treated females in the next 2 weeks. Overall inhibition of reproduction throughout the observation period was 91.5% for female treatment and 77.1% for male treatment.

The number of progeny/female and the inhibition of reproduction when German cockroach males were exposed to the pyriproxyfen-treated aluminium surface (100–400 $\mu\text{g}/\text{cm}^2$ as active ingredient) are shown in Table 4. Observation was done on first oviposition (up to 6 weeks after emergence) and second oviposition (from 7 to 12 weeks after emergence). Inhibitory effect on reproduction was dose-dependent and higher inhibition was obtained in 400 $\mu\text{g}/\text{m}^2$ treatment. No significant inhibition was obtained in 100 $\mu\text{g}/\text{m}^2$ treatment. Inhibition was still higher for second oviposition, although slight recovery was noted.

DISCUSSION

The inhibition effects of reproduction with JHMs are thought to be categorized into two groups, i.e., 1) the “delayed effect” by treat-

ment to larval or nymphal stages and 2) the effect by treatment to the adult stage. The former effect involves not only physiological aberration but also functional and morphological abnormalities as a result of the inhibition of emergence. The latter, on the other hand, is thought to be the direct effect against the physiological condition of adults.

The results in the present paper show that pyriproxyfen causes not only a decrease in the number of eggs but also a reduction in hatchability of eggs oviposited by treated females. Similarity in the development of eggs in ovaries of pyriproxyfen-treated and untreated housefly females suggests that pyriproxyfen does not cause the reduction of ovarian egg production. Hatakoshi and Hirano (1990) reported similar effects of a JHM (S-71639) against *Spodoptera litura* and they proposed two principal roles of S-71639 on adults, i.e., 1) inhibition of “oviposition stimulating hemolymph factor” to cause decrease in the number of eggs and 2) the direct effect against ovaries. The present results also support the above hypothesis.

The sexual or mechanical transmission of sterility by treated males was also observed, although the dosage required to cause sterility to partners was relatively higher. The results are comparable to observation by Masner *et al.* (1968), who reported that the dosage required to cause sterility in untreated females of *Pyrrhocoris apterus* by sexual contact with treated males was a hundred times higher than that required for treatment of females. Insect spermatogenesis is generally initiated in earlier larval or nymphal stages than oögenesis. For instance, spermatogonium of *Bombyx mori* starts differentiation at second instar larval stage and spermatogenesis is completed before adult emergence. The physiological effect of JHMs on males is, therefore, considered to be minor and sterilizing of females by JHM-treated males is thought to be merely a result by mechanical transmission of JHMs through sexual intercourse.

Several attempts to sterilize adult insects and to control population by insect growth regulators have recently been made. Among them, fewer experiments using JHMs as sterilants were carried out as compared with those using benzoyl urea type chitin synthesis

inhibitor (Wright and Harris, 1976; Wright and Spates, 1976; Weaver and Begley, 1982; Knapp and Herald, 1983; Weaver *et al.*, 1984; Broce and Gonzaga, 1987). The laboratory test using pyriproxyfen as a sterilant of the tsetse fly, *Glossina morsitans morsitans* (Langley *et al.*, 1990) is one of the former cases. The authors suggested that use of vegetable oil was an efficient means of treating adults, because entry of pyriproxyfen into the insect was slower and surface losses were lower with the oil than with an acetone solution. This seemed to be an advantage for transmission of sterility from male to female through sexual intercourse. The dosage required to cause sterility to the housefly and German cockroach by exposure to the pyriproxyfen-treated surface seemed to be relatively higher than that required for the tsetse fly. This is mainly due to the difference in mode of action of pyriproxyfen, that is, it affects to eggs in the former insects but in the latter case it can affect the final instar larvae which is generally known to be most sensitive to JHMs.

Baited traps, or spot treatments of biologically active chemicals, are becoming mandatory rather than widespread treatments because of increasing social requirements for environmentally clean techniques in pest control. The use of pyriproxyfen, which has high chemical stability as well as high biological activity, would lead to a new intelligent way in pest control programs. Further research concerning efficient techniques for attracting insects and the practical ways for administering sufficient chemicals to insect populations will be needed.

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摘 要

昆虫成長制御剤ピリプロキシフェン
のイエバエおよびチャバネゴキブリ成虫
の繁殖に及ぼす影響

昆虫成長制御剤ピリプロキシフェンのイエバエおよ

びチャバネゴキブリ成虫に対する不妊化効果について検討を行った。ピリプロキシフェンの処理により、産卵数および孵化率の低下、あるいは次世代の幼虫数の減少がイエバエ、チャバネゴキブリいずれにおいても観察された。また、雄成虫にピリプロキシフェンを処理することにより、これと交尾した雌成虫に不妊性を伝播することが確認されたが、この場合に必要とされる薬量は、雌成虫に直接処理することによってこれを不妊化する薬量に比べ、約10倍以上高かった。ナタネ油に溶かし込んだピリプロキシフェンをアルミニウム面に処理し、これに雄成虫を接触させた場合、イエバエでは $100 \mu\text{g}/\text{cm}^2$ 処理で10分間、チャバネゴキブリは $400 \mu\text{g}/\text{cm}^2$ 処理で60分間接触させた場合、これと交尾した雌成虫に不妊性を伝播しうることが明らかとなった。