AN EPISODE OF RESISTANCE TO PERMETHRIN IN LARVAE OF *SIMULIUM SQUAMOSUM* (DIPTERA: SIMULIIDAE) FROM CAMEROON, AFTER 3½ YEARS OF CONTROL

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ABSTRACT. Because of the biting nuisance from females of *Simulium squamosum*, a 30 km section of the Sanaga River (Cameroon) was treated since 1987 with permethrin for the control of larval populations. In 1990, resistance to permethrin occurred in a small proportion of the larvae, with a resulting 2–4× increase of the LC_{95} for dead larvae (moribund larvae considered as live). In 1991, after a 6-month interruption of the treatments, susceptibility to permethrin returned to the initial level, and was similar to the susceptibility of *S. squamosum* larvae from a non-treated section of the Sanaga. In the context of a small-scale control program, resistance to permethrin can be reversible, and it can be avoided by rotation with other types of insecticides such as *Bacillus thuringiensis* serovar. *israelensis*.

Simulium squamosum (Leuckart), a member of the *Simulium damnosum* complex, is a major vector of onchocerciasis in Cameroon. It breeds extensively in the largest river of the country, the Sanaga. In the 250 km river section between Mbandjock and Edéa, adult females are the cause of hyperendemic foci of human onchocerciasis and a major source of nuisance. In the vicinity of the Song-Loulou dam, onchocerciasis is not so severe (Ripert et al. 1977) but the nuisance, which can reach 8,000 bites/man/day (Chauvet et al. 1982), has justified the control of larval blackfly populations. Because of the resistance to temephos and chlorphoxim (Traoré-Lamizana et al. 1985), larval populations of *S. squamosum* have been controlled since 1987 by weekly ground treatments, mostly with permethrin (20% EC, 0.06 liter per m^3/sec of river discharge) and occasionally at lower discharges with *Bacillus thuringiensis* serovar. *israelensis*. At high discharges there is no alternative to the use of permethrin because of the costs involved and of the resistance to organophosphate insecticides. The control operations have substantially reduced the nuisance in the area of Song-Loulou (Lochouarn et al. 1987), in spite of the short section treated (30 km) and the strong recolonization potential of the upstream, non-treated, section of the Sanaga River.

We report below changes in the susceptibility of *S. squamosum* larvae to permethrin that occurred at Song-Loulou after 3 ½ years of treatments. The first bioassays were performed in 1987, at the start of the permethrin treatments of the Sanaga. Populations of *S. squamosum* were sampled in the treated section (Song Loulou dam), after a short interruption of the treatment, and in a non-treated section 200 km upstream (Nachtigal). These results were used as baseline data. The second series of tests took place in 1990, after 180 weekly cycles of treatment with permethrin. The third series was performed in March 1991, when treatments had been interrupted during 6 months for lack of larvicide.

The susceptibility test protocol is derived from that proposed by Mouchet et al. (1977). Larvae (instars 6–7) are sorted and distributed by lots of 25 in porcelain bowls filled with distilled water, where they are exposed to a solution of insecticide for 3 h at 23–25°C without agitation. There are 6–7 concentrations and 3 replicates per concentration. The stock solution of insecticide is prepared with ethanol, the end concentration of which is equal in all bowls, including the controls. After 3 h, pupae and young larvae are discarded, and the remaining mature larvae are sorted as dead, moribund or live (Guillet et al. 1980). Therefore the response is threefold, as opposed to the more common dichotomous type (Finney 1971). All percentages are corrected for control mortality using Abbott's formula, and 0 and 100% percentages are transformed using Berkson's adjustment by counting at least 0.1 larva dead, moribund or live (Finney 1971).

The susceptibility of the blackfly populations tested was evaluated by 2 methods. The first comprises determining the empirical LC_{100}, estimated as the lowest concentration killing all larvae (LC_{100} [dead only]) or leaving only dead or moribund larvae (LC_{100} [dead + moribund]). The second method, used for the analysis of results of the Onchocerciasis Control Programme (OCP), comprises estimating the LC_{95} or "diagnostic dose" and the LC_{50} by regression

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analysis. Experience in the OCP has shown that regression analysis by the probit method does not provide accurate estimates, especially for high mortalities, because of the nonlinearity of mortality percentages, once they have been transformed in probits or logits (Hewlett and Plackett 1979) and plotted against the log of concentrations. Therefore another method of regression analysis has been preferred: 1) ten extra logits are interpolated linearly between each pair of successive points and 10 points are extrapolated from all observed values for extremely high and low doses, and 2) two 4th-degree polynomial curves are fitted by multiple regression to the observed, interpolated and extrapolated points, one for the mortality logits of dead larvae only, and one for the mortality logits of dead plus moribund larvae. The resulting polynomes (concentration = *f* [mortality]) give estimates of LC_{50} and LC_{95}s for both curves that are more accurate than those obtained by classical methods, but confidence intervals cannot be calculated.

Results of the tests in Nachtigal (Table 1) show that in this untreated area there was no significant change in the susceptibility to permethrin over a 4-year period. Values of LC_{50}, LC_{95} and LC_{100} remained consistent throughout, for both [dead + moribund] and [dead only] mortality curves.

In 1987, susceptibility levels to permethrin were the same in Song-Loulouu as in Nachtigal. But in 1990 a few live larvae were observed at higher concentrations than usual, which resulted in higher LC_{100}s(10 μg/liter instead of 5 μg/liter) for the (dead + moribund) mortality curves. Due to the presence of moribund larvae at still higher concentrations there was a more noticeable increase (2–4×) in LC_{95}s for the [dead only] mortality curves. In one of the tests of 1990, the LC_{100} [dead only] was estimated between 50 and 250 μg/liter, compared to 5 μg/liter in 1987; in the 2 other tests it was estimated to be above 10 μg/liter. In 1991, after a 6-month interruption of permethrin treatments in Song-Loulou, susceptibility levels to permethrin were the same as those of 1987.

This is the first time that blackfly resistance to permethrin has been reported. Permethrin has been used by the OCP for blackfly control since 1985, and resistance was not noticed until now. It must be noted that on the Sanaga River the selective pressure for resistance to permethrin was relatively high, although limited in space. As a comparison, the OCP uses permethrin only for 6 wk per year, in rotation with other insecticides, and only at river discharges above 70 m^3/sec, with a lower dosage rate of 0.045 liter of 20% EC per m^3/sec of river discharge (Kurtak 1990). The quick return to normal levels of susceptibility on the Sanaga warrants the strategy adopted by the OCP as a method of limiting the risks of resistance.

Larvae of *S. squamosum* of the Sanaga are still strongly resistant to temephos and chlorphoxim, although organophosphate insecticides have not been used for blackfly control for several years. A permanent resistance has been recorded among populations of *Simulium sanctipauli* and *Simulium damnosum s.s.* of the Comoe River in southeastern Ivory Coast. A positive correlation was observed there between resistance to chlorphoxim and susceptibility to permethrin (Kurtak et al. 1987). This was not the case for the *S. squamosum* populations of the Sanaga, which were simultaneously resistant to permethrin and chlorphoxim, and highly resistant to temephos.

Only a small proportion of the *S. squamosum* population from Song-Loulou became resistant to permethrin, and just for a short time. The large populations breeding in the upstream, non-

Table 1. Susceptibility tests with permethrin on larvae of *Simulium squamosum* from the Sanaga River (Cameroon).

Location	Date	Number larvae tested	Lethal concentrations of permethrin (μg/liter)					
			Dead = dead + moribund larvae			Dead larvae only		
			LC_{50}	LC_{95}	LC_{100}	LC_{50}	LC_{95}	LC_{100}
Song-Loulou (treated)	Mar. 1987	811	1.65	2.46	5.0	2.40	4.95	>5.0
	Jan. 1990	431	1.22	3.73	10.0	1.88	9.18	>10.0
		420	1.70	4.90	10.0	2.92	11.97	>10.0
		525	1.61	4.30	10.0	2.33	19.03	50 ≪ 250
	Feb. 1991	544	1.32	3.66	3.5	2.91	4.66	>3.5
Nachtigal (not treated)	Mar. 1987	915	1.43	3.55	5.0	2.40	6.30	>5.0
	Oct. 1990	496	0.97	1.83	3.5	1.42	3.19	>3.5
	May 1991	440	0.60	2.31	3.5	1.16	5.03	>3.5

treated, portion of the Sanaga would suffice to explain this reversal. On operational grounds, this means that permethrin may still be used for local nuisance control in situations similar to the Song-Loulou dam, as long as there are interruptions in the treatment schedule, or rotations with insecticides belonging to different groups. *Bacillus thuringiensis* serovar. *israelensis*, a very selective bacterial insecticide (Guillet et al. 1990), can serve this purpose, especially at low waters, with the added advantage of reducing the insecticide pressure on non-target populations.

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