Reviews in Toxicology 2 (1998) 357–394
IOS Press

Pyriproxyfen and other juvenile hormone analogues

Masachika Hirano, Makoto Hatakoshi and Hitoshi Kawada
Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co., Ltd., 2–1, 4-chome, Takatsukasa, Takarazuka, Hyogo, 665 Japan

Yoshiyuki Takimoto
Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1–98, 3-chome, Kasugadenaka, Konohana, Osaka, 554 Japan

Abstract. Pyriproxyfen [4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether] is a new photo-stable insect growth regulator and active against whiteflies, thrips, aphids and scales as agricultural pests and against mosquitoes, flies, cockroaches and fleas as non-agricultural pests. Mode of action and selectivity and metabolism of pyriproxyfen are also discussed.

1. Introduction

Juvenile hormone (JH) is an important hormone regulating numerous insect functions including insect molting, metamorphosis, reproduction, egg development, migration, diapause and other developmental processes. Williams [160] was the first to attempt to extract and identify JH from the abdomens of the moth, *Platysamia cecropia*, but was unsuccessful. Röller [125] using a *Tenebrio molitor* bioassay was successful, and the compound was identified as methyl-(2E,6E)-*cis*-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (JH I). The *P. cecropia* extract contained another juvenizing component, which was later identified by Meyer et al. [90] as methyl-(2E,6E)-*cis*-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate (JH II). Judy et al. [58] identified a third JH active component (JH III) from *Manduca sexta*. JH0 and iso-JH0 (4-methyl JH I) was isolated by Bergot et al. [13] from embryos of *M. sexta*, and Richard et al. [120] isolated JH III bisepoxide (JH B3), the sixth naturally occurring JH so far discovered. The chemical structures of the natural JHs identified until now are shown in Fig. 1.

Interestingly, JH active compounds had been reported before JH was isolated and identified from insects. Schmialek [137] isolated farnesol and farnesal which possessed JH activity from the feces of *Tenebrio molitor*. Wigglesworth [158] recognized that farnesol and related compounds had a similar effect on *Rhodnius* to that of JH extracts. Sláma and Williams [142] found that paper made from Canadian balsam fir, *Abies balsamea*, showed JH like activity against *Pyrrhocoris apterus* and named the compound the paper factor. The chemical structure of the paper factor was identified as the methyl ester of todomaturic acid and was named juvabione [16].

1382-6980/98/$8.00

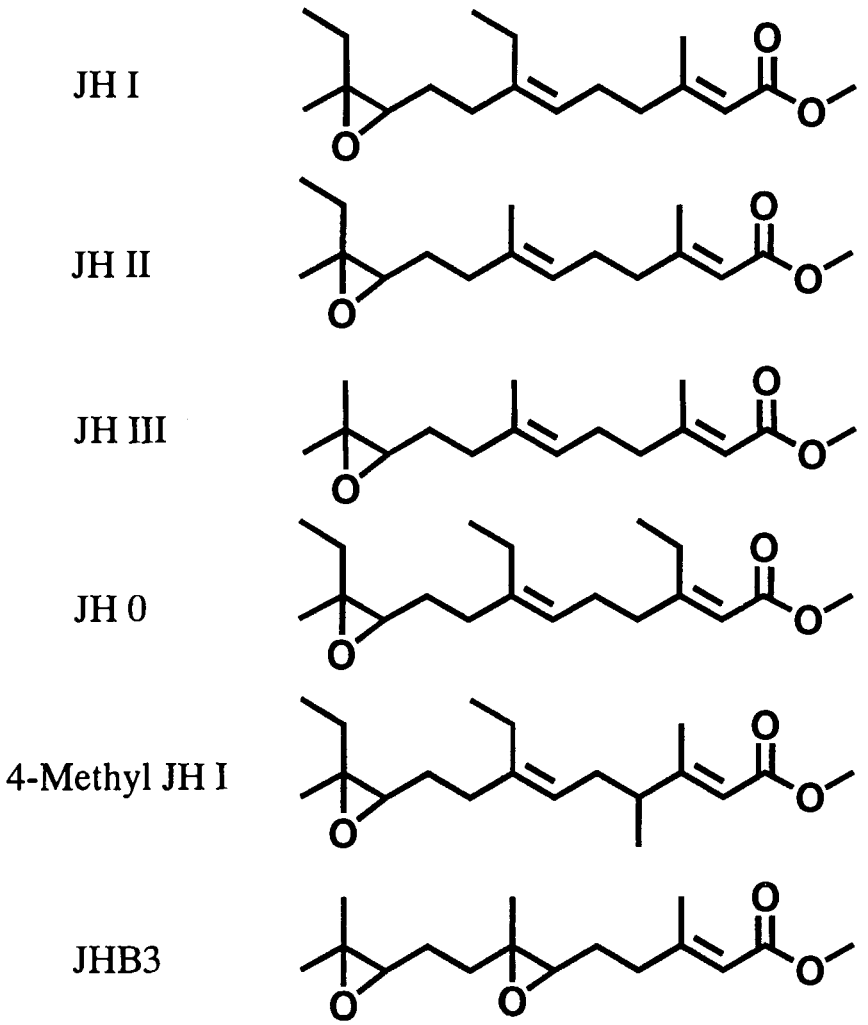

Fig. 1. Chemical structures for the natural juvenile hormones (JHs). See text for discription.

Numerous JH related compounds were synthesized, and the JH activities evaluated. Among the farnesol derivatives, the dihydrochloride of methyl farnesoate was 100,000 times more active than farnesol against 5th instars of *Pyrrhocoris apterus* [84]. Synthetic JH inhibited molting in full grown larvae of *Aedes aegypti* [144]. From these results, Williams [159] suggested that JH active compounds could be used as insecticides. As JH is active against only insects, JH-like compounds would be selective insecticides with low mammalian toxicity. Williams [159] referred to JH as 3rd generation insecticides.

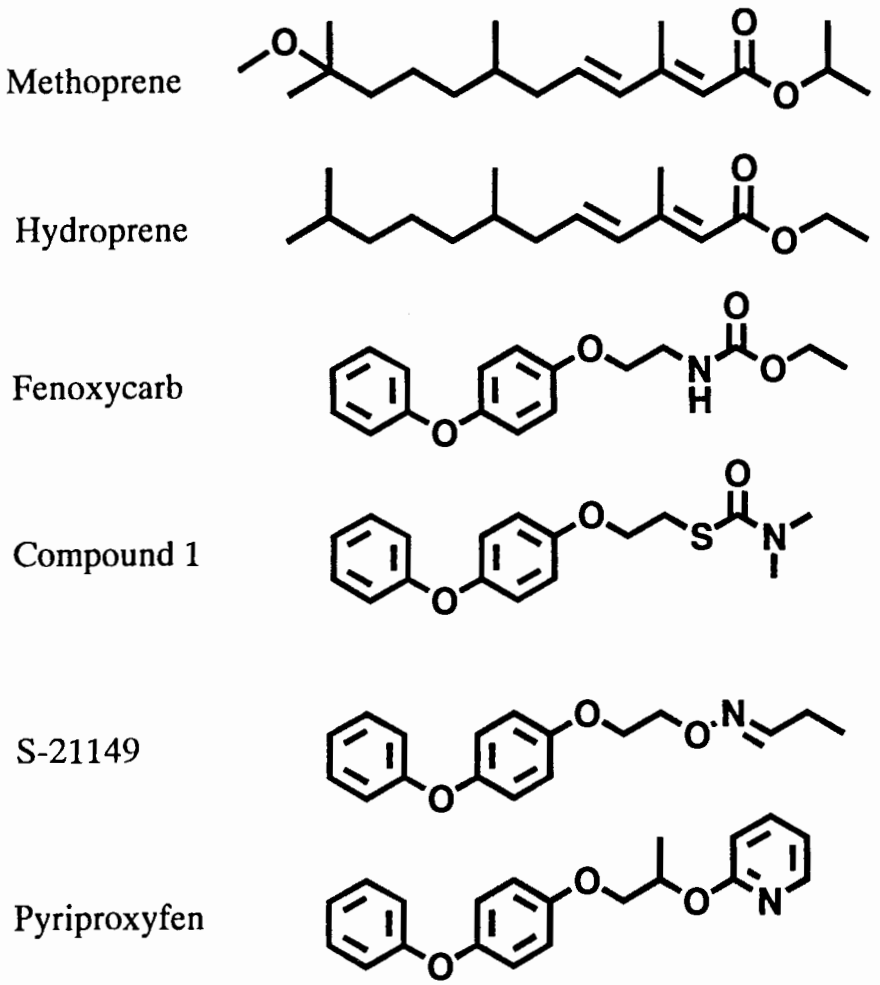

Fig. 2. Chemical structures for some juvenile hormone analogues (JHAs).

After Williams' suggestion, several synthetic JH analogues (JHAs) were reported such as ethyl or methyl 7,11-dichloro-3,7,11-trimethyl-2-dodecanoate [126], ethyl or methyl 11-chloro-3,7,11-trimethyl-2,6-dodecadienoate [56], RO 203600 [18] and R 20458 [110]. It was interesting that some insecticide-synergists such as sesoxame and piperonyl butoxide also possessed JH-like activity [17]. These compounds, however, were never commercialized for insect pest control. The first commercial successes were methoprene and hydroprene [46]. Methoprene is active against dipteran insects and fleas, and hydroprene is active against cockroaches. These compounds, however, were too unstable under field conditions to be used for agri-

culture. Dorn et al. [30] reported on the photostable JH analogue, fenoxycarb. Fenoxycarb was effective not only on household pests but also on agricultural pests such as leafrollers, the Codling moth and *Psylla pyricola*. The chemical structures of these JHAs are shown in Fig. 2.

We also synthesized numerous JHAs and evaluated them for the control of household and agricultural pests, finally discovering pyriproxyfen as one of the most potent JH analogues known to date. In this review, the basis for pyriproxyfen discovery, structural optimization, biological activity against household and agricultural pests, mode of action, resistance and the effect on non-target organisms are discussed.

2. Basis for discovery

During the course of insecticidal screening of newly synthesized compounds, Makoto Hatakoshi found that a compound synthesized as an acaricide, changed the color of treated larvae of the tobacco cutworm, *Spodoptera litura*. The compound did not show acaricidal and insecticidal activities but did induced larval color change. The chemical structure is shown as Compound 1 in Fig. 2. Although Compound 1 is a derivative of an acaricide, fenothiocarb, and the chemical structure is quite different from those of the natural JHs and well known JH analogues, it was assumed that Compound 1 might have JH activity. Numerous analogues of Compound 1 were synthesized and tested for insect growth regulator (IGR) activity, using last instars of *Culex pipiens pallens* and 4-day-old larvae of the housefly, *Musca domestica*. From these investigations, Compound 1 was the most potent. Parallel with these assays, *Galleria* wax moth bioassays for measuring JH activity were conducted. Compound 1 was twice, one-tenth and ten thousand times as active as methoprene against larval mosquitoes, housefly larvae and pupae of the greater wax moth, *Galleria mellonella*, respectively. However, the efficacy of Compound 1 against mosquito larvae in the field was insufficient.

To improve on compound 1, the carbamate moiety was changed to an oxime ether, and many oxime ether derivatives were synthesized and assessed for activity against mosquitoes and houseflies. From these studies, S-21149 (Fig. 2) and S-21150 were found to be highly active. In laboratory studies, S-21149 was

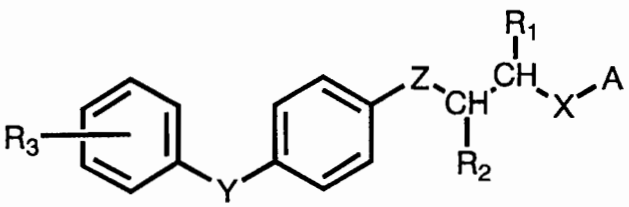

Fig. 3. General formula for pyriproxyfen derivatives.

five thousand, ten and 80 thousand times as active as methoprene against the mosquito, the housefly and the greater wax moth, respectively. S-21149, however, was only ten times more active than methoprene when both were sprayed in the field for mosquito control. This seemed to be due to instability of the oxime portion of the molecule in polluted water. Our chemist synthesized new compounds containing ring structures in the oxime ether moiety. We found that these compounds also possessed high activities against mosquito and the housefly and were stable in water. Pyriproxyfen (Fig. 2) was selected among these compounds.

3. Optimization/Structure activity

Optimization of the insecticidal activity of the pyriproxyfen derivatives (Fig. 3) was conducted using mosquito and housefly larvae [72]. When A in Fig. 3 is a benzene ring, the IC_{50} for mosquito larvae is 0.075 ppm while the IC_{50} for the housefly was > 3 ppm. By changing the benzene ring to a pyridine or pyrimidine ring, the activity increased. Among the pyridine compounds examined, the 2-pyridyl ring gave the highest activity. The activity of the 2-pyrimidyl analogue was about one-tenth for mosquitoes and equal for houseflies to the insecticidal activity of the 2-pyridyl compounds. The 2-pyridyl ring was selected as the most suitable substituent. Oxygen in X, Y and Z gave the highest activity against both insect species. For R1 and R2, methyl and hydrogen gave high activity. Furthermore, the introduction of fluorine to the 3- or 3,5-positions of R3 enhanced activity.

4. Biological activity against agricultural pests

In 1995, pyriproxyfen was registered as an agricultural insecticide in Japan. Target crops and insects are shown in Table 1. The main pests that pyriproxyfen controls are whiteflies, thrips, aphids and scales. Pyriproxyfen against these pests exhibits ovicidal activity, inhibits metamorphosis and produces sterility.

4.1. Whiteflies

The activity of pyriproxyfen against the whitefly was first reported in 1988 [7]. When a pyriproxyfen solution was sprayed onto cotton plants at concentrations as low as 1 ppm and adult tobacco whiteflies, *Bemisia tabaci*, allowed to oviposit on these plants, egg mortality was 100 %. The activity against larvae, pupae and adults was also examined. Some activity was noted against 1st instars but not against 2nd instars and pupae. Pyriproxyfen was non-toxic to adults.

The activity against the sweetpotato whitefly, *B. tabaci*, was also examined [51]. When 0–1 day old eggs were dipped into a solution of 2.5 ppm pyriproxyfen, hatchability was 0 %. Egg hatch increased when 2–3 day old eggs were treated.

Table 1
Target pests and recommended application dosage of pyriproxyfen in Japan

Crop	Pests	Rate (ppm)
Cucumber	*Trialeurodes vaporariorum*	100–200
	Thrips palmi	100–200
Eggplant	*Bemisia tabaci*	200
	Thrips palmi	100–200

Older eggs are less sensitive to pyriproxyfen. No lethality against larvae was observed. Adult emergence was inhibited completely when 2nd instars were treated with only a 0.04 ppm solution. But 5 ppm was necessary to inhibit adult emergence completely when 3rd instars were used. Similar stage specific activity of pyriproxyfen against the greenhouse whitefly, *Trialeurodes vaporariorum*, was reported [166]. Ishaaya et al. [52] reported that younger larvae of *T. vaporariorum* were less sensitive. At a concentration of 0.04 ppm, pyriproxyfen inhibited 60 % and 100 % adult emergence when 1st and 3rd instars were used, respectively. Although discrepancies in activity against the nymphal stage of *T. vaporariorum* and *B. tabaci* were observed, the activity against eggs was greater than that against larvae in both species.

When 100 ppm pyriproxyfen was sprayed on eggplant infested with the greenhouse whitefly, the adult population decreased gradually [166]. Furthermore, when 25 ppm pyriproxyfen was sprayed twice at one week interval on tomato plants infested with the greenhouse whitefly in greenhouses, the adult population showed no significant reduction after 7 days, but decreased after 14 days and continued at a lower level for at least one month. These results show that pyriproxyfen is slow acting as a result of its ovicidal activity and inhibitory effect on metamorphosis. To improve speed of action, formulations were made with fenpropathrin. When a mixture of 6.25 ppm of pyriproxyfen and 25 ppm fenpropathrin was sprayed on eggplant, only one application decreased the adult population in a short period.

Application timing was also studied. Changes in the larval and pupal population of the greenhouse whitefly was observed when 25 ppm of pyriproxyfen was treated 3 times at one week intervals. Also considered was the relationship between the size of the insect population at the time of the first treatment and efficacy of the insecticide treatment. The application just after infestation (almost no larva and pupa/leaf) gave the highest efficacy and low nymphal and pupal populations continued for two months. But the application after infestation (about 50 larvae and pupae/leaf) gave poor control.

Pyriproxyfen shows transovarial activity [52]. The contact of adult greenhouse whitefly to tomato seedlings treated with 25 ppm pyriproxyfen for 24 h decreased the hatchability of oviposited eggs to half that of controls.

4.2. Thrips

Against *Thrips palmi* as well as whiteflies, pyriproxyfen showed no lethality against larvae and prepupae, but showed an inhibitory activity on adult emergence [103]. When 100 ppm pyriproxyfen was sprayed one time on eggplant infested with *T. palmi* in a greenhouse, the population of larvae and pupae decreased with time after treatment. No larvae or pupae were observed after 10 days. No differences in the adult population were observed between treated and untreated insects. Although pyriproxyfen showed only an inhibitory activity on adult emergence for *T. palmi*, high efficacy was obtained by the combination of pyriproxyfen and the natural enemy, *Orius* spp.; pyriproxyfen has no adverse effect on the latter [103].

4.3. Aphids

When one-day-old nymphs of the insecticide sensitive green peach aphid, *Myzus persicae*, were dipped in 50 ppm pyriproxyfen, most of the treated nymphs developed to adults, but none of them oviposited [41]. When nymphs were dipped in 5 ppm, 26.7 % of the adults oviposited, but the number of offspring was one-third that of the untreated control. Longevity of the adults was shortened to 7 days, half that of the untreated control. This sterilizing activity of JH analogues against aphids is well known [11,80].

4.4. Scales

The activity of pyriproxyfen was examined on the arrowhead scale, *Unaspis yanonensis* [41], the tea scale, *Fiorinia theae* [27], the California red scale, *Aonidiella aurantti*, and the Florida wax scale, *Ceroplastes floridensis* [115]. Metamorphosis in scales is different among species. The former 3 species of scales belong to the Family Diaspididae, and the latter species of scale to the Coccidae. Males develop to prepupa, pupa and adults with wings. On the other hand, females lack a pupal stage and have no wings. The effects of pyriproxyfen on scales are different between males and females.

Cooper and Oetting [27] reported that when 60 to 300 ppm of pyriproxyfen was sprayed three times on potted *Camellia japonica* infested with the tea scale, its activity was the same as the activity of bendiocarb (600 ppm) and triflumuron (240–1200 ppm). Pyriproxyfen did not show any lethal activity against female adults even at 300 ppm, but decreased the number of nymphs oviposited. This seemed to be due to reduced fecundity and mortality of the first instars. The effect of pyriproxyfen was the most pronounced on first instars; more than 60 % developmental inhibition was observed at 60 ppm, and no adults were obtained in males and females. Males ceased development before prepupae, and females failed to develop from the 2nd instar to the adult.

First instars of the California red scale were very sensitive to pyriproxyfen. Female crawlers failed to molt to the 2nd instar when they were treated at 25 ppm. Male crawlers molted, but development stopped at the end of the 2nd instar. When early stage 2nd instars were treated with 25 ppm pyriproxyfen, male and female development was completely inhibited. Late 2nd instar females were less sensitive to pyriproxyfen and completed their development even at 200 ppm. Female adults that were treated as late 2nd instars at 100 ppm could not produce a next generation. Male nymphs in the late 2nd instar were sensitive to pyriproxyfen, but some completed their development when the treatment was early in the 2nd instar. Male pupae showed no sensitivity to pyriproxyfen even at 200 ppm. In the case of the Florida wax scale, the first and 2nd instars were sensitive to pyriproxyfen, and their development was completely inhibited at 50 ppm. The proportion of individuals that completed development to the 3rd instar decreased with increasing dosage, i.e., 40.9 % of the nymphs molted at 50 ppm, 26.3 % at 100 ppm and 0.15 % at 200 ppm. When young female adult (4–10 day old) were treated with pyriproxyfen, the percentage of females that oviposited, the number of eggs per female and the hatchability of oviposited eggs decreased depending on dosages. Twenty to 25 day old female adults were insensitive to pyriproxyfen even at 600 ppm since they oviposited eggs and the eggs hatched normally.

When a solution of 100 ppm of pyriproxyfen was sprayed on citrus trees infested with the arrowhead scale before the initiation of their reproductive cycle, high efficacy was obtained by inhibition of the next generation. As mentioned above, pyriproxyfen exhibited its efficacy by inhibiting reproduction and/or development of the 1st instar [41].

5. Biological activity against non-agricultural pests

Pyriproxyfen was also registered and widely used for controlling non-agricultural pests such as household and public hygiene insects.

5.1. Mosquitoes

One of the most important insect orders in respect to human health is the Order Diptera. Most of human tropical diseases, such as malaria, yellow fever, dengue, filariasis, etc., are transmitted by mosquitoes. Larval treatment seems to be the most suitable measure for the control of these diseases since most dipteran pests are noxious in their adult stages and the larval habitats are often limited to small and/or local areas. Most of the effects of JHAs are on the last instar which becomes deformed or dies at the pupal stage as a result of treatment.

Pyriproxyfen causes vacuolation and developmental inhibition of the imaginal buds of *Aedes aegypti* larvae, and histolysis, such as disrupted mitochondria, abundant vacuoles and poorly-structured cytoplasmic organelles were also observed

[148]. Similar histopathological effects on *Aedes aegypti* were reported for methoprene by Cocke et al. [26]. Adult mosquitoes, *Anopheles balabacensis*, which survived 48 h of immersion in 0.005 ppb (one eighth the LC_{50}) of pyriproxyfen during their last stadium, demonstrated considerable reduction in sperm and egg production and also reduced blood feeding and copulation activity [55]. When *Ae. aegypti* larvae were exposed to sublethal dosages of pyriproxyfen, adult emergence was reduced by 48.7 %, the hatching of eggs laid by these survivors was 36.8 % lower than normal, and 39.9 % of the eggs were sterile [86]. Methoprene was reported to reduce pupal glycogen levels in a dose dependent relationship, treated adult females had significantly lower glycogen levels than controls, and the longevity of the female stage was reduced [129]. JHAs act as a sterilant when adult insects are treated. Methoprene was reported to affect ovarian development and adult longevity in *Aedes aegypti* [57,73]. The number of eggs/female and the hatchability decrease with the application of pyriproxyfen to female *Ae. aegypti* [54,65].

There are a large number of laboratory studies on the larvicidal efficacy of pyriproxyfen against mosquitoes (Table 2). The IC_{50} (or LC_{50}) for pyriproxyfen ranged widely from 0.00042 ppb to 0.11 ppb. There was no cross resistance between pyriproxyfen and other insecticides, such as organophosphates, carbamates and pyrethroids [64]. Schaefer et al. [134] also reported a lack of cross resistance; the IC_{50}s of pyriproxyfen for susceptible and organophosphate resistant strains of *Culex quinquefasciatus* were 0.018 and 0.022 ppb and for *C. tarsalis* were 0.021 and 0.052 ppb, respectively. The relative activity of pyriproxyfen seems to be higher for *Anopheles* than *Culex* [64]. Ali et al. [2] evaluated the efficacy of three IGRs (pyriproxyfen, methoprene and diflubenzuron) against *Ae. albopictus* larvae in comparison with other insecticides and concluded that the IGRs showed exceptional activity. They also reported that pyriproxyfen was 2.23 and 21.5 times more active than diflubenzuron and methoprene, respectively. The higher potency of pyriproxyfen than other IGRs was also reported by Mulla [95] and Kawada et al. [64].

Methoprene [9,99,131], fenoxycarb [96,132,157] and pyriproxyfen are some of the most promising JH-based IGRs available as larvicides for mosquito control. Mulla [95] reported that the approximate field rates (lb/acre AI) for pyriproxyfen 0.5G, fenoxycarb 1G and methoprene SR10 were 0.005, 0.1 and 0.1 for *Culex* (*C. stigmatosoma* and *C. tarsalis*) and 0.005, 0.005 and 0.025 for *Aedes* (*Ae. nigromaculis* and *Ae. melanimon*), respectively. Field evaluations of pyriproxyfen as a mosquito control agent are shown in Table 3. A single, low application rate of pyriproxyfen ranging from 0.01 to 0.1 ppm or 0.0011 to 0.1 lb/acre resulted in good control in the field. This is due to the high activity and stability of pyriproxyfen under field conditions. Chemical stability is important especially for JHAs, since they have to be available at specific susceptible stages in the insect's development, i.e., late in the fourth stadium for mosquitoes [63,95].

Controlled release formulations that sustain a minimum level of active ingredient in water sufficient for control is an important factor in the efficacy for JH analogues

Table 2
Laboratory efficacy of pyriproxyfen against mosquito larvae

Species	Result	References
Aedes taeniorhynchus	LC_{50} = 0.010 ppb, LC_{95} = 0.052 ppb	Schaefer et al. (1988)
Ae. aegypti	IC_{50} = 0.0039 ppb	Henrick (1995)
Ae. aegypti	IC_{50} = 0,023 ppb	Hatakoshi et al. (1987)
Ae. aegypti	IC_{50} = 0.056 ppb	Itoh et al. (1994)
Ae. aegypti	IC_{50} = 0.011 ppb	Itoh et al. (1994)
Ae. albopictus	IC_{50} = 0.11 ppb	Ali et al. (1995)
Anopheles albimanus	IC_{50} = 0.016 ppb	Kawada et al. (1993)
An. albimanus (OP-Carbamate resistant)	IC_{50} = 0.00042 ppb	Kawada et al. (1993)
An. balabacensis	LC_{50} = 0.04 ppb	Iwanaga & Kanda (1988)
An. gambiae	IC_{50} = 0.025 ppb	Kawada et al. (1993)
An. gambiae (Diedrin resistant)	IC_{50} = 0.0098 ppb	Kawada et al. (1993)
An. gambiae (DDT resistant)	IC_{50} = 0.0040 ppb	Kawada et al. (1993)
An farauti	IC_{50} = 0.0017 ppb	Kawada et al. (1993)
An. stephensi	IC_{50} = 0.043 ppb	Hatakoshi et al. (1987)
An. stephensi (Malathion resistant)	IC_{50} = 0.025 ppb	Kawada et al. (1987)
Culex pipiens pallens	IC_{50} = 0.0046 ppb	Hatakoshi et al. (1987)
C. pipiens pallens (OP resistant)	IC_{50} = 0.016 ppb	Kawada et al. (1987)
C. pipiens molestus (OP resistant)	IC_{50} = 0.029 ppb	Kawada et al. (1994)
C. quinquefasciatus	LC_{50} = 0.04 ppb, LC_{90} = 0.4 ppb	Mulla et al. (1986)
C. quinquefasciatus	LC_{50} = 0.018 ppb, LC_{95} = 0.16 ppb	Schaefer et al. (1988)
C. quinquefasciatus (OP resistant)	LC_{50} = 0.022 ppb, LC_{95} = 0.42 ppb	Schaefer et al. (1988)
C. tarsalis	LC_{50} = 0.021 ppb, LC_{95} = 0.25 ppb	Schaefer et al. (1988)
C. tarsalis (OP resistant)	LC_{50} = 0.052 ppb, LC_{95} = 0.65 ppb	Schaefer et al. (1988)

as insecticides. With methoprene, both microencapsulated (SR10) and charcoal-based (10F) formulations had more residual activity than an EC formulation [130]. Granular formulation of pyriproxyfen (0.5G) showed the most stable activity in the field among several formulations studied [63,97]. Granular formulations and other types of slow release formulations, such as a resin strip, are applicable to running water [71], temporary water pools [105] and water jars [53], because the active ingredient remains in the formulation and is gradually released according to time and/or the removal of water. Okazawa et al. [105] reported that a 0.1 ppm treatment of a granular formulation of pyriproxyfen had continued activity after dry conditions for 50 days against *An. punctulatus* larvae, which are found mainly in unshaded temporary ground water accumulations in the mountainous regions of the Solomon Islands. Pyriproxyfen, incorporated into a synthetic polymer as a slow release formulation, demonstrated prolong activity against *Ae. aegypti* larvae even though the water in jars were partially used and replenished [53].

Table 3
Field studies of pyriproxyfen against mosquitoes

Species	Formulation	Dosage (as AI)	Results (Residual efficacy)	References
Aedes melanimon	0.5 % Granule	0.005–0.01 lb/acre	80–81 % inhibition of emergence at 4 d	Mulla et al. (1986)
Ae. nigromaculis	0.5 % Granule	0.0025–0.005 lb/acre	66–79 % inhibition of emergence	Mulla et al. (1986)
Ae. nigromaculis *Ae. melanimon*	10 % EC	0.0011–0.0056 kg/ha	73–100 % mortality at 48 h	Schaefer et al. (1988)
Ae. aegypti	0.5 % Granaule	0.025–0.05 ppm	82–100 % mortality	Adames & Rovira (1993)
Anopheles minimus *An. maculatus*	0.5 % Granule	1 ppm for 24 hr running water	> 4 wk control	Kerdpibule (1989)
An. farauti	1 % EC	0.1 ppm	> 3 mo control	Suzuki et al. (1989)
An. punctulatus	0.5 % Granule	0.01–0.1 ppm	> 5 mo control at 0.1 ppm, > 3 mo control at 0.02–0.05 ppm	Okazawa et al. (1991)
An. albimanus	0.5 % Granule	0.025–0.05 ppm	82–100 % mortality	Adames & Rovira (1993)
An subpictus *An. nigerrimus*	10 % EC	0.1 ppm	> 71 d control	Hemingway et al. (1988)
Culex tarsalis	0,5 % Granule	0.005–0.025 lb/acre	85–100 % inhibitions of emergence at 7 d	Mulla et al. (1986)
C. pipiens pallens	0,5 % Granule	0,05–0.1 ppm	5–6 wk control	Kamimura & Arakawa (1991)
C. pipiens pallens	0.5 % Tablet	0.03–0.1 ppm	100 % control for 10 d	Ishii et al. (1990)
C. pipiens molestus	0.5 % Water soluble granule	0.01 ppm	> 1 mo control	Kawada et al. (1994)
C. tritaeniorhynchus	0.5 % Granule	0.01 ppm	> 3 wk control	Kamimura & Arakawa (1991)
C. tritaeniorhynchus	0.5 % Granule	–	> 90 % reduction of population	Thongrungiat & Kanda (1991)
C. peus	0.5 % Granule	0.025–0.05 lb/acre	63–66 % inhibition of emergence at 2 d	Mulla & Darwazeh (1988)
Culex spp.	0.5 % Granule	0.1 kg AI/ha	28–68 d control	Mulligan & Schaefer (1990)
C. quinquefasciatus	10 % EC	0.1 ppm	4 wk control at rainy season & 11 wk control at dry season	Chavasse et al. (1995)
C. quinquefasciatus	0.5 % Granule	0.025–0.05 ppm	82–100 % mortality	Adames & Rovira (1993)

Wettable powder (WP), emulsifiable concentrate (EC) and other types of liquid formulations are applicable to mosquito breeding places that are too broad for operators to treat the whole area or that are inaccessible to humans, such as sewage storage areas [25,44,66,147]. Water soluble granules [66] or fizzy tablets [49,104] make treatment easier in such circumstances than conventional liquid-base treatments noted above. Water quality and other habitat parameters, such as water depth, vegetation, flow velocity, etc., also influence the field efficacy of IGRs. Polluted water requires several to more than 20 times the dosage for effective mosquito control as does clean water [95].

The attempt to use adult mosquitoes as a vehicle for the distribution of pyriproxyfen to larval habitats has also been considered [53,54,65]. When a blood-fed female of *Ae. aegypti* comes in contact with pyriproxyfen, some of the insecticide can be transferred from her body to the water adjacent to the site of oviposition [54,65]. Significant inhibition of emergence was observed in field trials in Bangkok, where the inside of resting traps were treated with an oil formulation of pyriproxyfen in a closed room [53]. A high margin of safety to fish, birds, mammals and most aquatic nontarget organisms [93,97,134] and presumably low selection pressure for resistance development [135] are some important features associated with pyriproxyfen as a mosquito control agent.

5.2. Flies and other dipteran pests

Synanthropic and symbovine muscid flies have been a potential menace to humans and animal health. The Stomoxyinae including the stable fly, *Stomoxys calcitrans*, and the horn fly, *Haematobia irritans*, are hematophagous muscids. They reduce milk yield, weight gain and feeding efficiency in stabled cattle and become the intermediate host of cattle parasites. The movement of the housefly, *Musca domestica*, between feces and food makes them ideal transmitters of human disease. Biting midges, sandflies, blackflies, horseflies, deer flies, tsetse flies and blowflies are also important pests. The emergence of chironomid midges in large numbers causes a variety of nuisance and economic problems [2]. Recently they have gained attention as an allergen and have been considered as one of the most important sources of human allergic diseases [87].

Topical application of pyriproxyfen to the housefly caused 50 % inhibition of emergence at 0.00033 μg/prepupa ([47] Table 4) and at 0.000732 μg/late 3rd instar [67]. Inhibition of emergence by topical application was exceptionally higher with pyriproxyfen than methoprene (> 160 times), permethrin (> 160 times) and fenitrothion (> 450 times) [67]. Pyriproxyfen was more effective against the housefly by short time dipping than methoprene [67]. IC_{50} values of pyriproxyfen seemed to be relatively higher for wild-type colonies of the housefly than for laboratory insects [67]. Bull and Meola [21] reported that cuticular penetration of topically applied pyriproxyfen was reduced and metabolic degradation enhanced in a wild strain (organophosphate resistant) of the housefly, which showed 32-fold tolerance to pyriproxy-

Table 4
Laboratory efficacy of pyriproxyfen against flies and other dipteran larvae

Species	Type of application	Results	References
Musca domestica	Topical application	ID_{50} = 0.00033 µg/prepupa	Henrick (1995)
M. domestica (WHO strain)	Mixing to rearing medium	IC_{50} = 0.0091 µg/g medium	Hatakoshi et al (1987)
M. domestica (CSMA strain)	Mixing to rearing medium	IC_{50} = 0.0031 µg/g medium	Hatakoshi et al (1987)
M. domestica (WHO strain)	Mixing to chicken medium	IC_{50} = 0.0030 µg/g medium	Hatakoshi et al (1987)
M. domestica (Field colony strain)	Mixing to rearing medium	IC_{50} = 0.053–0.36 µg/g medium	Kawada et al. (1987)
M. domestica (CSMA strain)	Immersion for 24 hr	IC_{50} = 0.016 ppm	Kawada et al. (1987)
M. domestica (CSMA strain)	Topical application	ID_{50} = 0.000732 µg/larvae	Kawada et al. (1987)
Haematobia irritans	Mixing to rearing medium	LC_{50} = 9.3 ppb	Bull & Meola (1993)
Stomoxys calcitrans	Mixing to rearing medium	IC_{50} = 12.8 ppb	Bull & Meola (1984)
Stomoxys calcitrans	Immersion	IC_{50} = 2.6 ppb	Bull & Meola (1984)
Sarcophaga stercoaria	Mixing to dung	IC_{50} = 0.0113 µg/g	Amano (1992)
Chironomus fusciceps	Water treatment	EI_{50} = 0.00177 ppm EI_{90} = 0.05369 ppm	Takagi et al. (1995)
Polypedilum nubifer	Water treatment	EI_{90} = 0.01 ppm	Trayler et al. (1994)

fen as compared with the laboratory standard strain. They also suggested microsomal monooxygenase had a major role in this metabolic transformation. The susceptibilities of the hematophagous flies, *Stomoxys calcitrans* and *Haematobia irritans*, and the dung fly, *Sarcophaga stercoraria*, to pyriproxyfen were in the same range as that for houseflies [3,20,22].

The number of field trials for pyriproxyfen against flies is relatively fewer than that against mosquitoes (Table 5). Kamimura [60] reported that almost complete inhibition of housefly (OP resistant) emergence was maintained for more than 3 weeks after treatment with 0.5 % granules of pyriproxyfen at the rate of 50 mg/m^2 of active ingredient. Furthermore, no adverse effect to the parasitic wasp was suggested. The absence of adverse effects to parasitic wasps was demonstrated by Shono et al. [139], who observed little influence on the emergence of two parasitic wasps, *Spalangia endius* and *Nasonia vitripennis*, with treatments of pyriproxyfen at the dose of 6.4 μg/g medium; this treatment caused 100 % inhibition of emergence of the houseflies. Kawada et al. [67] reported that more than 90 % control of a wild colony of the housefly (OP and pyrethroid resistant) was achieved by the treatment of several formulation of pyriproxyfen at the rate of 100 mg/m^2 of active ingredient under field conditions, while 10 times more diflubenzuron (25WP) and greater than 8-fold more methoprene (10F) were required for control at the same effect level.

The larva of the tsetse fly grows within the adult female body until it is ready to pupate. The full grown larva is deposited and immediately burrows into the soil and pupates. This larviposition makes larval control of the tsetse fly by conventional measures impossible. Chemosterilants with lower toxicity and higher stability have been required for the effective control of the tsetse fly. Langley et al. [82,83] found that pyriproxyfen treatments were sufficient to prevent emergence of any offspring for the life of the tsetse fly, *Glossina morsitans morsitans*. The possibility of using pyriproxyfen as a safe chemosterilant with high stability was demonstrated by Langley et al. [81]. They also found that doses as low as 0.01 μg in 10 μl vegetable oil/cm^2 caused females to produce non-viable offspring for at least two reproductive cycles following tarsal contact for 1 min, and a dose 10 times this amount was necessary for an exposed male to disrupt the reproductive potential of his mate [83]. The emergence rate of naturally occurring pupae in an area where pyriproxyfen treated bait traps were located fell by 30 % for *G. morsitans* [37]. Application of pyriproxyfen to housefly adults reduced the hatchability of eggs and the number of progeny [68]. They also reported male houseflies treated with a rather higher dose of pyriproxyfen were able to transfer it to females and caused a 70 % reduction in the number of progeny. The development of highly effective trapping devices will result in an important use for pyriproxyfen as a novel and effective chemosterilant for flies.

Methoprene has long been used for the control of chironomid midges [100,149,150]. A more recent evaluation of methoprene in different formulations against chironomids in ponds showed the superiority of sustained release formula-

Table 5
Field studies of pyriproxyfen against flies and other dipteran larvae

Species	Formulation	Dosage (as AI)	Result	References
Musca domestica	0.5 % Tablet	50–100 mg/m²	> 2 mo control	Kamei et al. (1990)
M. domestica	Feed-through	1 ppm/food (hens) 5 ppm/food (pigs)	> 80 % inhibition of emergence	Miller (1989)
M. domestica	0.5 % Granule 5 % WP 5 % EC	100–200 mg/m²	> 90 % control	Kawada et al. (1987)
M. domestica	0.5 % Granule	50–100 mg/m²	3–4 wk control (50 mg/m²) > 2 mo control (100 mg/m²)	Kamimura (1991)
M. domestica *M. autumnalis*	Feed-through	0.004–0.1 mg/kg/day (cattle)	23.6–100 % inhibition of emergence	Miller (1989)
Polypedilum parvum *Apedilum elachistus* *Tnanytarsini sp.*	3 % Granule 10 % EC	0.05–0.2 kg/ha	1 wk control at 0.05 kg/ha 2 wk control at 0.2 kg/ha (EC) 5 wk control at 0.2 kg/ha (Granule)	Ali et al. (1993)
Polypedilum nubifer *Kiefferulus intertinctus*	0.5 % Granule	0.01 ppm	> 24 d control	Trayler et al. (1994)
Chironomus yoshimatsui *Glyptotendipes pallens*	0.5 % Granule	0.05 ppm to the amount of running water/hr	7–10 days control	Morikawa et al. (1990)

tions, such as granules, briquets and pellets over liquid formulation. The briquet formulation of methoprene at 0.82 kg AI/ha suppressed emergence of midges by 38–98 % for 7 weeks, and the pellet formulation at 0.22 kg AI/ha suppressed emergence by 64–98 % for 7 weeks [6]. Pyriproxyfen was far superior in activity to methoprene against chironomids in the same ponds with 81–100 % suppression of adult emergence for 9 weeks post-treatment by granules applied at a rate as low as 0.05 kg AI/ha [4]. They also reported higher residual efficacy with a granular formulation of pyriproxyfen than with emulsifiable concentrate against several chironomid species. Trayler et al. [154] conducted a field trial of pyriproxyfen at 0.01 ppm against chironomid midges. Pyriproxyfen significantly reduced the emergence of *Polypedilum nubifer* and *Kiefferulus intertinctus* for more than 24 days. They also reported that larval abundances of these species were not affected by pyriproxyfen treatment. This was highly desirable since these larvae are used as food for other organisms in the wetland environment, and in this respect, pyriproxyfen is more preferable than the action of organophosphate larvicides [5]. Morikawa et al. [94] reported that 0.05 ppm of a 0.5 % granule formulation to the total volume of running water/hr resulted in 7 to 10 days control of *Chironomus yoshimatsui* and *Glyptotendipes pallens.*

5.3. Cockroaches

Cockroaches are one of the most important household pests. Nymphs and adults are noxious, and they sometimes live in places not readily accessible to humans, which makes them extremely difficult to control with conventional insecticides. Insecticide resistance in the German cockroach, *Blattella germanica*, has been a persistent problem for 40 years. Insecticide resistance has been reported for 30 insecticides in German cockroach populations from 20 countries [136]. Recently, behavioral resistance associated with a food component in bait formulations of hydramethylnon was reported [141]. New chemicals with new modes of action and new approaches to cockroach control are constantly being sought [114]. One of the most promising alternatives to conventional insecticides is the use of insect growth regulators. Das and Gupta [28,29] first reported that cockroaches treated with JHAs as nymphs emerged as adultoids. Dark coloration, twisted wings or wing abnormality are outstanding characteristics of cockroaches affected by JHAs. The most important effect is that treated individuals fail to reproduce, resulting in prolonged suppression of population growth.

Most studies on the use of JH analogues for cockroach control targeted the German cockroach and the Oriental cockroach, *Blatta orientalis* [33,40]. Hydroprene was first reported by Henrick et al. [46]. Kramer et al. [78] found that male German cockroaches treated as nymphs with hydroprene were unable to copulate because of a malformed left phallomere and incomplete differentiation of the phallic groove. Field populations of German cockroaches were controlled in ca. 7–8 months with applications of hydroprene [12,113]. Hydroprene was reported to have a volatile effect on cockroaches, while fenoxycarb did not [8].

One of the earliest reports on the effects of pyriproxyfen on cockroaches was a laboratory study by Kawada et al. [69]. Topical application of pyriproxyfen to last stadium females induced higher inhibition of emergence than by hydroprene, and a high correlation was observed between the degree of inhibition of female emergence and the inhibition of reproduction. They also reported that population growth of the German cockroach, consisting of various nymphal stages and adults, was suppressed and that insect populations were minimized in less than a year when harborage sites were treated with pyriproxyfen at the rate of 3.8 mg/m^2. Higher inhibitory activity of reproduction by pyriproxyfen than other JHAs was also reported by Reid et al. [118]. They reported that mortality due to ecdysial failure in response to topical treatment of late 5th stadium nymphs was outstandingly lower for pyriproxyfen than fenoxycarb, which may suggest differences in their modes of action. Ross and Cochran [128] evaluated the relative effects of single dietary treatments and combinations of three insect growth regulators, pyriproxyfen, fenoxycarb and diflubenzuron, against older German cockroach nymphs. With pyriproxyfen and fenoxycarb, the effects on reproduction appeared at 3 ppm and female sterility was complete after exposure to 100 ppm, while diflubenzuron at 100 ppm had no effect. No eggs hatched after males and females were exposed by tarsal contact to low concentrations of pyriproxyfen (2 ng/cm^2) and fenoxycarb (6 ng/cm^2) and then allowed to mate [127]. Kawada et al. [68] reported that topical treatment of pyriproxyfen to virgin female adult German cockroaches at the dose of 10 µg/insect resulted in 92 % inhibition of reproduction when she mated with normal, untreated males, while a 10-fold increase in dose was needed for the inhibition of reproduction when a virgin male was treated and mated with a normal female. Pyriproxyfen and acephate were used in a German cockroach management program [75]. Monthly application of pyriproxyfen resulted in 74.6–78.5 % of the adults having twisted wings. Compared to apartments treated with acephate, the German cockroach populations in apartments treated with pyriproxyfen were significantly reduced in 12 to 18 months after the initial treatment.

Population suppression with JHAs takes time when its the sole active ingredient. This delayed effect in cockroach control can be reinforced by the addition of a rapid acting adulticide. Since the insecticidal activity of JHAs is lower in earlier instars, it is necessary that the insecticide be formulated so that the active ingredient is present during the reproductive period of the cockroach. More advanced formulation design and the development of a delivery system that can maintain exogenous JH analogues at a high level within the cockroach body, are required to maximize the utility of pyriproxyfen and other JHAs [63].

5.4. Fleas

Fleas occur on a wide range of terrestrial mammals. The main fleas of medical importance are the tropical rat flea, *Xenopsylla cheopis*, the main vector of plague and murine typhus to humans. The human flea, *Pulex irritans*, and the sand flea,

Tunga penetrans, are also important as human parasites. Fleas of veterinary importance include the pests of poultry, *Echidnophaga gallinacea* and *Ceratophyllus gallinae* and the cat flea, *Ctenocephalides felis*. The cat flea, *Ct. felis*, is a major pest of humans and companion animals because they are more synanthropic and have a wider host range than other flea species. The larvae are found in carpets and other areas of the house and high populations of larvae are also found in the yards surrounding homes, which provide a constant source of flea infestation [112]. The larva appears to be the best life stage to target for control because they are often distributed in the pets bedding area [108].

JH analogues are highly effective as growth inhibitors for flea control. Chamberlain and Becker [23] and Olsen et al. [106] reported that methoprene at an extremely low concentration inhibited the emergence of the oriental rat flea, *X. cheopis*, and the cat flea, *Ct. felis*. The higher inhibitory activity of pyriproxyfen as compared with methoprene or fenoxycarb was reported by Senbo et al. [138] and Kobayashi et al. [74]. Hinkle et al. [48] reported that the survival of cat flea larvae exposed to commercial, total release aerosol formulations of methoprene (a mixture of 0.075 % S-methoprene, 1.0 % propoxur, 0.47 % dichlorvos) became insignificant when compared with controls by the 3rd month while 0.025 % pyriproxyfen showed a significant reduction in survival by the 7th month. Kawada and Hirano [70] evaluated residual effectiveness of pyriproxyfen and methoprene as a spray formulation against cat flea larvae under simulated household conditions. Pyriproxyfen provided control of larvae for more than 12 months when applied at 1 mg and 0.2 mg/m^2, and more than 3 months at the rate of 0.04 mg/m^2. Methoprene applied at 1 mg/m^2 provided control for more than 12 months and 6 months at 0.2 mg/m^2. No significant mortality was observed when methoprene was applied at 0.04 mg/m^2. The relative activity ratio of pyriproxyfen to methoprene was approximately more than 5 times. These results demonstrate the higher persistence of pyriproxyfen on carpet under household conditions than methoprene, although the relative activity of pyriproxyfen had been reported to be less than twice the activity of methoprene [74]. High stability of pyriproxyfen in the carpet matrix or in house dust is more likely to be the reason for the long-term residual effectiveness in carpet treatments. Carpets seem to be one of the more difficult substrates to be treated effectively with an insecticide because of their increased surface area [48]. Osbrink et al. [108] reported the lack of residual effectiveness of pressurized aerosols containing pyrethrins and tetramethrin against cat flea larvae. They also found that the combination of IGRs with chemicals noted above reinforced their residual effectiveness. High stability of chemicals is essential for treatment in yards around homes. An emulsifiable concentrate formulation of pyriproxyfen at a dose of 32 mg/m^2 in 0.82 liter of water/m^2 prevented development in 80 % of the fleas for a period of 3 weeks in field conditions [112]. The use of IGRs with high stability and high inhibitory activity, such as pyriproxyfen, would minimize the total amount of chemicals used in houses and yards and reduce the operational cost in the household flea control program.

Ovicidal activity and sterilization with IGRs have been expected as one of the most efficient control strategies for flea control. Olsen [107] found that methoprene applied to flea eggs prevented larval hatching. Kobayashi et al. [74] reported that inhibition of egg hatch in.the cat flea was more than 4 times higher with pyriproxyfen than with methoprene. Eggs laid by pyriproxyfen-treated fleas within 70 h after exposure were often devoid of yolk and frequently collapsed after oviposition. Minimal amounts of yolk were deposited in eggs laid after 70 h and no blastoderm was formed with treatment of pyriproxyfen, while eggs laid by methoprene-treated fleas showed no gross morphological effects [111]. Treating the fur of cats with methoprene at 2–10 mg/kg body weight prevented the eggs from developing into normal adult fleas for at least 43 days [107]. Treating the fur of cats with pyriproxyfen prevented 50 % of the eggs from developing into normal adult fleas for at least 1 month at 4 mg/cat and for more than 2 months at 12 mg/cat [74]. Furthermore, Meola et al. [88] found that adult cat fleas exposed continuously to pyriproxyfen died within 8–10 days. The death appears to be caused by histopathological damage to fat body, Malpighian tubules, midgut epithelium, salivary gland cells and other internal tissues. These laboratory results suggest that continuous exposure of fleas to pyriproxyfen on a host animal could prevent deposition of viable eggs and eventually kill adults, thereby controlling all stages of flea development.

5.5. Other insect pests

Several laboratory and field studies have been conducted to consider the use of pyriproxyfen and other JH analogues for the control of non-agricultural insect pests other than the species noted above. Methoprene and fenoxycarb that were formulated in baits have provided control for the Pharaoh ant, *Monomorium pharaonis* [31,162,163] Vail and Williams [155] reported that Pharaoh ant colonies were effectively controlled following ingestion of pyriproxyfen formulated in peanut butter oil. Pyriproxyfen reduced egg production in the queens, decreased the amount of brood and caused death of pupae about 3 weeks after treatment. Queens eventually died due to a lack of workers. At concentrations of 0.25, 0.5 and 1.0 %, pyriproxyfen was more effective than the commercially available baits which contain methoprene at 0.5 % active ingredient. Pyriproxyfen caused 80–85 % reductions in colony size in laboratory colonies of the red imported fire ant, Solenopsis invicta, within 4 weeks after treatment [10]. The baits containing pyriproxyfen at concentration of 0.5–1.0 % were as effective in field tests as the commercially available baits which contain fenoxycarb. Among the termite species tested with pyriproxyfen, the eastern subterranean termite, *Reticulitermes flavipes*, appears to be an appropriate target for pyriproxyfen [43]. In a no-choice experiment, pyriproxyfen induced presoldier formation more effectively in the eastern subterranean termite, *R. flavipes*, than in the Formosan subterranean termite, *Coptotermes formosanus* [146]. Feeding of *R. flavipes* exposed to wood cubes containing 30 or 150 ppm of pyriproxyfen caused ca. 80 % worker mortality, while 300 ppm of pyriproxyfen did not increase presoldier

formation in *C. formosanus*. Langley et al. [82] reported that pyriproxyfen was 28 times more effective than S-methoprene at inducing supernumerary molts following topical application to 5th instars of *Rhodnius prolixus*, although the practical formulation, such as the trapping device which was invented for control of tsetse fly [37,83] has not been developed. Pyriproxyfen and other JHAs were reported to inhibit metamorphosis of *Tribolium castaneum* [156] and *T. freemani* [76]. Recently, Teel et al. [152] reported that the number of ovipositing, newly engorged females of the lone star tick, *Amblyomma americanum*, was affected by continuous contact to pyriproxyfen on a glass surface at 16 $\mu g/cm^2$, and that complete inhibition of egg hatch occurred when the ticks were in contact with 4 $\mu g/cm^2$ for more than 7 d. The authors concluded that pyriproxyfen might provide a new means of tick control. The stored grain pests, human and animal louse, bed bugs and other hematophagous arthropod pests will be the next target for pyriproxyfen in future investigations.

6. Mode of action

Pyriproxyfen is a JH agonist but its precise mode of action is not clear. This is also true for JH; the molecular basis for the mode of action of juvenile hormone has not yet been clarified. In this section, our current knowledge of JH mode of action is considered, followed by the action mechanism for pyriproxyfen. It should be noted that this is an active research area in several laboratories throughout the world, and our knowledge of this area is rapidly evolving.

6.1. Molecular mode of action of JH

Since most of the available studies on JH binding protein, JH receptors and related research has been conducted mainly on lepidopteran insects, the results obtained for the tobacco hornworm, *Manduca sexta*, are discussed here (also see reviews by Prestwich et al. [117] and Riddiford [121]). JH secreted from corpora allata is a highly lipophilic compound that binds with hemolymph JH binding protein to be dissolve and carried to target organs. By binding with JH binding protein, JH is protected from non-specific absorption to organs and non-specific metabolism by esterase, but is metabolized by JH esterase. JH binding protein isolated from the hemolymph of the tobacco hornworm, *M. sexta*, has a molecular weight of 32,000 [77,89] and does not bind with methoprene or metabolites of JH [35]. JH reaching target organs penetrates the cell wall entering the cytosol and binds with cytosolic JH binding protein. A 38,000 molecular weight protein in the cytosol was identified as JH binding protein by photoaffinity labeling [109]. After reaching the nuclei, JH appears to bind with receptors in the nucleus. Although two 29,000 molecular weight proteins were isolated from the larval epidermis and fat body of *M. sexta* as nuclear JH receptors [109], subsequent detailed studies showed that the specific

Table 6
Effect of pyriproxyfen on the development of *Spodoptera litura*[a]

Dose (μg/larva)	No.	% Pupation	% Eclosion	% Supernumerary larval molt
100	14	0	0	100
30	15	40	0	60
10	14	93	0	7
3	15	93	16	7
1	15	100	20	0
0.3	15	100	72	0

[a] Day 0 last studium larvae were topically applied.

binding was an artifact of contaminating esterases [24]. Another series of studies on the JH receptor have been conducted in *Locusta migratoria* and demonstrated a component with high affinity for JH III, as the fat body JH nuclear receptor [14]. Characterization of this component has not yet been undertaken.

JH has two actions on insects, morphogenetic and reproductive effects. Pyriproxyfen also exhibits these same two actions on the various species of insects that have been tested. In the following section, results on morphogenetic [40] and reproductive action [40] is discussed for the tobacco cutworm, *Spodoptera litura*.

6.1.1. Morphogenetic action

When pyriproxyfen is applied to day 0 last stadium larvae of *S. litura*, various responses were observed depending on the dosage (Table 6). One hundred μg/larva of pyriproxyfen by topical application induced 100 % extra molting to a 7th stadium, while insects treated with one μg/larva pupated normally. However, adult emergence in treated pupae was only 20 %; apparently emergence was inhibited. The larval period of the 6th stadium for insects that molted to a 7th stadium, was about 4 d, irrespective of dosages as was also the case for *M. sexta* [42]. The prothoracicotropic hormone (PTTH) released in these larvae was observed at 52.5 h after molting, head capsule slippage (HCS) 14.5 h later and larval molting to the 7th stadium after an additional 18.5 h (Table 7). PTTH release in the untreated larvae was observed at 80.3 h after molting. The ecdysteroid titer in the larvae destined to a 7th stadium was almost the same as that in the untreated 5th instars [39]. The 6th stadium in the-treated insects (85.5 h) was longer than the 5th stadium in the untreated larvae (64.1 h). This was due to a delay of PTTH release in treated insects (Table. 7). The application of 100 μg pyriproxyfen to day 2, 6th stadium larvae that were neck-ligated on the same day, inhibited pupation completely, although all neck-ligatured larvae without pyriproxyfen treatment and all larvae starved and applied with 100 μg pyriproxyfen pupated. These results indicate that pyriproxyfen inhibits the function of the prothoracic glands in the absence of the brain, but makes

Table 7
Effect of pyriproxyfen on the events during larval development of *Spodoptera litura*

Larva	Time (h) between each event			
	Larval molt	PTTH release	Head cap slippage	Larval molt
5th instar	37.0	12.5	14.6	
6th instar	80.3			
Pyriproxyfen treated 6th instar	52.5	14.5	18.5	

the brain release PTTH to activate the prothoracic glands in the presence of the brain. The extra molting seems to be due to maintenance of the brain in the presence of pyriproxyfen for some period which allows the release of PTTH followed by ecdysteroid release under a high JH (pyriproxyfen) concentration.

The changes in pyriproxyfen in the hemolymph were observed by Hatakoshi and Takahashi (unpublished results). The amount of pyriproxyfen in the hemolymph was calculated in experiments where 100 μg of ^{14}C-pyriproxyfen (0.359 mCi/mg, radiochemical purity was more than 99 %, chemical purity was more than 99 %) and pyriproxyfen (0.125 μg and 99.875 μg, respectively) was applied topically to day 0 last stadium larvae. Just after application, ^{14}C-insecticide equivalent to 8,696 ng pyriproxyfen/ml was present in the hemolymph. After 3 days, the amount of ^{14}C-label decreased to 315 ng pyriproxyfen/ml. These studies were also conducted using cold pyriproxyfen (100 μg) applied by the same method, and the amount of pyriproxyfen in the hemolymph was detected by GC-MS. Pyriproxyfen concentration decreased linearly with time, and its half-life was about 20 h. Approximately 40 and 93 % were metabolized after 24 and 72 h, respectively.

The changes of naturally occurring JH in 5th and 6th stadium larvae were observed by GC-MS (Hatakoshi and Takahashi, unpublished results). JH II was predominant, but small amounts of JH I and JH III were also observed. JH titer decreased to an undetectable level just after molting to the 6th stadium. It was shown that pyriproxyfen acts on the insect brain. Two types of neurosecretory cells in the pars intercerebralis were stained by para aldehyde fuchsin, two pair of big cells (23 μm) and four pairs of small cells (13 μm). The relationship between changes of stainability of these cells and timing of PTTH release was observed. The changes of stainability of the big cells were correlated to the timing of PTTH release in pyriproxyfen treated and untreated 6th instars. These results indicate that pyriproxyfen acts on neurosecretory cells in the brain directly or indirectly.

In another study, the brains from day 0 last stadium larvae were incubated with 10^{-7} M pyriproxyfen for some period in Grace's medium to examine the incorporation of pyriproxyfen. After incubation, the brains were washed with Grace's medium thoroughly, homogenized in buffer and the radioactivity measured. Pyriproxyfen was shown to penetrate into the brain. No metabolite of pyriproxyfen was de-

tected in methanol extracts of tissue homogenates analyzed by TLC for brains incubated with 10^{-7} M pyriproxyfen for one hour.

The above mentioned results were summarized as follows. Topically applied pyriproxyfen penetrates the cuticle promptly, then after reaching the brain, stimulates the brain to secrete PTTH. Pyriproxyfen seems to inhibit the function of the prothoracic glands, but are activated by PTTH to release ecdysteroid. The simultaneous presence of pyriproxyfen (a JH mimic) and ecdysteroid results in the retention of larval proteins produced by the insect cells.

6.1.2. Reproductive action

It is known that the application of JH analogues to adult insects decreases the hatchability of oviposited eggs. When pyriproxyfen was applied to larvae or pupae of *S. litura*, the hatchability of eggs oviposited by these treated insects also decreased [40]. The inhibition of egg hatching by adult treatment may be explained by effects on the development of the ovary in the female pupae. The topical application of pyriproxyfen to larvae or pupae of *S. litura* also causes a reduction in the number of eggs oviposited, depending on dosage (Table 8). The application of 0.3 μg pyriproxyfen to day 0 female pupae decreased the number of eggs about one-third as compared to the untreated control. This effect was recognized when the application was made between day 0 to day 3 of the pupal stage.

The effect of pyriproxyfen on ovarian development was examined and no differences were noted in ovarian weight and egg maturation. The relationship between ovarian development and hormone titer was studied by neck-ligation of female adults within 15 min after emergence. Ovarian development occurred for one day before and after emergence, and JH release after emergence was necessary to develop the ovary normally. JH in *M. sexta* appeared only for 7 h around emergence [145]. As the ovarian development of female adults of *S. litura* that were neck-ligatured within 15 min after emergence was stimulated by pyriproxyfen and methoprene, this means that JH also in *S. litura* presented only for a short period around adult emergence is sufficient for ovarian development.

Table 8
Effect of pyriproxyfen on day 0 female pupae in the eclosion and oviposition by *Spodoptera litura*

Dose (ng/pupa)	No.	% Eclosion	Average number of eggs per female that oviposited[a]
1.0	20	30	73
0.3	20	65	309
0.1	10	80	597
0.03	16	100	902
Untreated	15	100	1036

[a] Female adults were mated with untreated male moths.

It was speculated that the inhibition of oviposition may be due to the lack of some factor in the hemolymph. Ringer's solution and hemolymph from normal mated females (2-3 days old) and males (2-3 days old) were injected into day 0 virgin females which were subsequently allowed to mate with untreated males; the oviposition rate was increased only when hemolymph from the female was injected. On the other hand, hemolymph from normal untreated females (2-3 days old) injected into day 0 female adults that received 0.3 μg pyriproxyfen as day 0 pupae also increased the oviposition rate. These results indicated that the hemolymph from mated females could enhance the oviposition rate, irrespective of the application of pyriproxyfen during the pupal stage. Hemolymph from virgin females also increased the rate to some extent, and it was shown that unmated virgin females just after emergence also contain a factor that affects oviposition rate. Apparently, day 2 mated female moths contain a factor which stimulates oviposition. Changes in the content of this factor in female hemolymph were correlated with the timing of oviposition, i.e., the content until day 1 after emergence was the same as that in the pupal stage and increased at 2 days after emergence. This factor was not prostaglandin E2, F2α, 20-hydroxyecdysone and JH. Hemolymph from day 2 female moths were studied to try to identify this factor. The factor was thought to be a peptide with a molecular weight greater than 14,000. Using gel filtration chromatography, the peptide was partially purified and its molecular size estimated as 21,000-27,000. The presence of factors which correspond to this oviposition stimulating factor is known in other lepidopteran insects, such as the cecropia silkworm, *Hyalophora cecropia* [122], and *G. mellonella* [89].

When pyriproxyfen is applied to oviposited eggs, the hatchability of the treated eggs decreased. When eggs of *S. litura* were dipped in 100 ppm pyriproxyfen, hatchability changed with elapsed time after oviposition and increased after 30 h (Fig. 4). This effect is thought to be due to inhibition of embryo development by pyriproxyfen which penetrates the egg shell. It is well known that JH and JH analogues have ovicidal activity against many species of insects. Slama and Williams [143] first reported that JH showed an ovicidal activity against the European bug, *Pyrrhocoris apterus*, with the sensitivity of eggs to JH being higher in young eggs as compared to older eggs. JH is known to have ovicidal activity when applied to eggs directly. Riddiford and Williams [124] topically applied synthetic JH to eggs of *H. cecropia* and *Antheraea pernyi* and showed that JH had an ovicidal activity that was greater for young eggs. Quantitative and qualitative changes of JH in eggs scarcely had been studied when these studies were conducted. Bergot et al. [13] identified the egg JHs and measured JH titer. Almost no JH was found in eggs just after oviposition, but at 2 to 3 days after oviposition, the amount of JH 0 and JH I reached peak levels. Also in the eggs of the cockroach, *Nauphoeta cinerea* [15], and the African migratory locust, *Locusta migratoria* [116], no JH was found early in embryogenesis. These reports show that JHAs affect embryo development only when the natural JH is absent.

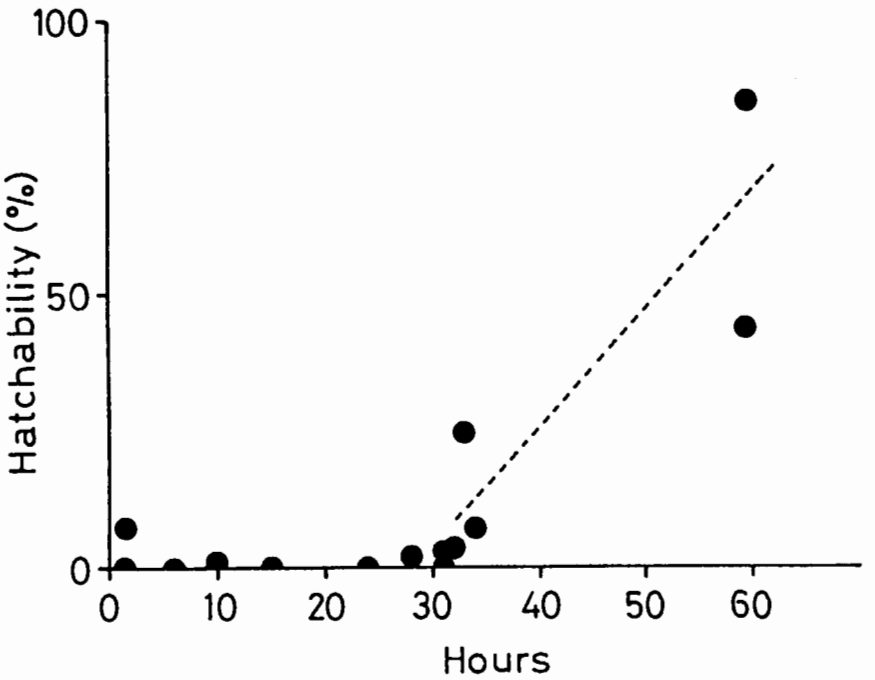

Fig. 4. A plot of percentage hatchability of *Spodoptera litura* larvae as a function of hours after oviposition when eggs were dipped in 100 ppm of a solution of pyriproxyfen for 1 min. Each point represents 30–100 eggs.

7. Resistance

Williams [159] suggested that one advantage of JH analogues over other insect control chemicals was the fact that insects could not develop resistance to their own hormones. On the other hand, it is widely known that the repeated use of a single insecticide on field populations of insects usually results in insecticide resistance. Can insects develop resistance to JH analogues even whenthe same JHA is repeatedly used? The answer is not always no.

Brown and Brown [19] reported that methoprene-selected *Culex pipiens pipiens* became resistant to methoprene and also to the similar compound, hydroprene (resistance ratios of 3.9 and 15.2, respectively) but not to other JHAs like R 20458 (Table 9). Georghiou et al. [34] reported that high levels of resistance (> 1,000 fold) to methoprene have been induced in a dimethoate-resistant laboratory strain as well as in a field multi-resistant strain of *Musca domestica* by continuous selection. In *Drosophila melanogaster*, a JH mutant, Methoprene-tolerant (Met), which is resistant to JH III and methoprene was found and genetically characterized [164,165]. These reports showed that even JHAs could cause resistance by continuous selection.

Table 9
Cross resistance and cross-tolerance spectrum of the methoprene-selected strain to certain insecticides and JH mimics[a]

Compound	EC_{50} ppm			
	Control Stock	Methoprene-selected strain	Generation	Resistance ratio
Methoprene	0.004	0.051	F8	13.0
Hydroprene	0.046	0.700	F8	15.2
R 20458	0.0041	0.0027	F7	0.65
Malathion	0.037	0.067	F8	1.70
Carbaryl	0.66	0.70	F9	1.60

[a] Brown and Brown (1974)

As we mentioned earlier, pyriproxyfen possesses high ovicidal and eclosion inhibiting activity against various species of insect pests and is widely used to control agricultural and household pests. This JHA also possesses high efficacy not only to susceptible strains but also to insecticide-resistant colonies. Only a few reports on pyriproxyfen-resistant insects were found. Riddiford and Ashburner [123] reported that the methoprene-resistant mutants, Met^1 and Met^2, were 10- and 6-fold resistant to pyriproxyfen in the white puparial assay and about 20-fold resistant in larval feeding experiments than the wild-type *D. melanogaster.* They also reported that pyriproxyfen was 23 times more active than methoprene against *D. melanogaster.* The efficacy level of pyriproxyfen against the methoprene-resistant strain is the same as that of methoprene against the susceptible strain.

Against the housefly, Kawada et al. [67] reported that pyriproxyfen showed higher efficacy than methoprene, diflubenzuron and conventional insecticides, but that the IC_{50} values for 4 day old larvae were different among five different housefly strains with varying levels of resistance to organophosphorus or pyrethroid insecticides. Bull and Meola [21] reported that a methoprene-resistant strain (Rmp) demonstrated decreased penetration and enhanced metabolic degradation of pyriproxyfen in comparison with insecticide-susceptible houseflies. These results showed that the susceptibility of housefly colonies selected with conventional insecticides to pyriproxyfen or other JH mimics might be decreased.

Fortunately, there are no reports of resistance to pyriproxyfen by mosquitoes, which are the most important target pest for IGRs. Kawada et al. [64] reported that pyriproxyfen showed no cross resistance among IGRs and other conventional insecticides in the Anopheline mosquitoes, and that IC_{50} values for pyriproxyfen against insecticide-resistant strains of 3 *Anopheles* spp. were lower than to a susceptible strain of the same species. Schaefer and Mulligan [135] selected an OP-resistant strain of *Culex quinquefasciatus* with pyriproxyfen for 17 generations and had no indication of increased tolerance to this IGR (Table 10).

Table 10
Susceptibility of the OP-R strain of Culex quinquefasciatus before and after pressuring with pyriproxyfen (in ppm)[a]

Generation	LC_{50} (ppm)
Parent	0.000022
F5	0,000015
F10	0.000032
F15	0.000015
F17	0.000018

[a] Schaefer and Mulligan (1991)

Horrowitz and Ishaaya [50] monitored insecticide resistance in *Bemisia tabaci* in a rose greenhouse during 1991 and 1992, and they found that pyriproxyfen caused a relatively high level of resistance after three successive applications but no resistance after one treatment. This is the only report of resistance to pyriproxyfen against agricultural pests. This experiment, however, was conducted in a greenhouse using continuous applications. So, the possibility of causing resistance to pyriproxyfen seems to be low. Nevertheless, it is important to alternate between insecticide classes and IGRs with different modes of action to prevent the development of resistance.

8. Selectivity and metabolism of pyriproxyfen in target and nontarget organisms

As discussed previously, pyriproxyfen affects larval development on almost all insect species with the early phase of the last stadium being most sensitive. Furthermore, pyriproxyfen affects ovarian and embryo development as well as diapause and phase variation in some species of insects. The mode of action of the IGRs differ profoundly from conventional insecticides such as the organochlorinated, organophosphorus, carbamate and pyrethroid pesticides, and differences in environmental effects might also be expected for insecticides like pyriproxyfen.

The environmental effects of pyriproxyfen will be briefly discussed from the viewpoint of arthropod selectivity, since vertebrates and invertebrates other than the Arthropoda are less susceptible to IGRs. Further detailed information on effects of IGRs is available from other review articles [36,91,101,119].

Pyriproxyfen was registered in Japan in 1989 for controlling mosquitoes and the housefly, both important to public health. This compound has also been registered and used as an agricultural insecticide. The clearest picture on selectivity has been shown for aquatic organism studies, which are also highly relevant to the usage of pyriproxyfen and to possible concerns about environmental impact. The following discussion concentrates on the aquatic environment.

8.1. Sensitivity of aquatic organisms to pyriproxyfen

Pyriproxyfen is active with an EC_{95} (inhibition of emergence of 95 %) at the sub-ppb levels against 4th instars of the mosquitoes, *Anopheles quadrimaculatus*, *Aedes aegypti*, *Aedes taeniorhynchus*, *Culex tarsalis* and *Culex quinquefaciatus* [55,134]. Nontarget aquatic insect larvae and crustaceans are less sensitive (more than 100 times) than mosquitoes. Different from insect larvae which show molting defects, crustaceans such as *Daphnia pulex* and *D. magna* produce shorter body lengths and fewer numbers of young, but survival and molting rates were not affected even at high exposure levels [93]. Furthermore, the minimal effects that were observed are quickly reversed when *D. pulex* are transferred to fresh water free from pyriproxyfen for 1 week.

8.2. Sensitive stage of nontarget insect larvae

To determine the most susceptible stage, last instars as well as pre-last instars of the dragonfly, *Orthetrum albistrum speciosum*, and non-biting midge, *Chironomus yoshimatsui*, were exposed to pyriproxyfen. Last instars demonstrated a more than 1000 times higher susceptibility than early instars. Furthermore, the initial 8 days for a last stadium that is 40 days in duration, was the most susceptible time period.

8.3. Metabolism in aquatic organisms

To investigate selectivity from the viewpoint of metabolism, insect larvae were exposed to ^{14}C-pyriproxyfen at 10 ppb in flowing water at 25 °C for 48 h (for the dragonfly, *O. albistrum speciosum*, and the mosquito, *Culex pipiens pallens*) or 24 h (for the midge, *Chironomus yoshimatsui*). Pyriproxyfen concentrations in the larvae plateau on day 1 of the exposure, and the maximum bioaccumulation ratios and biological half-lives for ^{14}C and pyriproxyfen are shown in Table 11. These bioaccumulation ratios and half-life values are 1/3 to 1/10 and 1/3 to 1/8 of those in the fish, *Cyprinus carpio*, respectively. In these insect larvae, the major metabolic pathways in common were hydroxylation at the 4'-position of the phenoxyphenyl group and cleavage of the pyridyloxy ether bond.

8.4. Bioavailability from sediment

Although pyriproxyfen applied to ponds disappeared rapidly with half-lives of 1.6 days in the water phase and 10 days in sediment [93], the efficacy of this compound to the mosquito is prolonged for 1-2 months. Since the compound is very active to mosquito larvae, low concentrations of pyriproxyfen adsorbed onto organic matter in sediment might be available to the mosquito larvae by ingested, thus permitting prolong control in the field.

Table 11
Bioaccumulation ratios and half-lives of pyriproxyfen in aquatic insects

Insect larva	Maximum bioaccumulation ratio		Biological half-life (Days)	
	^{14}C	PYR[a]	^{14}C	PYR
Dragonfly	120	40	0.52	0.13
Midge	500	47	0.28	0.07
Mosquito	280	73	0.32	0.13

[a] Pyriproxyfen

To investigate this point, a water/sediment system was treated with ^{14}C-pyriproxyfen and maintained for 1 day under static conditions. Then water was passed through the system for 8-10 days. Thereafter, one group of dragonfly, midge and mosquito larvae were placed free on the sediment and the other group kept in a cage away from the sediment. A 2-day exposure resulted in 3-9 times higher concentrations of pyriproxyfen in midges and mosquitoes on the sediment than those that were caged away from the sediment (Table 12). The calculated bioaccumulation ratios for caged larvae based on the water concentrations are comparable to those exposed in a flow through system.These results demonstrate that uptake of pyriproxyfen adsorbed on the sediment is likely in mosquito and midge larvae which are known detritus feeders, but not for the dragonfly which is a carnivore.

Table 12
Uptake of pyriproxyfen in aquatic insects

Compound	Concentration (μg/kg)							
	Water	Sediment	Dragonfly		Midge		Mosquito	
			Free	Caged	Free	Caged	Free	Caged
^{14}C	2.0	338	58.7	69.0			508	202
PYR[a]	0.2	152	4.2	4.1			148	34.3
^{14}C	2.1	653			753	256		
PYR	0.04	424			40.7	4.4		

[a] Pyriproxyfen

8.5. *Field monitoring*

To clarify the selectivity of aquatic organisms to pyriproxyfen, field monitoring studies were conducted. Pyriproxyfen was applied twice at 50 and 110 g a.i./ha (8 and 20 times greater than the effective dose of 5.6 g/ha) to experimental plots.

Minor suppression of the reproductive capacity of cladocerans and ostracods were observed, and a low degree of induction of morphogenetic aberrations in Odonata at adult emergence was exhibited. Aquatic beetle adults, dragonfly nymphs and lycosid spiders showed no adverse effects from these treatments [133]. Field application rates of 5.6, 11.2 and 28.0 g a.i./ha resulted in no marked ill effects during a 21-days test period on nontarget organisms prevalent in the experimental ponds, i.e., mayfly naiads, *Callibaetis pacificus*, dragonfly naiads, *Tarnetrum corruptom* and *Anex jubius*, several species of diving beetle larvae and adults, Hydrophilidae and Dytiscidae, and two species of ostracods, *Cypridopsis sp.* and *Cyprinotus sp.* [32].

Based on these studies, pyriproxyfen selectivity is dependent on taxonomic division, species differences within the Insecta, the developmental stage of insect larvae, the developmental age within the last stadium and feeding behavior, but is not greatly dependent on metabolism. In addition to these selectivities, the environmental impact is related to the developmental stage of the insect larvae when the application is made. Although vertebrates and invertebrates except for the Arthropoda appear considerably less susceptible to pyriproxyfen due to its mode of action, a wide range of precise information is required with regard to effects on variety of arthropods for exact assessment of its environmental impact.

9. Future directions

From the foregoing discussion, pyriproxyfen shows high reproduction and metamorphosis inhibiting activity against agricultural, household and public hygiene insect pests. As its effect is limited to arthropods, this IGR shows low mammalian toxicity. Pyriproxyfen has been used in many countries, and it was registered in 1996 by the US EPA for controlling cotton whiteflies in addition to non-agricultural use. The contribution of this IGR to agricultural pest control will become more significant in the near future as new US EPA registrations become available. However, more work is needed on new pyriproxyfen formulations and applications methods for agricultural, household and public hygiene pest control. It is especially important to develop application programs to prevent the development of resistance. In order to develop more ideal JH mimics, further studies are needed on the mechanisms controlling JH action and the physiology of JHs in arthropods. Such research will contribute to the development of the next generation of IGRs.

References

[1] E. Adames and J. Rovira, Evaluation of the juvenile growth regulator pyriproxyfen (S-31183) against three species of mosquitoes in Panama, *J. Am. Mosq. Control Assoc.* **9** (1993), 452–453.

[2] A. Ali, J.K. Nayar and R. Xue, Comparative toxicity of selected larvicides and insect growth regulators to a Floridsa laboratory population of *Aedes albopictus*, *J. Am. Mosq. Control Assoc.* **11** (1995), 72–76.

[3] K. Amano, Juvenile hormone activity of pyriproxyfen on the yellow dung fly, *Scatophaga atercoraria*, *Ann. Rept. Plant Prot. North Japan* **43** (1992), 175–176 (in Japanese).
[4] A. Ali, R. Xue and R. Lobinske, Efficacy of two formulations of the insect growth regulator, pyriproxyfen (Nylar or Sumilarv), against nuisance Chironomidae (Diptera) in man-made ponds, *J. Am. Mosq. Control Assoc.* **9** (1993), 302–307.
[5] A. Ali, Perspectives on management of pestiferous Chironomidae (Diptera), an emerging global problem, *J. Am. Mosq. Control Assoc.* **7** (1991a), 260–281.
[6] A. Ali, Activity of new formulations of methoprene against midges (Diptera: Chironomidae) in experimental ponds, *J. Am. Mosq. Control Assoc.* **7** (1991b), 616–620.
[7] K.R.S. Ascher and M. Eliyahu, The ovicidal properties of the juvenile hormone mimic Sumitomo S-31183 (SK-591) to insects, *Phytoparasitica* **16** (1988), 15-21.
[8] T.H. Atkinson, P.G. Koehler and R.S. Patterson, Volatile effects of insect growth regulators against the German cockroach (Dictyoptera: Blattellidae), *J. Med. Entomol.* **29** (1992), 364–367.
[9] R.C. Axtel, D.R. Rutz and T.D. Edwards, Field evaluation of insecticides and insect growth regulators for the control of *Culex quinquefasciatus* in anaerobic animal waste lagoons, *Mosq. News* **40** (1980), 36–42.
[10] W.A. Banks and C.S. Lofgren, Effectiveness of the insect growth regulator pyriproxyfen against the red imported fire ant (Hymenoptera: Formicidae), *J. Entomol. Sci.* **26** (1991), 331–338.
[11] R.J. Bauernfeind and R. K. Chapman, Effect of some insect growth regulators on green peach aphids (Homoptera: Aphididae) under greenhouse conditions, *J. Econ. Entomol.* **77** (1984), 211-215.
[12] G.W. Bennett, J.W. Yonker and E.S. Runstrom, Influence of hydroprene on German cockroach (Dictyoptera: Blattellidae) populations in public housing, *J. Econ. Entomol.* **79** (1986), 1032–1035.
[13] B.J. Bergot, F.C. Baker, D.C. Cerf, G. Jamieson and D.A. Schooley, Qualitative and quantitative aspects of juvenile hormone titers in developing embryos of several insect species: Discovery of a new JH-like substance extracted from eggs of *Manduca sexta*, in: *Juvenile Hormone Biochemistry*, 1981, pp. 33-45.
[14] R.P. Braun, G.C. Edwards, K.J. Yagi, S.S. Tobe and G.R. Wyatt, Juvenile hormone binding components of locust fat body, *Arch. Insect Biochem. Physiol.* **28** (1995), 291–309.
[15] E.A. Bruning, A. Aaxer and B. Lanzrein, Methyl farnesoate and juvenile hormone III in normal and precocene treated embryos of the ovoviviparous cockroach *Nauphoeta cinerea*, *Int. J. Invert. Reprod. Dev.* **8** (1985), 269-278.
[16] W.S. Bowers, H.M. Fales, M.J. Thompson and E.C. Uebel, Juvenile hormone: Identification of an active compound from balsam fir, *Science* **154** (1966), 1020-1021.
[17] W.S. Bowers, Juvenile hormone activity of natural and synthetic synergists, *Science* **161** (1968), 895-897.
[18] W.R. Bransby-Williams, Activity of two juvenile hormone analogues with *Heliothis armigera* (Hubner), Sitophilus zeamais Motshulsky and Ephestia cautella (Walker), *East Afr. Agr. Forest. J.* **38** (1972), 170-174.
[19] T.M. Brown and A.W.A. Brown, Experimental induction of resistance to a juvenile hormone mimic, *J. Econ. Enomol.* **67** (1974), 799-801.
[20] D.L. Bull and R.W. Meola, Effect and fate of the insect growth regulator pyriproxyfen after application to the horn fly (Diptera: Muscidae), *J. Econ. Entomol.* **86** (1993), 1754–1760.
[21] D.L. Bull and R.W. Meola, Efficacy and toxicodynamics of pyriproxyfen after treatment of insecticide-susceptible and -resistant strains of the house fly (Diptera:Muscidae), *J. Econ. Entomol.* **87** (1994a), 1407–1415.
[22] D.L. Bull and R.W. Meola, Interactions of the insect growth regulator pyriproxyfen with immature and adult stages of the stable fly, *Southwest Entomol.* **19** (1994b), 257–263.
[23] W.F. Chamberlain and J.D. Becker, Inhibition of cocoon formation and adult emergence of oriental rat fleas, *Xenopsylla cheopis*, by insect growth regulators, *Southwest Entomol.* **2** (1977), 179–182.

[24] J.-P. Charles, H. Wojtasek, A.J. Lentz, B.A. Thomas, B.C. Bonning, S.R. Palli, A.G. Parker, G. Dorman, B.D. Hammock, G.D. Prestwich and L.M. Riddiford, Purification and reassessment of ligand binding by the recombinant, putative juvenile hormone receptor of the tobacco hornworm, *Manduca sexta*, *Arch. Insect Biochem. Physiol.* **31** (1996), 371–393.

[25] D.C. Chavasse, J.D. Lines, K. Ichimori, A.R. Majara, J.N. Minjas and J. Marijani, Mosquito control in Dar Es Salaam. II. Impact of expanded polystyrene beads and pyriproxyfen treatment of breeding sites on *Culex quinquefasciatus* densities, *Med. Vet. Entomol.* **9** (1995), 147–154.

[26] J. Cocke, A.C. Bridges, R.T. Mayer and J.K. Olson, Morphological effects of the insect growth regulating compounds on *Aedes aegypti* (Diptera: Culicidae) larvae, *Life Sci.* **24** (1979), 817–832.

[27] R.M. Cooper and R. D. Oetting, Effects of the juvenile hormone mimic S-31183 and trifluron on development of tea scale (Homoptera: Diaspididae), *J. Entomol. Sci.* **20** (1985), 429-434.

[28] Y.T. Das and A.P. Gupta, Effects of three juvenile hormone analogs on the female German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), *Experientia* **30** (1974), 1093–1095.

[29] Y.T. Das and A.P. Gupta, Abnormalities in the development and reproduction of *Blattella germanica* (L.) (Dictyoptera: Blattellidae) treated with insect growth regulators with juvenile hormone activity, *Experientia* **33** (1977), 968–970.

[30] S. Dorn, M.L. Frischknecht, V. Martinez, R. Zyrfluh and U.Z. Fisher, Pflanzenkrankh., *Pflanzenschutz* **88** (1981), 269.

[31] J.P. Edwards, The effects of a juvenile hormone analogue on laboratory colonies of pharaos ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae), *Bull. Entomol. Res.* **65** (1975), 75–80.

[32] J.G. Estrada and M.S. Mulla, Evaluation of two new insect growth regulators against mosquitoes in the laboratory, *J. Am. Mosq. Control Assoc.* **2** (1986), 57.

[33] R.G. Evans, A. Sunley, C. Bradford and R.I. Patmore, Effects of fenoxycarb on development and reproduction of the Oriental cockroach, *Blatta orientalis*. *Med. Vet. Entomol.* **9** (1995), 235–240.

[34] G. P. Georghiou, S. Lee and D.H. DeVries, Development of resistance to the juvenoid methoprene in the housefly, *J. Econ. Entomol.* **71** (1978), 544–547.

[35] W.G. Goodman and E. S. Chang, Juvenile hormone cellular and hemolymph binding proteins, in: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, G.A. Kerkut and L.I. Gilbert, eds., 1985, pp. 491–510.

[36] B.D. Hammock and G.B. Quistad, Metabolism and mode of action of juvenile hormone, juvenoids, and other insect growth regulators, in: *Progress in Pesticide Biochemistry*, D.H. Hutson, and T.R. Roberts, eds., John Wiley and Sons, Ltd., New York, Vol. 1, 1981, pp. 2–88.

[37] J.W. Hargrove and P. A. Langley, Sterilizing tsetse in the field: a successful trial, *Bull. Ent. Res.* **80** (1990), 397–403.

[38] M. Hatakoshi, H. Kawada, S. Nishida, H. Kishida and I. Nakayama, Laboratory evaluation of 2-[1-methyl-2- (4-phenoxy-phenoxy) ethoxy] pyridine against larvae of mosquitoes and housefly, *Jpn. J. Sanit. Zool.* **38** (1987), 271–274.

[39] M. Hatakoshi, N. Agui and I. Nakayama, 2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine as a new insect juvenile hormone analogue: Induction of supernumerary larvae in *Spodoptera litura* (Lepidoptera: Noctuidae), *Appl. Ent. Zool.* **21** (1986), 351–353.

[40] M. Hatakoshi, An inhibitory mechanism over oviposition in the tobacco cutworm, *Spodoptera litura* by juvenile hormone analogue pyriproxyfen, *J. Insect Physiol.* **38** (1992), 793–801.

[41] M. Hatakoshi, Y. Shono, H. Yamamoto and M. Hirano, Effects of the juvenile hormone analog pyriproxyfen, on *Myzus persicae* and *Unaspis yanonensis*, *Appl. Ent. Zool.* **26** (1991), 412–414.

[42] M. Hatakoshi, I. Nakayama and L.M. Riddiford, The induction of an imperfect supernumerary larval moult by juvenile hormone analogues in *Manduca sexta*, *J. Insect Physiol.* **34** (1988), 373–378.

[43] M. Haverty, N. Su, M. Tamashiro and R. Yamamoto, Concentration-dependentpresoldier induction and feeding deterrency: Potential of two insect growth regulators for remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae), *J. Econ. Entomol.* **82** (1989), 1370–1374.

[44] J. Hemingway, B.C. Bonning, K.G.I. Jayawardena, I.S. Weerasinghe, P.R.J. Herath and H. Oouchi, Possible selective advantage of Anopheles spp. (Diptera: Culicidae) with the oxidase- and acetylcholinesterase- based insecticide resistance genes after exposure to organophosphates or an insect growth regulator in Sri Lankan rice fields, *Bull. Entomol. Res.* **78** (1988), 471–478.

[45] C.A. Henrick, G.B. Staal and J.B. Siddall, Alkyl 3,7,11-trimethyl-2,4-dodecadienoates, a new class of potent insect growth regulators with juvenile hormone activity, *J. Agr. Food Chem.* **21** (1973), 354–359.

[46] C.A. Henrick, G.B. Staal and J.B. Siddal, Alkyl 3,7,11-trimethyl-2,4-dodecadienoates, a new class of potent insect growth regulators with juvenile hormone activity, *J. Agric. Food Chem.* **21** (1973), 354–359.

[47] C.A. Henrick, Juvenoids, (*Conf. Proc.*) *Agrochem. Nat. Prod.*, (1995), pp. 147–213.

[48] N.C. Hinkle, P.G. Koehler and R.S. Patterson, Residual effectiveness of insect growth regulators applied to carpet for control of cat flea (Siphonaptera: Pulicidae) larvae, *J. Econ. Entomol.* **88** (1995), 903–906.

[49] T. Ishii, Y. Utsumi, A. Kamata and S. Kamei, Field trials of BCP-8702 against mosquito larvae in ditches, *J. Sci., Univ. Tokushima* **23** (1990), 9–19 (in Japanese).

[50] A.R. Horowitz and I. Ishaaya, Managing resistance to insect growth regulators in the sweetopotato whitefly (Homoptera : Aleyrodidae), *J. Econ. Entomol.* **87** (1994), 866–871.

[51] I. Ishaaya and A.R. Horowitz, Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweetpotato whitefly (Homoptera: Aleyrodidae), *J. Econ. Entomol.* **85** (1992), 2113–2117.

[52] I. Ishaaya, A. De Cock and D. Degheele, Pyriproxyfen, a potent suppressor of egg hatch and adult formation of the greenhouse whitefly (Homoptera: Aleyrodidae), *J. Econ. Entomol.* **87** (1994), 1185–1189.

[53] T. Itoh, Control of DF/DHF vector, *Aedes* mosquito, with insecticides, *Trop. Med.* **35** (1993), 259–267.

[54] T. Itoh, H. Kawada, Y. Abe, Y. Eshita, Y. Rongsriyam and A. Igarashi, Utilization of blood-fed females of *Aedes aegypti* as a vehicle for the transfer of the insectgrowth regulator pyriproxyfen to larval habitats, *J. Am. Mosq. Control Assoc.* **10** (1994), 344–347.

[55] K. Iwanaga, and T. Kanda, The effects of a juvenile hormone active oxime ether compound on the metamorphosis and reproduction of an Anopheline vector, Anopheles balabacensis, *Appl. Ent. Zool.* **23** (1988), 186–193.

[56] V. Jarolim, K. Hejuno, F. Sehnal and F. Sorm, Natural and synthetic materials with insect hormone activity 8. Juvenile activity of the farnesane-type compounds on *Galleria mellonella*, *Life Sci.* **8** (1969), 831–841.

[57] C.L. Judson and H.Z. Lumen, Some effects of juvenile hormone and analogs on ovarian follicles of the mosquito *Aedes aegypti* (Diptera: Culicidae), *J. Med. Entomol.* **13** (1976), 197–201.

[58] K.J. Judy, Isolation, structure and absolute configuration of a new natural insect juvenile hormone from *Manduca sexta*, *Proc. Nat. Acad. Sci. USA* **70** (1973), 1509–1513.

[59] S. Kamei, A. Kamata, Y. Utsumi and T. Ishii, Field trials of BCP-8702 against housefly larvae in hen and pig house, *Jpn. J. Environ. Entomol. Zool.* **2** (1990), 81–83 (in Japanese).

[60] K. Kamimura, Field evaluation of an insect growth regulator pyriproxyfen, against the housefly, *Musca domestica*, *Jpn. J. Environ. Entomol. Zool.* **3** (1991), 1–6.

[61] K. Kamimura, and R. Arakawa, Field evaluation of an insect growth regulator, pyriproxyfen, against *Culex pipiens pallens* and *Culex tritaeniorhynchus*, *Jpn. J. Sanit. Zool.* **42** (1991), 249–254.

[62] H. Kawada, K. Dohara and G. Shinjo, Laboratory and field evaluation of an insect growth regulator, 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, as a mosquito larvicide, *Jpn. J. Sanit. Zool.* **39** (1988), 339–346.

[63] H. Kawada, An insect growth regulator against cockroaches, *SP World No.11* (*Sumitomo Chemical Co., Ltd.*), 1988, pp. 2–4.

[64] H. Kawada, Y. Shono, T. Itoh and Y. Abe, Laboratory evaluation of and insect growth regulators against several species of Anopheline mosquitoes, *Jpn. J. Sanit. Zool.* **44** (1993), 349–353.

[65] H. Kawada, T. Itoh, Y. Abe and M. Horio, Can mosquito be a carrier of larvicides?, in: *Proceedings of the 1st International Congress on Insect Pests in the Urban Environment*, Cambridge, UK, 1993, p. 497.

[66] H. Kawada, T. Kohama and Y. Abe, Larvicidal activity of a water-soluble granular formulation of the insect growth regulator, pyriproxyfen, against *Culex* mosquitoes, *Jpn. J. Environ. Entomol. Zool.* **6** (1994), 68–77 (in Japanese).

[67] H. Kawada, K. Dohara and G. Shinjo, Evaluation of larvicidal potency of insect growth regulator, 2-[1-methyl-2-(4-phenoxy-phenoxy) ethoxy] pyridine, against the housefly, *Musca domestica*, *Jpn. J. Sanit. Zool.* **38** (1987), 317–322.

[68] H. Kawada, S. Senbo and Y. Abe, Effects of pyriproxyfen on the reproduction of the housefly, *Musca domestica*, and the german cockroach, *Blattella germanica*, *Jpn. J. Sanit. Zool.* **43** (1992), 169–175.

[69] H. Kawada, I. Kojima and G. Shinjo, Laboratory evaluation of a new insect growth regulator pyriproxyfen, as a cockroach control agent, *Jpn. J. Sanit. Zool.* **40** (1989), 195–201.

[70] H. Kawada, and M. Hirano, Insecticidal effects of an insect growth regulators methoprene and pyriproxyfen on the cat flea (Siphonaptera: Pulicidae), *J. Med. Entomol.* **33** (1996), 819–822.

[71] V. Kerdpibule, A field test of 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine against principal vectors of malaria in a foot-hill area in Thailand, *Japan J. Trop. Med. Hyg.* **17** (1989), 175–183.

[72] H. Kishida, S. Nishida, M. Hatakoshi and N. Matsuo, Pyriproxyfen: A novel insect growth regulator, in: *Book of abstracts, 7th International Congress of Pesticide Chemistry*, 1990, p. 33.

[73] M.J. Klowden and G. M. Chambers, Ovarian development and adult mortality in Aedes aegypti treated with sucrose, juvenile hormone and methoprene, *J. Insect Physiol.* **35** (1989), 513–517.

[74] Y. Kobayashi, Y. Ono, Y. Yoshioka, T. Okano and K. Buei, Effect of juvenile hormone analogues, pyriproxyfen and methoprene, against the cat flea, *Ctenocephalides felis* (Bouchè), *Jpn. J. Sanit. Zool.* **45** (1994), 245–251 (in Japanese).

[75] P.G. Koehler and R.S. Patterson, Incorporation of pyriproxyfen in a German cockroach (Dictyoptera: Blattellidae) management program, *J. Econ. Entomol.* **84** (1991), 917–921.

[76] T. Kotaki, H. Nakakita and M. Kuwahara, Crowding inhibits pupation in *Tribolium freemani* (Coleoptera: Tenebrionidae): Effects of isolation and juvenile hormone analogues on development and pupation, *Appl. Ent. Zool.* **28** (1993), 43–52.

[77] K.J. Kramer, P.E. Dunn, R.C. Peterson, H.L. Seballos, L.L. Sanburg and J.H. Law, Purification and characterization of the carrier protein for juvenile hormone from the hemolymph of the tobacco hornworm, *Manduca sexta* Johannson (Lepidoptera: Sphingidae), *J. biol. Chem.* **251** (1976), 4979–4985.

[78] R.D. Kramer, P.G. Koehler and R.S. Patterson, Morphogenetic effects of hydroprene on German cockroaches (Orthoptera: Blattellidae), *J. Econ. Entomol.* **82** (1989), 163–170.

[79] P.A. Langley, T. Felton and H. Oouchi, Juvenile hormone mimics as effective sterilants for the tsetse fly *Glossina morsitans morsitans*, *Med. Vet. Entomol.* **2** (1988), 29–35.

[80] R.J. Kuhr and J. S. Cleere, Toxic effects of synthetic juvenile hormones on several aphid species, *J. Econ. Entomol.* **66** (1973), 1019–1022.

[81] P.A. Langley, J.W. Hargrove, B. Mauchamp, C. Royer, and Oouchi, H. Prospects for using pyriproxyfen-treated targets for tsetse control, *Entomol. Exp. Appl.* **66** (1993), 153–159.

[82] P.A. Langley, V. Howl and H. Oouchi, Regulation of reproduction in *Rhodnius prolixus* by the juvenile hormone mimic pyriproxyfen, *Entomol. Exp. Appl.* **57** (1990), 271–279.

[83] P.A. Langley, T. Felton, K. Stafford and H. Oouchi, Formulation of pyriproxyfen, a juvenile hormone mimic, for tsetse control, *Med. Vet. Entomol.* **4** (1990), 127–133.

[84] J.H. Law, C. Yuan and C.M. Williams, Synthesis of a material with high juvenile hormone activity, *Proc. Nat. Acad. Sci. USA* **55** (1966), 576–578.

[85] K.A. Lerro and G.D. Prestwich, Cloning and sequencing of a cDNA for the hemolymph juvenile hormone binding protein of larval *Manduca sexta*, *J. biol. Chem.* **265** (1990), 19800–19806.

[86] P.Y. Loh and H.H. Yap, Laboratory studies on the efficacy and sublethal effects of an insect growth regulator, pyriproxyfen (S-31183) against *Aedes aegypti*, *Trop. Biomedicine* **6** (1989), 7–12.

[87] G. Marcer, B. Saia, C. Zanetti, C. Giacomin, F. Acietto, S. Della Sala and F. D'Andrea, Aspetti sanitari dellinfestazione da Chironomidi, in: *Chironomidi, Culicidi, Simulidi-aspetti sanitari ed ecologici*, F. D'Andrea and G. Marchese, eds., Regione Veneto, ULSS 16, S.I.P., Venezia, Italy, 1990, pp. 89–99.

[88] R. Meola, S. Pullen and S. Meola, Toxicity and histopathology of the growthregulator pyriproxyfen to adults and eggs of cat flea (Siphonaptera: Pulicidae), *J. Med. Entomol.* **33** (1996), 670–679.
[89] M. Mesnier, Recherches sur le determinisme de la ponte chez *Galleria mellonella* (Lepidopteres), *C. r. Acad. Sci. Paris* **D274** (1972), 708–711.
[90] A.S. Meyer, H.A. Schneiderman and E. Hanzman, The two juvenile hormones from cecropia silk moth, *Proc. Nat. Acad. Sci. USA* **60** (1968), 853.
[91] L.S. Mian and M.S. Mulla, Biological and environmental dynamics of insect growth regulators (IGRs) as used against Diptera of public health importance, *Residue Rev.* **84** (1982), 28.
[92] R.W. Miller, Evaluation of S-31183 for fly (Diptera: Muscidae) control as a feed-through compound for poultry, cattle, and swine, *J. Agric. Entomol.* **6** (1989), 77–81.
[93] J. Miyamoto, M. Hirano, Y. Takimoto and M. Hatakoshi, Insect growth regulators for pest control, with emphasis on juvenile hormone analogs. Present status and future prospects., in: *Pest Control with Enhanced Environmental Safety*, S.O. Duke, J.J. Menn and J.R. Plimmer, eds., ACS Symposium Series 524, American Chemical Society, Washington, DC., 1993, pp. 144–168.
[94] K. Morikawa, S. Yano, K. Kuwata, K. Ishikawa and M. Shiba, Seasonal appearance and control of the midges at the Miyamae river in Matsuyama, *Rep. Res. Matsuyama Shinonome Jr. Col.* **21** (1990), 175–193 (in Japanese).
[95] M.S. Mulla, The future of insect growth regulators in vector control, *J. Am. Mosq. Control Assoc.* **11** (1995), 269–273.
[96] M.S. Mulla, H.A. Darwazeh, L. Ede and B. Kennedy, Laboratory and field evaluation of the IGR fenoxycarb against mosquitoes, *J. Am. Mosq. Control Assoc.* **1** (1985), 442–448.
[97] M.S. Mulla, H.M. Darwazeh, B. Kennedy and D.M. Dawson, Evaluation of new insect growth regulators against mosquitoes with notes on nontarget organisms, *J. Am. Mosq. Control Assoc.* **2** (1986), 314–320.
[98] M.S. Mulla and H.A. Darwazeh, Efficacy of new insect growth regulators against mosquito larvae in dairy waste water lagoons, *J. Am. Mosq. Control Assoc.* **4** (1988), 322–325.
[99] M.S. Mulla and H.A. Darwazeh, New insect growth regulators against flood and stagnant water mosquitoes – Effects on nontarget organisms, *Mosq. News* **39** (1979), 746–755.
[100] M.S. Mulla , R. Lee, T. Ikeshoji and W.L. Kramer, Insect growth regulators for the control of aquatic midges, *J. Econ. Entomol.* **67** (1974), 165–170.
[101] M.S. Mulla, G. Majori and A.A. Arata, Impact of biological and chemical mosquito control agents on nontarget biota in aquatic ecosystems, *Residue Rev.* **71** (1979), 121.
[102] F.S. Mulligan and C.H. Schaefer, Efficacy of a juvenile hormone mimic, pyriproxyfen (S-31183), for mosquito control in dairy wastewater lagoons, *J. Am. Mosq. Control Assoc.* **6** (1990), 89–92.
[103] K. Nagai, Effects of a juvenile hormone mimic material, 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, on *Thrips palmi* Karny (Thysanoptera: Thripidae) and its predator *Orius sp.* (Hemiptera: Anthocoridiae), *Appl. Ent. Zool.* **25** (1990), 199–204.
[104] M. Ogawa, H. Kawada, T. Ohtsubo, S. Tsuda, T. Itoh, Y. Abe and K. Tsuji, Efficient vector control using pyriproxyfen water fizzy formulations, in: *Proceedings of the 1st International Congress on Insect Pests in the Urban Environment*, Cambridge, UK, 1993, p. 496.
[105] T. Okazawa, B. Bakote'e, H. Suzuki, H. Kawada and N. Kere, Field evaluation of an insect growth regulator, pyriproxyfen, against *Anopheles punctulatus* on north Guadalcanal, Solomon islands, *J. Am. Mosq. Control Assoc.* **7** (1991), 604–607.
[106] A. Olsen, M. Brandt and O. Skovmand, Fleas, *Dan. Pest Infest. Lab. Ann. Rep.*, **1981** (1981), 48–50.
[107] A. Olsen, Ovicidal effect on the cat flea, *Ctenocephalides felis* (Bouchè), of treating fur of cats and dogs with methoprene, *Int. Pest. Control* **27**(16) (1985), 10–13.
[108] W.L.A. Osbrink, M.K. Rust and D.A. Reierson, Distribution and control of cat fleas in homes in southern California (Siphonaptera: Pulicidae), *J. Econ. Entomol.* **79** (1986), 135–140.
[109] S.R. Palli, E.O. Osir, W. Eng, M.F. Boehm, M. Edward, P. Kulcsar, I. Ujvary, K. Hiruma, G.D. Prestwich and L.M. Riddiford, Juvenile hormone receptors in insect larval epidermis: Identification by photoaffinity labeling, *Proc. Nat. Acad. Sci. USA* **87** (1990), 769–800.

[110] F.M. Pallos, J.J. Menn, P.E. Letchworth and J.B. Miaullis, Synthetic mimics of insect juvenile hormone, *Nature* **232** (1971), 486–487.
[111] K.G. Palma, S.M. Meola and R.W. Meola, Mode of action of pyriproxyfen and methoprene on eggs of *Ctenocephalides felis* (Siphonaptera: Pulicidae), *J. Med. Entomol.* **30** (1993), 421–426.
[112] K.G. Palma and R.W. Meola, Evaluation of Nylar for control of cat fleas (Siphonaptera: Pulicidae) in home yards, *J. Med. Entomol.* **27** (1990), 1045–1049.
[113] R.S. Patterson and P.G. Koehler, Sterility: A practical IPM approach for German cockroach (*Blattella germanica*) control, in: *Proceedings of the First Insect Growth Regulator Symposium*, 24 July 1985, Dallas, Tex., pp. 48–60.
[114] R.S. Patterson and P.G. Koehler, Cockroach biology, ecology and control in the United States of America, in: *Proc. 5th Sem. Control Vectors and Pests*, Taipei, Taiwan, 1992, pp. 67–81.
[115] B.A. Peleg, Effect of a new phenoxy juvenile hormone analog on California red scale (Homoptera: Diaspididae), Florida wax scale (Homoptera: Coccidae) and the ectoparasite *Aphytis holoxanthus* DeBache (Hymenoptera: Aphelinidae), *J. Econ. Entomol.* **81** (1988), 88–92.
[116] M.P. Pener, D. Dessberg, P. Lazarovici, C.C. Reuter, L.W. Tsai, and E.C. Baker, The effect of a synthetic precocene on juvenile hormone III titer in late *Locusta* eggs, *J. Insect Physiol.* **32** (1986), 853–857.
[117] G.D. Prestwich, K. Touhara, L.M. Riddiford and B.D. Hammock, Larva light: A decade of photoaffinity labeling with juvenile hormone analogues, *Insect Biochem. Molec. Biol.* **24** (1994), 747–761.
[118] B.L. Reid, V.L. Brock and G.W. Bennett, Developmental morphogenetic and reproductive effects of four polycyclic non-isoprenoid juvenoids in the german cockroach (Dictyoptera: Blattellidae), *J. Entomol. Sci.* **29** (1994), 31–42.
[119] A. Retnakaran, J. Granett and T. Ennis, Insect Growth Regulators, in: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, G.A. Kerkut and L.I. Gilbert, eds., Pergamon Press, Oxford, Vol. 12, 1985, pp. 530–601.
[120] D. Richard, S.W. Applebaum, T.J. Sliter, F.C. Baker, D.A. Schooley, C.C. Reuter, V.C. Henrich and L.I. Gilbert, Juvenile hormone bisepoxide biosynthesis in vitro by the ring gland of *Drosophila melanogaster*: a putative juvenile hormone in the higher Diptera, *Proc. Natl. Acad. Sci. U.S.A.* **86** (1989), 1421–1425.
[121] L.M. Riddiford, Cellular and molecular actions of juvenile hormone I. General considerations and premetamorphic actions, *Adv. Insect Physiol.* **24** (1994), 213–274.
[122] L.M. Riddiford and J.B. Ashenhurst, The switchover from virgin to mated behavior in female cecropia moths: The role of the bursa copulatrix, *Biol. Bull.* **144** (1973), 162–171.
[123] L.M. Riddiford and M. Ashburner, Effects of juvenile hormone mimics on larval development and metamorphosis of *Drosophila melanogaster*, *Gen. Comp. Endocrinol.* **82** (1991), 172–183.
[124] L.M. Riddiford and C.M. Williams, The effect of juvenile hormone analogues on the embryonic development of silkworms, *Proc. Natl. Acad. Sci. USA* **53** (1967), 595–601.
[125] H. Roller, K.H. Dahm, C.C. Sweeley and B.M. Trost, The structure of the juvenile hormone, *Angew. Chem. Int. Ed.* **6** (1967), 179–180.
[126] M. Romanuk, K. Slama and F. Sorm, Constitution of a compound with a pronounced juvenilehormone activity, *Proc. Natl. Acad. Sci. USA* **57** (1967), 349–352.
[127] M.H. Ross and D.G. Cochran, Effects on German cockroach nymphs of contact exposure to IGRs singly and in combination, *Entomol. Exp. Appl.* **61** (1991), 117–122.
[128] M.H. Ross and D.G. Cochran, Response of late-instar *Blattella germanica* (Dictyoptera: Blattellidae) to dietary insect growth regulators, *J. Econ. Entomol.* **83** (1990), 2295–2305.
[129] R. Sawby, M.J. Klowden, R.D. Sjogren, Sublethal effects of larval methoprene exposure on adult mosquito longevity, *J. Am. Mosq. Control Assoc.* **8** (1992), 290–292.
[130] C.H. Schaefer, T. Miura, F.S. Mulligan III and R.M. Takahashi, Insect developmental inhibitors. Biological activity of RE-17565, RE-17937 and RE-18286 against mosquitoes (Diptera: Culicidae) and nontarget organisms, *Proc. Calif. Mosq. Control Assoc.* **42** (1974), 147.

[131] C.H. Schaefer, and W.H. Wilder, Insect developmental inhibitors: A practical evaluation as mosquito control agents, *J. Econ. Entomol.* **65** (1972), 1066–1071.
[132] Schaefer, C. H., Wilder, W. H., Mulligan III, F. S. and Dupras Jr., E. F. Efficacy of fenoxycarb against mosquitoes (Diptera: Culicidae) and its persistence in the laboratory and field, *J. Econ. Entomol.* **80** (1987), 126–130.
[133] C.H. Schaefer and T. Miura, Chemical persistence and effects of S-31183, 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine, on aquatic organisms in field tests, *J. Econ. Entomol.* **83** (1990), 1768.
[134] C.H. Schaefer, T. Miura, E.F. Dupras Jr., F.S. Mulligan and W.H. Wilder, Efficacy, nontarget effects, and chemical persistence of S-31183, a promising mosquito (Diptera: Culicidae) control agent, *J. Econ. Entomol.* **81** (1988), 1648–1655.
[135] C.H. Schaefer and F.S. Mulligan III, Potential for resistance to pyriproxyfen: A promising new mosquito larvicide, *J. Am. Mosq. Control Assoc.* **7** (1991), 409–411.
[136] M.E. Scharf, J. Hemingway, B.L. Reid, G.J. Small and G.W. Bennett, Toxicological and biochemical characterization of insecticide resistance in a field-collected strain of *Blattella germanica* (Dictyoptera: Blattellidae), *J. Econ. Entomol.* **89** (1996), 322–331.
[137] P. Schmialek, Die Identifizierung zweier in Tenebriokot und in Hefe vorkommender Substanzen mit Juvenilhormonwirkung, *Z. Naturforsch.* **B 16** (1961), 461–464.
[138] S. Senbo, H. Kawada, T. Itoh and Y. Abe, Susceptibility of Cat flea, *Ctenocephalides felis* to several insecticides and use of insect growth regulator, pyriproxyfen, as a flea control agent, *Kaokugaityu* **15** (1993), 94–98 (in Japanese).
[139] Y. Shono, R. Arakawa, M. Hirano and Y. Abe, Effect of pyriproxyfen, fenitrothion and permethrin on the fly pupal parasitoids, *Spalangia endius* and *Nasonia vitripennis*, *Jpn. J. Sanit. Zool.* **44** (1993), 29–32.
[140] J.E. Short and J.P. Edwards, Effects of hydroprene on development and reproduction in the Oriental cockroach, *Blatta orientalis*, *Med. Vet. Entomol.* **6** (1992), 244–250.
[141] J. Silverman and M.H. Ross, Behavioral resistance of field-collected German cockroaches to baits containing glucose, *Environ. Entomol.* **23** (1994), 425–430.
[142] K. Slama and C.M. Williams, *Paper factor* as an inhibitor of the embryonic development of the European bug, *Pyrrhocoris apterus*, *Nature* **210** (1966), 329–330.
[143] K. Slama and C.M. Williams, Juvenile hormone activity for the bug *Pyrrhocoris apterus*, *Proc. Natl. Acad. Sci. USA* **54** (1965), 411–414.
[144] A. Spielman and C.M. Williams, Lethal effects of synthetic juvenile hormone on larvae of the yellow fever mosquito, *Aedes aegypti*, *Science* **154** (1966), 1043–1044.
[145] P. Sroka and L.I. Gilbert, The timing of juvenile hormone release for ovarianmaturation in *Manduca sexta*, *J. Insect Physiol.* **20** (1974), 1173–1180.
[146] N. Su and R.H. Scheffrahn, Comparative effects of an insect growth regulator, S-31183, against the Formosan subterranean termite and Eastern subterranean termite (Isoptera: Rhinotermitidae), *J. Econ. Entomol.* **82** (1989), 1125–1129.
[147] H. Suzuki, T. Okazawa, N. Kere and H. Kawada, Field evaluation of a new insect growth regulator, pyriproxyfen, against *Anopheles farauti*, the main vector of malaria in the Solomon Islands, *Jpn. J. Sanit. Zool.* **40** (1989), 253–257.
[148] Syafruddin, R. Arakawa, K. Kamimura and F. Kawamoto, Histopathological effects of an insect growth regulator, 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether (pyriproxyfen), on the larvae of *Aedes aegypti*, *Jpn. J. Sanit. Zool.* **41** (1990), 15–22.
[149] Y. Tabaru, Studies on chemical control of a nuisance chironomid midge (Diptera: Chironomidae). 4. Efficacy of two insect growth regulators to *Chironomus yoshimatsui* in laboratory and field, *Jpn. J. Sanit. Zool.* **36** (1985), 306–313.
[150] Y. Tabaru, K. Moriya and A. Ali, Nuisance midges (Diptesra: Chironomidae) and their control in Japan, *J. Am. Mosq. Control Assoc.* **3** (1987), 45–49.
[151] M. Takagi, Y. Tsuda and Y. Wada, Laboratory evaluation of a juvenile hormone mimic pyriproxyfen, against *Chironomus fusciceps* (Diptera: Chironomidae), *J. Am. Mosq. Control Assoc.* **11** (1995), 474–475.

[152] P.D. Teel, W.A. Donahue, O.F. Strey and R.W. Meola, Effects of pyriproxyfen on engorged female and newly oviposited eggs of the lone star tick (Acari: Ixodidae), *J. Med. Entomol.* **33** (1996), 721–725.

[153] S. Thongrungiat and T. Kanda Efficacy of pyriproxyfen for the control of *Culex tritaeniorhynchus* at an open rice field in Bang Len, Nakhon Pathom province, Thailand, *Trop. Biomedicine* **8** (1991), 113–116.

[154] K.M. Trayler, A.M. Pinder and J.A. Davis, Evaluation of the juvenile hormone mimic pyriproxyfen (S-31183) against nuisance Chironomids (Diptera: Chironomidae), with particular emphasis on *Polypedilum nubifer* (Skuse), *J. Aust. Ent. Soc.* **33** (1994), 127–130.

[155] K.M. Vail and D.F. Williams, Pharaoh ant (Hymenoptera: Formicidae) colony development after consumption of pyriproxyfen baits, *J. Econ. Entomol.* **88** (1995), 1695–1702.

[156] E. Vinuela, J. Ondracek, J. Jacas, A. Adan, M. Rejzek and Z. Wimmer, Laboratory evaluation of five new JHA derivatives from 2-(4-hydroxybenzyl)-1-cyclohexanone against Tribolium castaneum (Herbst.), *J. Stored Prod. Res.* **30** (1994), 149–155.

[157] E.D. Walker and J.D. Edman, Evaluation of fenoxycarb against spring mosquitoes in Massachusetts, *J. Am. Mosq. Control Assoc.* **6** (1990), 725–726.

[158] V.B. Wigglesworth, The juvenile hormone effect of farnesol and some related compounds: Quantitative experiments, *J. Insect Physiol.* **9** (1963), 105–119.

[159] C.M. Williams, Third-generation pesticides, *Sci. Am.* **217** (1967), 13–17.

[160] C.M. Williams, The Juvenile hormone of insects, *Nature* **178** (1956), 212–213.

[161] D.F. Williams and K.M. Vail, Control of a natural infestation of the Pharaoh ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae) with a corn grit bait of fenoxycarb, *J. Econ. Entomol.* **87** (1994), 108–115.

[162] Williams, D.F. and K.M. Vail, The Pharaoh ant (Hymenoptera: Formicidae): Fenoxycarb baits affect colony development, *J. Econ. Entomol.* **86** (1993), 1136–1143.

[163] D.F. Williams, Effects of fenoxycarb baits on laboratory colonies of the pharaos ant, *Monomorium pharaonis*, in: *Applied Myrmecology*, R.K. Vander Meer, K. Jaffe and A. Cedeno, eds., Westview, Boulder, CO, 1990, pp. 676–683.

[164] T.G. Wilson and J.A. Fabian, Drosophola melanogaster mutant resistant to a chemical analogue of juvenile hormone, *Dev. Biol.* **118** (1986), 190–201.

[165] T.G. Wilson and J. Fabian, Selection of methoprene resistant mutants of *Drosophila melanogaster*, in: *Molecular Entomology*, Vol. 49, UCLA Symposia on Molecular and Cellular Biology, New Series, 1987, pp. 179-188.

[166] H. Yamamoto and K. Kasamatsu, Effects of a juvenile hormone mimic, S-71639, on the greenhouse whitefly, Trialeurodes vaporariorum, and the green peach aphid, Myzus persicae, in the greenhouse, *Adv. Inverteb. Reprod.* **5** (1990), 393–398.