Efficacy of Pyriproxyfen-Treated Nets in Sterilizing and Shortening the Longevity of *Anopheles gambiae* (Diptera: Culicidae)

KAZUNORI OHASHI,[1,2] KAZUHIDE NAKADA,[1] TAKAO ISHIWATARI,[1,3] JUN'ICHI MIYAGUCHI,[1] YOSHINORI SHONO,[1,3] JOHN R. LUCAS,[4] AND NOBUAKI MITO[1,5]

J. Med. Entomol. 49(5): 1052–1058 (2012); DOI: http://dx.doi.org/10.1603/ME12006

ABSTRACT Pyrethroid-resistant malaria vectors have become a serious threat for malaria control, and bed nets that reduce the development of resistance are urgently needed. Here, we tested the effects of bed nets treated with the insect growth regulator pyriproxyfen against adult female *Anopheles gambiae* Giles (Diptera: Culicidae) under laboratory conditions. Noninsecticidal nets made of 195 denier monofilament polyethylene with a mesh size of 75 holes per square inch (equivalent to the Olyset Net) were dipped in a 0.1, 0.01, or 0.001% (wt:vol) alcohol solution of pyriproxyfen and dried overnight. Adult females of an insecticide-susceptible *An. gambiae* strain were exposed to treated and untreated nets before and after a bloodmeal. Bioassays showed that females were completely sterilized after exposure to 0.1% (35 mg [AI]/m^2) and 0.01% pyriproxyfen-treated nets both before and after a bloodmeal. In addition, adult longevity decreased after exposure to the pyriproxyfen-treated nets in a concentration-dependent manner. The sterilizing and life-shortening effects of pyriproxyfen on the vector mosquito indicate that the combined use of pyriproxyfen and pyrethroids on bed nets has the potential to provide better malaria control and prevent the further development of pyrethroid resistance in malaria vectors.

KEY WORDS insecticide-treated bed net, malaria control, pyriproxyfen, resistance management, sterilizing effect

Malaria is a major public health problem, particularly in sub-Saharan Africa. The malaria burden in 2009 was estimated to be at least 225 million clinical cases and nearly one million deaths, of which 91% occurred in the African region (WHO 2010). Vector control using insecticides is an important component of malaria prevention, and the most effective ways to prevent malaria transmission are to reduce vector longevity and provide personal protection from mosquito bites. The most widely used vector control methods are indoor residual sprays (IRSs) and long-lasting insecticidal nets (LLINs) (Guillet et al. 2001a, WHO 2010). IRSs can shorten vector longevity, thereby preventing the transmission of malaria. Pyrethroid-treated bed nets are effective for personal protection and can provide community protection through a mass impact on vector populations when used at a high coverage rate in villages (Hawley et al. 2003, Lindblade et al. 2004, Henry et al. 2005, Bhattarai et al. 2007, Fegan et al. 2007). Mass distribution of LLINs has been scaled up in African countries with support from the Global Fund Partnership (WHO 2010).

Pyrethroid resistance in malaria vectors has become widespread in several malaria-endemic parts of the world (Chandre et al. 1999, Santolamazza et al. 2008, Ranson et al. 2011), perhaps due to the agricultural use of pyrethroids and DDT (Dabire et al. 2008) and the increasing exposure to insecticide-treated bed nets (Czeher et al. 2008, Mathias et al. 2011). Pyrethroid resistance has become a problem in all major malaria vector mosquitoes, such as *Anopheles gambiae* Giles, *Anopheles arabiensis* Giles, and *Anopheles funestus* Giles (all Diptera: Culicidae) in African countries (Martinez-Torres et al. 1998, Hargreaves et al. 2000, Müller et al. 2008, Kawada et al. 2011). Pyrethroids are the only class of insecticides recommended by the World Health Organization (WHO) for bed-net impregnation to prevent malaria transmission because of their fast knockdown action, excito-repellency, and relative safety to humans (Zaim et al. 2000). Thus, pyrethroid-resistant malaria vectors have become a serious threat, and resistance management bed nets using alternative chemicals are urgently needed to control malaria and prevent the further spread of pyrethroid resistance genes (Zaim and Guillet 2002).

The insect growth regulator (IGR) pyriproxyfen is a juvenile hormone analog (JHA) with extremely low toxicity to mammals (FAO 1999). Pyriproxyfen inhibits metamorphosis and embryogenesis in several in-

[1] Health and Crop Sciences Research Laboratory, Sumitomo Chemical Company, Ltd., Hyogo 665-8555, Japan.
[2] Corresponding author, e-mail: ohashik@sc.sumitomo-chem.co.jp.
[3] Technical and Product Development Department, Vector Control Division, Sumitomo Chemical Company, Ltd., Tokyo 104-8260, Japan.
[4] Sumitomo Chemical (UK) Plc. Horatio House, 77-85 Fulham Palace Rd., London W6 8JA, United Kingdom.
[5] Planning and Coordination Office, Health and Crop Sciences Sector, Sumitomo Chemical Company, Ltd., Tokyo 104-8260, Japan.

0022-2585/12/1052–1058$04.00/0

sects (Dhadialla et al. 1998), and has no cross-resistance to older class chemicals, including pyrethroids. Extremely low doses of pyriproxyfen delivered into mosquito larval breeding sites can inhibit adult emergence; for example, the LC_{50} has been reported to be 0.043, 0.023, and 0.0046 μg/liter against *Anopheles stephensi* Liston, *Aedes aegypti* (L.), and *Culex pipiens pallens* Coquillett, respectively (Hatakoshi et al. 1987). Thus, pyriproxyfen has been widely used in the contexts of public health and agriculture (Hirano et al. 1998). Moreover, evidence suggests that tarsal contact with a pyriproxyfen-treated substrate induces reduction of fecundity in adult females of *Ae. aegypti* (Itoh et al. 1994).

In theory, using a combination of insecticides with different modes of action can delay the development of insecticide resistance (Curtis 1985, Mani 1985, Tabashnik 1989). Thus, the use of a pyrethroid and a nonpyrethroid insecticide in combination on the same bed net has been proposed for the management of resistant vectors (Guillet et al. 2001b). Because pyriproxyfen induces sterilization in adult mosquitoes (Itoh et al. 1994), mixing it in a LLIN formulation with a pyrethroid has the potential to prevent the spread of pyrethroid-resistant mosquitoes. Here, we investigated the effect of netting treated with pyriproxyfen on fecundity, egg fertility, and adult longevity in female *An. gambiae* after contact with the netting before and after blood feeding, under laboratory conditions.

Materials and Methods

Test Insect. Adult females of insecticide-susceptible *An. gambiae* s.s. Kisumu strain derived from a culture in Kisumu, Kenya, were used in all laboratory experiments. All experiments were approved by Animal Care and Use Committee of Sumitomo Chemical.

Preparation of Net Samples. We used noninsecticidal nets made of 195 denier monofilament polyethylene with a mesh size of 75 holes per sq. in. that were equivalent to the Olyset Net (Sumitomo Chemical Co. Ltd., Tokyo, Japan). Technical-grade pyriproxyfen (Sumilarv, Sumitomo Chemical Co. Ltd.), was diluted with isopropyl alcohol at concentrations of 0.1, 0.01, and 0.001% (wt:vol). Small pieces (15 by 15 cm) of netting were dipped in each concentration of the pyriproxyfen solution for 1 h and dried overnight. Chemical analysis using gas chromatography showed that the net treated with 0.1% pyriproxyfen retained $\approx$35 mg/m^2 of the chemical. The 0.01 and 0.001% pyriproxyfen-treated nets retained $<$10 mg/m^2 pyriproxyfen, which as below the detection limit of gas chromatography.

Pyriproxyfen-Induced Sterilization. We tested the effects of the pyriproxyfen-treated net on egg production and egg hatchability. Laboratory-reared females (2- to 4-d-old) from the same cohort were divided into two groups to compare susceptibility before and after a bloodmeal. One group was allowed to take a bloodmeal overnight from mice in a rearing cage (21 by 21 by 28 cm) maintained at 25°C before exposure to the pyriproxyfen-treated net. The other group was allowed to take a bloodmeal after exposure to the pyriproxyfen-treated net. Samples of the 0.1, 0.01, and 0.001% pyriproxyfen-treated and untreated nets (15 by 15 cm) were kept on plastic panels inclined at 45°. Batches of 10 blood-fed or unfed females were exposed to the pyriproxyfen-treated net in standard WHO cones for 3 min. In total, 60 fed and 60 unfed females per each concentration were exposed to the net and released into separate cages with access to 5% sugar solution. The unfed females were then allowed to take a bloodmeal from two mice per cage overnight at 25°C. Forty engorged blood-fed females from each concentration in both groups exposed before and after bloodmeal were randomly selected 24 h after exposure to the pyriproxyfen-treated net and individually introduced into 200-ml plastic cups (9 cm in diameter) containing a filter paper moistened with a 1% sugar solution to allow sugar feeding and laying eggs. The individual rearing cups were kept in an environment-controlled room at 25 $\pm$ 1°C, 60 $\pm$ 10% RH, and a photoperiod of 14:10 (L:D) h.

Oviposition was observed for a maximum of 6 d post-bloodmeal. When a female deposited eggs in the cup, she was removed and water was put in the cup for egg hatching. The number of eggs and egg-hatching rate were recorded for each female. Hatched larvae were counted for at least 7 d after oviposition.

Survival Rate and Lifetime Fecundity. We tested the prolonged effects of a single exposure to a pyriproxyfen-treated net on the physiological status of mosquitoes as measured by survival rate and lifetime fecundity. Two- to 4-d-old females were given a bloodmeal from mice in a rearing cage overnight before the exposure. Engorged blood-fed females were used for the experiment. Batches of 10 females were exposed to 0.1, 0.01, and 0.001% pyriproxyfen-treated nets and an untreated control net in WHO cones for 3 min. Exposed females were then released into separate cages (21 by 21 by 28 cm) with access to 5% sugar solution. In total, 80 females were kept in each cage. Two cages were prepared ($\approx$160 females tested in each concentration) and pooled data were used in the analyses. Females were maintained at 27 $\pm$ 1°C, 90 $\pm$ 10% RH, and a photoperiod of 14:10 (L:D) h. Dead females were removed from the cages and counted nearly every day to record the survival rate. Two days after the bloodmeal, two to four 200-ml plastic cups (9 cm in diameter) lined with filter paper and filled with a small amount of water were placed in the cages for laying eggs. Three days later, the plastic cups were removed from the cages, and the number of eggs deposited was counted. Seven days after the bloodmeal, the females were again allowed to feed on mice overnight. Two days after the bloodmeal, new cups for laying eggs were introduced into the cage. This gonotrophic cycle was maintained at 7-d intervals to record lifetime fecundity until all females were dead.

Statistical Analysis. The proportion of egg-laying females after exposure to the chemically-treated and control nets were analyzed using a multivariate logistic regression analysis to test differences in the sensitivity to pyriproxyfen between two explanatory vari-

ables: blood-feeding status (pre- and postfeeding) and pyriproxyfen concentration on the net. The number of eggs per female was analyzed using the Kruskal–Wallis test, followed by the Steel–Dwass test for multiple comparisons between pyriproxyfen concentrations. Egg hatchability was analyzed using an analysis of variance (ANOVA) after arcsine square-root transformation.

Survival rates were analyzed using the Kaplan–Meier survival analysis and log-rank tests were used to compare longevity between treated and untreated females. Lifetime fecundity after exposure was compared among treatments using repeated measures ANOVA after log transformation [log $(x+1)$]. All statistical analyses were performed using the JMP 8.0.1 software package (SAS Institute 2009) except on Steel–Dwass test which was conducted with R 2.10.1 (R Development Core Team 2009).

Results

Pyriproxyfen-Induced Sterilization. No female laid eggs after exposure to the 0.01 and 0.1% pyriproxyfen-treated nets either before or after a bloodmeal, indicating that *An. gambiae* was completely sterilized after contact with the higher concentrations of pyriproxyfen (Fig. 1A and B). The logistic regression analysis showed that the proportion of egg-laying females was significantly affected by pyriproxyfen concentration ($\chi^2 = 195.0$; df = 3, 7; $P < 0.001$; Fig. 1A) but not by blood-feeding status ($\chi^2 = 0.5$; df = 1, 7; $P = 0.47$), and there was no significant interaction between the two variables ($\chi^2 = 1.3$; df = 3, 7; $P = 0.72$). The proportion of egg-laying females exposed to 0.001% pyriproxyfen-treated nets was significantly decreased in groups exposed before the bloodmeal ($P < 0.05$) but not in groups exposed after bloodmeal (Fig. 1A). The number of eggs per female (Fig. 1B) differed significantly among pyriproxyfen concentration in both groups exposed before the bloodmeal (Kruskal–Wallis test: $\chi^2 = 73.7$, df = 3, $P < 0.001$) and after the bloodmeal ($\chi^2 = 94.3$, df = 3, $P < 0.001$). The difference in egg hatchability (Fig. 1C) between females exposed to the 0.001% pyriproxyfen-treated and control net were significant in groups exposed after the bloodmeal (one-way ANOVA: $F = 13.0$; df = 1, 60; $P < 0.001$), but not in groups exposed before the bloodmeal ($F = 1.2$; df = 1, 45; $P = 0.28$).

Survival Rate and Fecundity. Figure 2A shows the survival curve of *An. gambiae* females exposed to the pyriproxyfen-treated and untreated control nets. Survival rates decreased as the pyriproxyfen concentration increased. Females exposed to the 0.1% pyriproxyfen-treated net died within 8 d after exposure. The mean longevity of females exposed to the 0.1, 0.01, and 0.001% pyriproxyfen-treated and untreated nets were 5.6 ± 1.3, 10.2 ± 7.7, 14.2 ± 7.4, and 22.9 ± 7.4 (days ± SD), respectively. The Kaplan–Meier survival analysis indicated that the longevity of the females exposed to the treated nets significantly decreased compared with those exposed to the untreated net

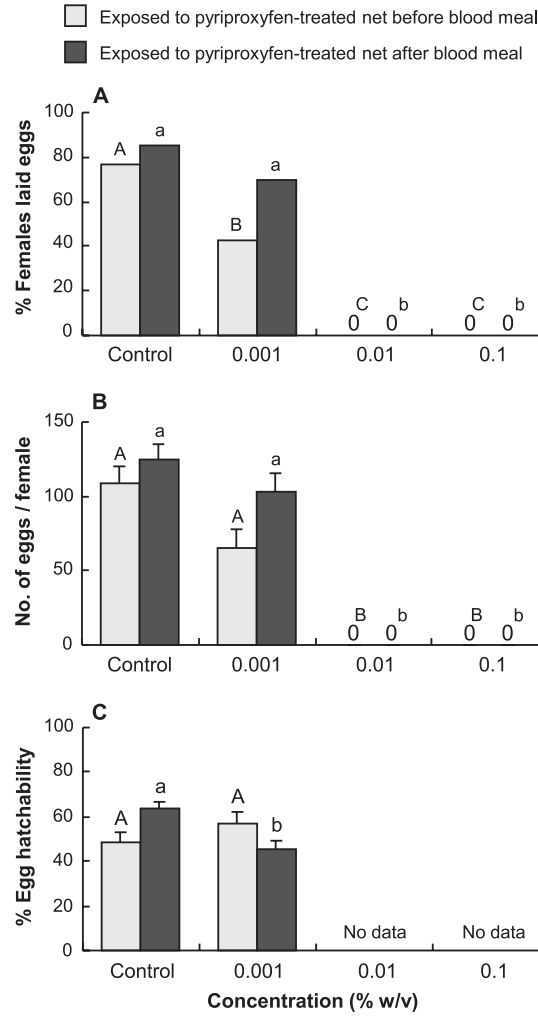

Fig. 1. Fecundity and egg fertility of *An. gambiae* females exposed to nets treated with various concentrations of pyriproxyfen before and after a bloodmeal. (A) Percentage of females that laid eggs. (B) Number of eggs per female tested. (C) Percentage of egg hatchability. Error bars represent the standard error. Uppercase letters indicate significant differences among females exposed before blood feeding ($P < 0.05$); lowercase letters denote significant differences among females exposed after blood feeding ($P < 0.05$).

(log-rank test, 0.1%: $\chi^2 = 255.4$, $P < 0.001$; 0.01%: $\chi^2 = 124.9$, $P < 0.001$; and 0.001%: $\chi^2 = 85.5$, $P < 0.001$).

Figure 2B shows the number of eggs per live female in each cage after exposure to the treated and untreated nets. Females exposed to the 0.1% pyriproxyfen-treated net laid no eggs. Females exposed to the 0.01 and 0.001% pyriproxyfen-treated nets produced significantly fewer eggs compared with control females (repeated measures ANOVA: $F = 4.9$; df = 2, 9; $P = 0.037$). In addition to decreased survival rate, the overall number of eggs laid by females differed significantly among the treatment groups ($F = 4.7$; df = 2, 9; $P = 0.040$) (Fig. 2C). The mean lifetime fecundities of females exposed to the 0.1, 0.01, and 0.001%

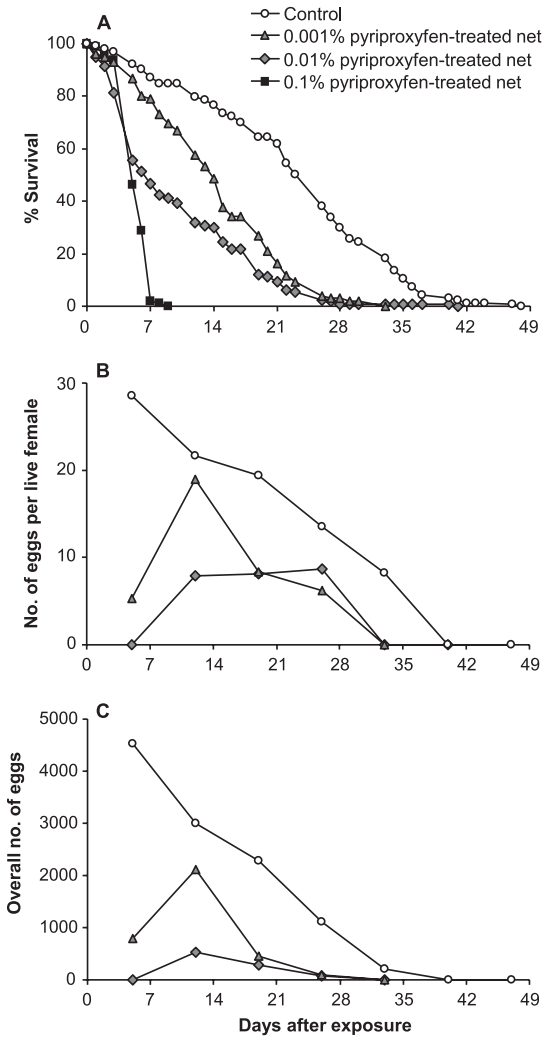

Fig. 2. Survival rates (A) and lifetime fecundity (B and C) of *An. gambiae* females after exposure to nets treated with various concentrations of pyriproxyfen. In total, 160–163 females for each treatment were maintained in two cages after exposure to pyriproxyfen-treated or untreated control nets.

pyriproxyfen-treated and untreated control nets were 0, 5.5, 21.5, and 68.3 eggs, respectively.

Discussion

The current study demonstrated that tarsal contact with pyriproxyfen-treated nets sterilized and shortened the longevity of adult female *An. gambiae* under laboratory conditions. Pyriproxyfen showed a remarkably high efficacy for sterilizing *An. gambiae*, even at the lower dose of 0.01%, which retained <10 mg/m^2 on the net surface. Topical application of JHAs onto the mosquito abdomen has been reported to reduce fecundity (Patterson 1974). However, Miler (1994) reported that exposure of a susceptible and a pyrethroid-resistant strain of *An. stephensi* to a net treated with 0.5 g/m^2 pyriproxyfen produced only a 33 and 55% reduction in the number of eggs, respectively, whereas Aiku et al. (2006) found no significant difference in fecundity between *An. stephensi* females exposed to pyriproxyfen-treated and untreated control nets. Although it is not known exactly why our results differ from those of previous investigations, the monofilament polyethylene netting treated with an isopropyl alcohol solution of pyriproxyfen may have a higher bioavailability than the netting used in these earlier studies. Furthermore, our investigations showed that sensitivity to the sterilizing effect of pyriproxyfen was higher in *Anopheles* mosquitoes than in *Aedes* and *Culex* mosquitoes (unpublished data).

Juvenile hormone has been known to regulate insect ovarian development. Shapiro et al. (1986) showed that a decline in juvenile hormone levels in *Ae. aegypti* mosquitoes occurs in the 36-h period after a bloodmeal, and this is necessary for normal egg development. Methoprene, a juvenile hormone analog, applied to *An. gambiae* was shown to inhibit vitellogenesis by modulating ecdysteroid action, causing abnormal development of the ovary (Bai et al. 2010). We also confirmed that sterile *An. gambiae* females exposed to pyriproxyfen had abnormally developed ovarian follicles (data not shown). Thus, exposure to a pyriproxyfen-treated net causes a disturbance in hormonal regulation of ovarian development, resulting in sterilization.

Itoh et al. (1994) reported that tarsal contact of *Ae. aegypti* with pyriproxyfen before a bloodmeal induced a large decrease in the number of eggs compared with contact after a bloodmeal. We also observed that at the low dose of 0.001% pyriproxyfen females exposed to the chemical before a bloodmeal were slightly more sensitive than those exposed within the 12 h after a bloodmeal. At the higher doses there were no apparent differences in sensitivity between mosquitoes exposed before or after a bloodmeal, due to the high efficacy of pyriproxyfen that caused complete sterilization, masking any time-dependent dosing effects. Furthermore, the effect of pyriproxyfen exposure in the first gonotrophic cycle continued in later gonotrophic cycles, affecting fecundity and longevity, suggesting that pyriproxyfen treatment produces irreversible damage in mosquitoes. For example, pyriproxyfen in the adult cat flea has been shown to damage internal tissues such as Malpighian tubules, midgut epithelia, and salivary gland cells, and to cause death (Meola et al. 1996).

The potential for malaria transmission by vector populations, referred to as vectorial capacity, is determined by mosquito abundance, human blood preference, and survival rate (Garrett-Jones 1964a,b; Dye 1992). The reduced fecundity and longevity in *An. gambiae* found in the current study has the potential to significantly reduce the vectorial capacity of mosquito populations. Malaria parasites require a significant incubation period in the mosquito vector before they can be transmitted to a new human host. The time period between a mosquito ingesting a bloodmeal containing parasites and when that mosquito becomes

infectious is known as the extrinsic incubation period, which is typically 10–14 d (Charlwood et al. 1997). Longevity of the adult mosquito is therefore a critical component in determining the ability of that mosquito to transit malaria (Dye 1992). The life-shortening effect of pyriproxyfen observed here may reduce the transmission of malaria parasites to a new human host, even after the mosquito has obtained a bloodmeal from an infected person.

Pyrethroid-treated nets have been shown to still be effective against *An. gambiae* carrying the *kdr* mutation in West Africa (Corbel et al. 2004, Dabire et al. 2006). However, better control tools are necessary to prevent further development of pyrethroid resistance in malaria vectors. One proposed way to delay the development of insecticide resistance is to use mixtures of different chemicals (Curtis 1985, Mani 1985, Tabashnik 1989). Insects developing resistance to one insecticide should theoretically still be killed by the second insecticide and thus prevent the resistance genes from passing to the next generation. The mixture of a pyrethroid with pyriproxyfen on a bed net is a potential candidate for resistance management because their modes of action are quite different.

Previous studies have investigated the efficacy of bed nets treated with pyrethroid and nonpyrethroid insecticides to control pyrethroid resistant vectors (Guillet et al. 2001b, Corbel et al. 2002, Hougard et al. 2003, Asidi et al. 2005). Bed nets treated with carbosulfan (a carbamate) and chlorpyrifos-methyl (an organophosphate), either alone or in combination with pyrethroids, were shown to be effective against pyrethroid-resistant mosquitoes (Guillet et al. 2001b, Corbel et al. 2002, Hougard et al. 2003, Asidi et al. 2005, Malima et al. 2009). Recently, the pyrrole chlorfenapyr (N′Guessan et al. 2007a, Mosha et al. 2008, N′Guessan et al. 2009) and the oxadiazine indoxacarb (N′Guessan et al. 2007b) also were considered for use in resistance management nets. Pyriproxyfen is a potential candidate for resistance management because it shows good contact efficacy and has long residual activity and low mammalian toxicity.

The current study demonstrated that adult female *An. gambiae* that came into contact with pyriproxyfen-treated netting were sterilized or had reduced fecundity and shortened longevity. These effects can reduce the vectorial capacity of malaria vectors. Furthermore, the combined use of pyriproxyfen and pyrethroids on bed nets may help prevent the development of pyrethroid-resistant mosquitoes because pyrethroid-resistant vectors that survive contact with a net containing both chemicals would not produce offspring with pyrethroid-resistant genes due to the sterilizing effect of pyriproxyfen. These findings need to be confirmed using pyrethroid-resistant malaria vectors in both the laboratory and in the field.

Acknowledgments

We thank Y. Kuroda and K. Irogawa for assisting with the laboratory experiments. This project was supported by the Innovative Vector Control Consortium, a Product Development Partnership established as a not-for-profit company and registered charity with funding from the Bill & Melinda Gates Foundation.

References Cited

Aiku, A. O., A. Yates, and M. Rowland. 2006. Laboratory evaluation of pyriproxifen treated bednets on mosquito fertility and fecundity. A preliminary study. West Afr. J. Med. 25: 22–26.

Asidi, A. N., R. N′Guessan, A. A. Koffi, C. F. Curtis, J. M. Hougard, F. Chandre, V. Corbel, F. Darriet, M. Zaim, and M. W. Rowland. 2005. Experimental hut evaluation of bednets treated with an organophosphate (chlorpyrifos-methyl) or a pyrethroid (lambdacyhalothrin) alone and in combination against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes. Malar. J. 4: 25.

Bai, H., D. B. Gelman, and S. R. Palli. 2010. Mode of action of methoprene in affecting female reproduction in the African malaria mosquito, *Anopheles gambiae.* Pest Manag. Sci. 66: 936–943.

Bhattarai, A., A. S. Ali, S. P. Kachur, A. Martensson, A. K. Abbas, R. Khatib, A. W. Al-Mafazy, M. Ramsan, G. Rotllant, J. F. Gerstenmaier, et al. 2007. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Med. 4: e309.

Chandre, F., F. Darrier, L. Manga, M. Akogbeto, O. Faye, J. Mouchet, and P. Guillet. 1999. Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bull. W.H.O. 77: 230–234.

Charlwood, J. D., T. Smith, F. F. Billingsley, W. Takken, E.O.K. Lyimo, and J.H.E.T. Meuwissen. 1997. Survival and infection probabilities of anthropophagie anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. Bull. Entomol. Res. 87: 445–453.

Corbel, V., F. Darriet, F. Chandre, and J. M. Hougard. 2002. Insecticide mixtures for mosquito net impregnation against malaria vectors. Parasite 9: 255–259.

Corbel, V., F. Chandre, C. Brengues, M. Akogbeto, F. Lardeux, J. M. Hougard, and P. Guillet. 2004. Dosage-dependent effects of permethrin-treated nets on the behaviour of *Anopheles gambiae* and the selection of pyrethroid resistance. Malar. J. 3: 22.

Curtis, C. F. 1985. Theoretical models of the use of insecticide mixtures for the management of resistance. Bull. Entomol. Res. 75: 259–265.

Czeher, C., R. Labbo, I. Arzika, and J. B. Duchemin. 2008. Evidence of increasing Leu-Phe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticide-treated nets implementation. Malar. J. 7: 189.

Dabire, K. R., A. Diabate, L. Djogbenou, A. Ouari, R. N′Guessan, J. B. Ouedraogo, J. M. Hougard, F. Chandre, and T. Baldet. 2008. Dynamics of multiple insecticide resistance in the malaria vector *Anopheles gambiae* in a rice growing area in South-Western Burkina Faso. Malar. J. 7: 188.

Dabire, R. K., A. Diabate, T. Baldet, L. Pare-Toe, R. T. Guiguemde, J. B. Ouedraogo, and O. Skovmand. 2006. Personal protection of long lasting insecticide-treated nets in areas of *Anopheles gambiae* s.s. resistance to pyrethroids. Malar. J. 5: 12.

Dhadialla, T. S., G. R. Carlson, and D. P. Le. 1998. New insecticides with ecdysteroidal and juvenile hormone activity. Annu. Rev. Entomol. 43: 545–569.

Dye, C. 1992. The analysis of parasite transmission by bloodsucking insects. Annu. Rev. Entomol. 37: 1–19.

[FAO] Food and Agricultural Organization of the United Nations. 1999. Pesticide residues in food–1999. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection, paper 153. Food and Agricultural Organization of the United Nations, Rome, Italy.

Fegan, G. W., A. M. Noor, W. S. Akhwale, S. Cousens, and R. W. Snow. 2007. Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study. Lancet 370: 1035–1059.

Garrett-Jones, C. 1964a. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. Nature 204: 1173–1175.

Garrett-Jones, C. 1964b. The human blood index of malaria vectors in relation to epidemiological assessment. Bull. W.H.O. 30: 241–261.

Guillet, P., D. Alnwick, M. K. Cham, M. Neira, M. Zaim, D. Heymann, and K. Mukelabai. 2001a. Long-lasting treated mosquito nets: a breakthrough in malaria prevention. Bull. W.H.O. 79: 998.

Guillet, P., R. N'Guessan, F. Darriet, M. Traore-Lamizana, F. Chandre, and P. Carnevale. 2001b. Combined pyrethroid and carbamate 'two-in-one' treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus.* Med. Vet. Entomol. 15: 105–112.

Hargreaves, K., L. L. Koekemoer, B. D. Brooke, R. H. Hunt, J. Mthembu, and M. Coetzee. 2000. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med. Vet. Entomol. 14: 181–189.

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

Hawley, W. A., P. A. Phillips-Howard, F. O. ter Kuile, D. J. Terlouw, J. M. Vulule, M. Ombok, B. L. Nahlen, J. E. Gimnig, S. K. Kariuki, M. S. Kolczak, et al. 2003. Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. Am. J. Trop. Med. Hyg. 68: 121–127.

Henry, M. C., S. B. Assi, C. Rogier, J. Dossou-Yovo, F. Chandre, P. Guillet, and P. Carnevale. 2005. Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Cote d'Ivoire. Am. J. Trop. Med. Hyg. 73: 859–864.

Hirano, M., M. Hatakoshi, H. Kawada, and Y. Takimoto. 1998. Pyriproxyfen and other juvenile hormone analogues. Rev. Toxicol. 2: 357–394.

Hougard, J. M., V. Corbel, R. N'Guessan, F. Darriet, F. Chandre, M. Akogbeto, T. Baldet, P. Guillet, P. Carnevale, and M. Traore-Lamizana. 2003. Efficacy of mosquito nets treated with insecticide mixtures or mosaics against insecticide resistant *Anopheles gambiae* and *Culex quinquefasciatus* (Diptera: Culicidae) in Cote d'Ivoire. Bull. Entomol. Res. 93: 491–498.

Itoh, T., H. Kawada, A. Abe, Y. Eshita, Y. Rongsriyam, and A. Igarashi. 1994. Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. J. Am. Mosq. Control Assoc. 10: 344–347.

Kawada, H., G. O. Dida, K. Ohashi, O. Komagata, S. Kasai, T. Tomita, G. Sonye, Y. Maekawa, C. Mwatele, S. M. Njenga, et al. 2011. Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae* s.s., *Anopheles arabiensis,* and *Anopheles funestus* s.s. in Western Kenya. PLoS ONE 6: e22574.

Lindblade, K. A., T. P. Eisele, J. E. Gimnig, J. A. Alaii, F. Odhiambo, F. O. ter Kuile, W. A. Hawley, K. A. Wannemuehler, P. A. Phillips-Howard, D. H. Rosen, et al. 2004. Sustainability of reductions in malaria transmission and infant mortality in western Kenya with use of insecticide-treated bednets: 4 to 6 years of follow-up. JAMA 291: 2571–2580.

Müller, P., M. Chouaïbou, P. Pignatelli, J. Etang, E. Walker, M. Donnelly, F. Simard, and H. Ranson. 2008. Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in *Anopheles arabiensis* sampled from an area of cotton fields in northern Cameroon. Mol. Ecol. 17: 1145–1155.

Malima, R. C., R. M. Oxborough, P. K. Tungu, C. Maxwell, I. Lyimo, V. Mwingira, F. W. Mosha, J. Matowo, S. M. Magesa, and M. W. Rowland. 2009. Behavioural and insecticidal effects of organophosphate-, carbamate- and pyrethroid-treated mosquito nets against African malaria vectors. Med. Vet. Entomol. 23: 317–325.

Mani, G. S. 1985. Evolution of resistance in the presence of two insecticides. Genetics 109: 761–783.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Berge, A. L. Devonshire, P. Guillet, N. Pasteur, and D. Pauron. 1998. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol. Biol. 7: 179–184.

Mathias, D. K., E. Ochomo, F. Atieli, M. Ombok, M. Nabie Bayoh, G. Olang, D. Muhia, L. Kamau, J. M. Vulule, M. J. Hamel, et al. 2011. Spatial and temporal variation in the *kdr* allele L1014S in *Anopheles gambiae* s.s. and phenotypic variability in susceptibility to insecticides in western Kenya. Malar. J. 10: 10.

Meola, R., S. Pullen, and S. Meola. 1996. Toxicity and histopathology of the growth regulator pyriproxyfen to adults and eggs of the cat flea (Siphonaptera: Pulicidae). J. Med. Entomol. 33: 670–679.

Miler, J. E. 1994. Can pyriproxyfen (an insect growth regulator) be used to prevent selection of permethrin resistance by impregnated bed nets? Trans. R. Soc. Trop. Med. Hyg. 88: 281.

Mosha, F. W., I. N. Lyimo, R. M. Oxborough, R. Malima, F. Tenu, J. Matowo, E. Feston, R. Mndeme, S. M. Magesa, and M. Rowland. 2008. Experimental hut evaluation of the pyrrole insecticide chlorfenapyr on bed nets for the control of *Anopheles arabiensis* and *Culex quinquefasciatus.* Trop. Med. Int. Health 13: 644–652.

N'Guessan, R., P. Boko, A. Odjo, M. Akogbeto, A. Yates, and M. Rowland. 2007a. Chlorfenapyr: a pyrrole insecticide for the control of pyrethroid or DDT resistant *Anopheles gambiae* (Diptera: Culicidae) mosquitoes. Acta Trop. 102: 69–78.

N'Guessan, R., V. Corbel, J. Bonnet, A. Yates, A. Asidi, P. Boko, A. Odjo, M. Akogbeto, and M. Rowland. 2007b. Evaluation of indoxacarb, an oxadiazine insecticide for the control of pyrethroid-resistant *Anopheles gambiae* (Diptera: Culicidae). J. Med. Entomol. 44: 270–276.

N'Guessan, R., P. Boko, A. Odjo, B. Knols, M. Akogbeto, and M. Rowland. 2009. Control of pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes with chlorfenapyr in Benin. Trop. Med. Int. Health 14: 389–395.

Patterson, J. W. 1974. A comparison of the morphogenetic and sterilizing activities of juvenile hormone mimics on *Aedes aegypti.* J. Insect Physiol. 20: 2095–2106.

Ranson, H., R. N'Guessan, J. Lines, N. Moiroux, Z. Nkuni, and V. Corbel. 2011. Pyrethroid resistance in African

anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 27: 91–98.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org).

Santolamazza, F., M. Calzetta, J. Etang, E. Barrese, I. Dia, A. Caccone, M. J. Donnelly, V. Petrarca, F. Simard, J. Pinto, et al. 2008. Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. Malar. J. 7: 74.

SAS Institute. 2009. JMP user's guide, release 8. SAS Institute, Cary, NC.

Shapiro, A. B., G. D. Wheelock, H. H. Hagedorn, F. C. Baker, L. W. Tsai, and D. A. Schooley. 1986. Juvenile hormone and juvenile hormone esterase in adult females of the mosquito *Aedes aegypti*. J. Insect Physiol. 32: 867–877.

Tabashnik, B. E. 1989. Managing resistance with multiple pesticide tactics: theory, evidence, and recommendations. J. Econ. Entomol. 82: 1263–1269.

[WHO] World Health Organization. 2010. World malaria report 2010. (http://www.who.int/malaria/world_malaria_report_2010/en/index.html).

Zaim, M., and P. Guillet. 2002. Alternative insecticides: an urgent need. Trends Parasitol. 18: 161–163.

Zaim, M., A. Aitio, and N. Nakashima. 2000. Safety of pyrethroid-treated mosquito nets. Med. Vet. Entomol. 14: 1–5.

Received 15 January 2012; accepted 26 May 2012.