DETECTION OF RESISTANCE TO TEMEPHOS AND CHLORPHOXIM IN *SIMULIUM DAMNOSUM* S.L. BY TOPICAL APPLICATION TO ADULTS

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ABSTRACT. Resistance to temephos and chlorphoxim has been found in larvae of some cytospecies of the *Simulium damnosum* complex in West Africa. Topical application of these chemicals to adult females caught near rivers containing resistant larvae reveals a clear reduction in susceptibility. This fact is very useful in monitoring resistance.

INTRODUCTION

The Onchocerciasis Control Programme (OCP) being executed by the World Health Organization has been described in several publications (Davies et al. 1978, Walsh et al. 1979). The goal of this vast vector program is to reduce the transmission of onchocerciasis to the point where the disease is no longer a serious public health problem in 7 West African countries (Benin, Ghana, Ivory Coast, Mali, Niger, Togo and Upper Volta). This effort is based on weekly applications of larvicides to the riverine breeding sites of the immature stages of *Simulium damnosum* s.l., the only vector in West Africa.

It is estimated that these larvicide treatments must continue for 10 to 15 years to achieve the desired level of disease reduction. Resistance is thus a serious threat. Resistance to temephos (Abate®) occurred in two "forest" cytospecies (*S. soubrense* and *S. sanctipauli*) of the vector complex[2] in one part of the OCP area in 1980 (Guillet et al. 1980), 5 years after the beginning of the OCP and a little over one year after the beginning of treatments in that area. Resistance to chlorphoxim occurred and cross-resistance to several other organophosphate compounds was demonstrated one year later (Kurtak et al. 1982). This has led to the widespread use of formulations of *Bacillus thuringiensis* (H-14), sometimes in alternance with organophosphates.

These changes in the susceptibility of the target populations have made it extremely important to continually monitor susceptibility to several compounds, both within and without the zone currently affected by resistance. This monitoring is now based on a larval susceptibility test (Mouchet et al. 1977). Nearly 150 of these tests were carried out in the OCP area in the last 6 months of 1983. However, in the rainy season collecting larvae for tests is not easy as access to the flooded rivers is difficult and sometimes dangerous. Even when boats are used, the larvae are often so widely scattered in the abundant trailing vegetation that it is impossible to gather sufficient numbers for a test. Larvae can also be reared from wildcaught female flies (Raybould et al. 1979), but it is difficult and several weeks may pass before the larvae reach the age required for testing.

These problems led the authors to attempt to determine if the resistance seen in the larvae could be detected in the adults. Frequently, a treatment failure in the rainy season is only detected by the presence of hundreds of biting females on the river banks and no or very few larvae for the reasons given above. In previous work on testing insecticides against adult *S. damnosum* s.l., Guillet et al. (1982) indicated that topical application was impractical and extensively investigated exposure to treated surfaces. After initial trials, the present authors found that topical application could be carried out if the volume of solvent did not exceed 0.05 microliter. At the same time, exposure to treated surfaces gave variable results according to the exact method of treating the surfaces.

This paper gives details of the topical application technique that was developed and illustrates its practical use.

MATERIALS AND METHODS

The general subject of topical application has been reviewed by Busvine (1971). The technique developed for blackflies by the authors is described in some detail since there is little or no published work on the subject.

Flies were obtained from human bait collecting as described in the general papers cited in

[1] World Health Organization Onchocerciasis Control Programme, OMS/ONCHO, B.P. 549, Ouagadougou, Haute Volta, West Africa.

[2] In the OCP area, *S. damnosum* s.l. is a complex which includes 6 common cytospecies. They can be separated broadly in "forest" and "savannah" groups according to their ecological associations (Vajime and Dunbar 1975, Vajime and Quillévéré 1978). Operationally, they can be roughly separated on the basis of adult morphology (Kurtak et al. 1981).

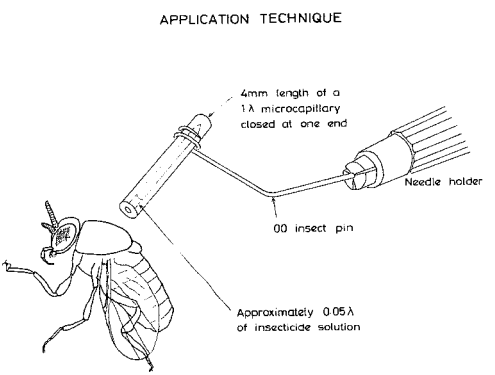

Fig. 1. Application technique.

the introduction. The sample thus obtained contained a mixture of parous and nulliparous females. To evaluate the possible variations with physiological age, all flies were dissected after testing and scored as parous or nulliparous.

Flies were usually tested on the day after they were caught. They were held in 15 × 55 mm polystyrene tubes (1 to 10 per tube) before the test. These tubes were wrapped in moist cotton wool and kept between 22 and 28°C. In most individual tests, the variation during the 24 hour observation period did not exceed 2°C.

Just before the test, the flies were transferred in groups of 20 into a cylindrical holding cage (120 × 50 mm). Flies which were abnormal in any way were eliminated. Carbon dioxide was administered for a few seconds, and the anesthetized flies then deposited in a holding tray with a weak flow of CO_2. Individual flies were picked up by the wings with a flexible forceps and transferred to the stage of a stereomicroscope for the application of the insecticide. The application (Fig. 1), was made with a 4 mm length of 1 microliter microcapillary (Drummond Microcap®) melted at one end and fixed in a holder. When touched to the surface of the solution desired, this tube picked up a constant volume of solution which was then transferred to the scutum of the fly. By optical measurement, this volume was approximately 0.05 microliter. Since our laboratory does not have facilities for direct calibration of small volumes, it was not possible to determine the volume precisely. However, it is reasonably consistent between applications and between duplicate capillaries; and, since all the tests are compara-

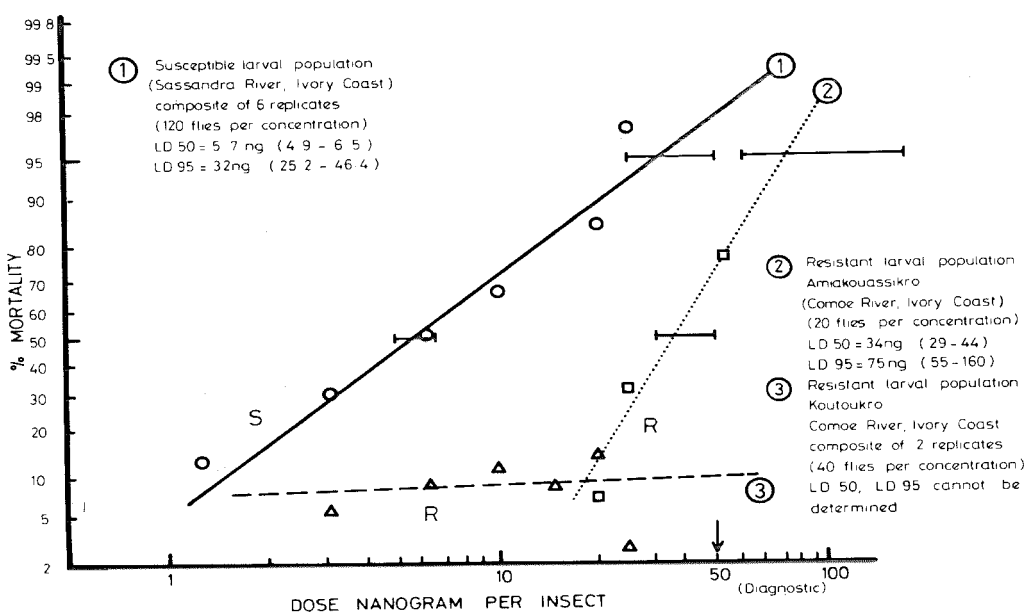

Fig. 2. Dose – mortality curves for adult female *Simulium damnosum* s.l. (= forest cytospecies *S. soubrense/ sanctipauli*) exposed to temephos. Comparison of flies from areas with resistant and susceptible larval populations. 95% confidence intervals in parentheses. 24 hr observation. Parous and nulliparous flies together.

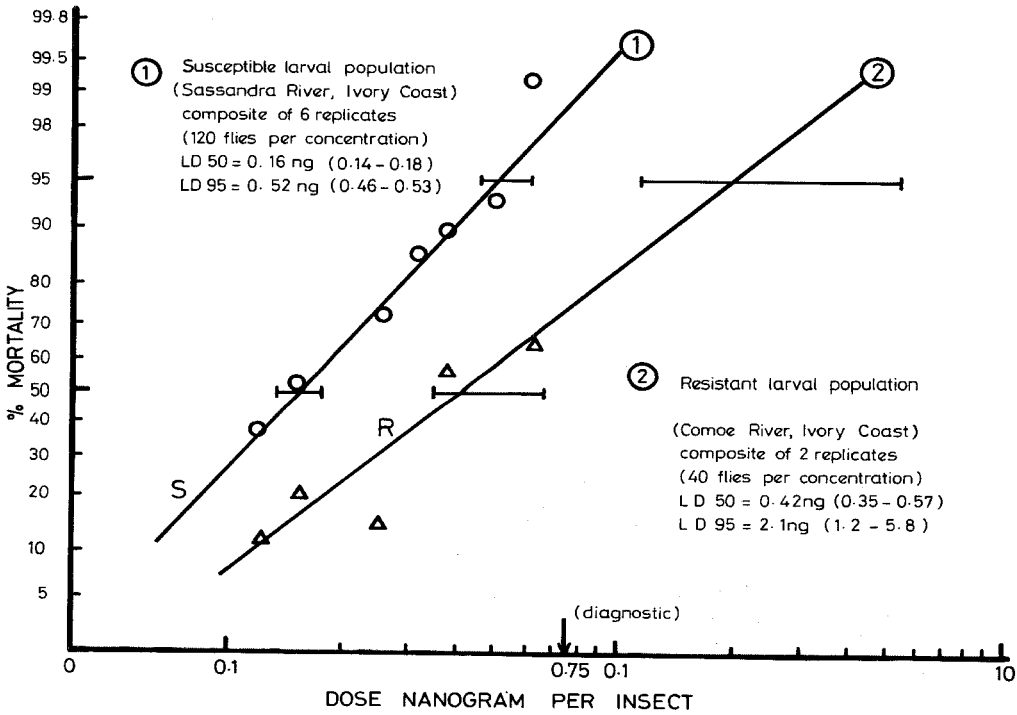

Fig. 3. Dose — mortality curves for adult female *Simulium damnosum* s.l. (= forest cytospecies *S. soubrense/sanctipauli*) exposed to chlorphoxim. Comparison of flies from areas with resistant and susceptible larval populations. 95% confidence intervals in parentheses. 24th observation. Parous and nulliparous flies together.

tive, it is not absolutely necessary to know the precise volume.

Technical material of the insecticide (temephos or chlorphoxim)[3] was dissolved in absolute ethanol for application. A series of solutions of increasing concentration was prepared to get a range of doses since the volume applied was constant. Absolute alcohol (0.05 microliter) was applied to the control group.

After application, the flies were transferred to individual tubes of the same type as used for the capture, but with a wick moistened with sucrose solution, a piece of applicator stick for a support, and a liner of slightly dampened filter paper. They were observed 5 minutes after application and those not recovering from the CO_2 were eliminated. The total exposure to CO_2 was about 5 minutes. The tubes were then stored in an insulated box at 25–30°C. Mortality was observed at 3, 6 and 24 hours after application. All flies were dissected to determine parous rate and infections, either as they died or at the end of the observation period.

In general, 20 flies were treated at each of 6 doses. For temephos, 1 to 100 nanograms per insect were applied and for chlorphoxim 0.1 to 10 nanograms per insect. This required about 3 hours for a team of 3 persons. Six replicates were carried out with flies in an untreated area where no resistance was present in the larvae and 3 replicates with flies caught along a river containing resistant larvae. Results were analyzed by probit analysis using a Hewlett-Packard HP 41CV programmable calculator. The program was written by Dr. B. Grab, formerly of the Health Statistical Methods Section of WHO, Geneva.

RESULTS

The composite results for flies supposed to be "resistant" and "susceptible" to temephos and

[3] Furnished by the Pesticide Development and Safe Use Unit of the Vector Biology and Control Division, WHO Headquarters, Geneva.

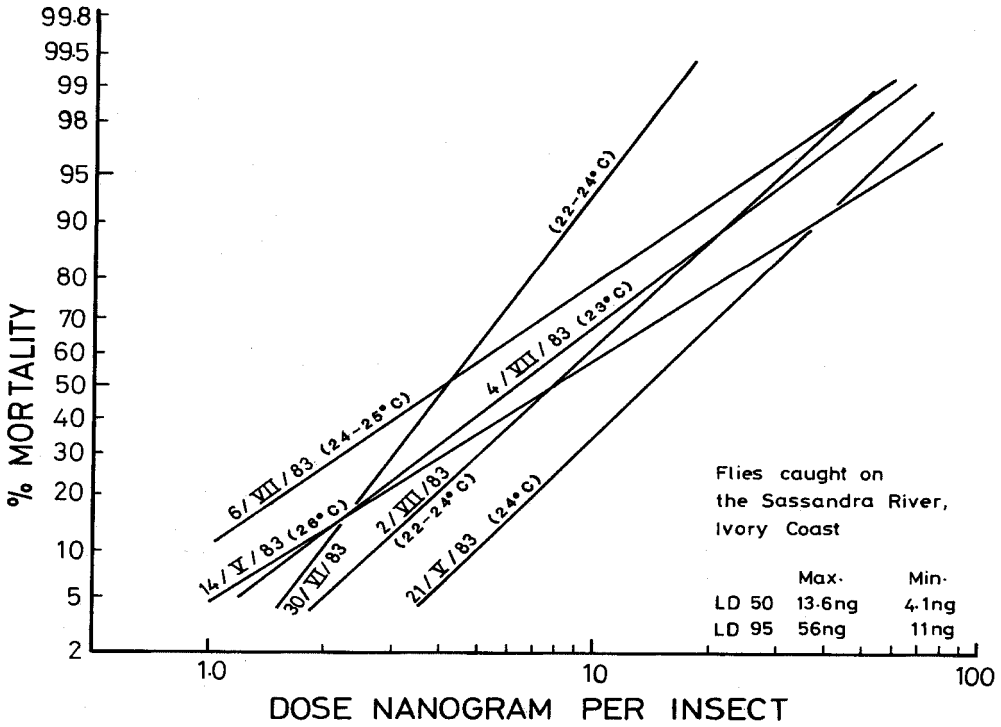

Fig. 4. Dose – mortality curves for adult female *Simulium damnosum* s.l. (= forest cytospecies *S. soubrense/sanctipauli*) exposed to temephos. Individual curves for 6 replicates with flies from an area with susceptible larvae (20 flies per dose per replicate). 24 hr observation. Parous and nulliparous flies together. Temperature during observation period in parentheses.

chlorphoxim are presented in Figs. 2 and 3. These data group parous and nulliparous flies observed after 24 hours. There is a significant difference between the two groups for both insecticides.

Figures 4 and 5 show the dose-mortality lines determined in the same way for the individual tests (20 flies/concentration) with the two products. The range of the individual tests is much larger than the confidence interval of the composite, showing the necessity for replicates. Nonetheless, the data with "resistant" flies are significantly different. There is virtually no overlap between the lower limit of the 95% confidence interval of the "resistant" data with even the highest LD_{50} and LD_{95} values obtained in the individual replicates with "susceptible" flies.

Although the temperature during the holding period varied somewhat from test to test and during certain tests, these variations have no consistent relationship to the results, since the highest and lowest LC50 values in each group were obtained at nearly the same temperature.

Figures 6 and 7 show the comparison of parous and nulliparous flies for the "susceptible" group for both chlorphoxim and temephos. There is no significant difference between parous and nulliparous in either case.

Finally, Figs. 8 and 9 present the results obtained with the "susceptible" group 3, 6 and 24 hours after application for the two compounds. The mortality increases only slightly after 3 hours with chlorphoxim but is significantly higher at 6 and 24 hours with temephos.

DISCUSSION AND CONCLUSION

The results clearly demonstrate that resistance to temephos and chlorphoxim in larval

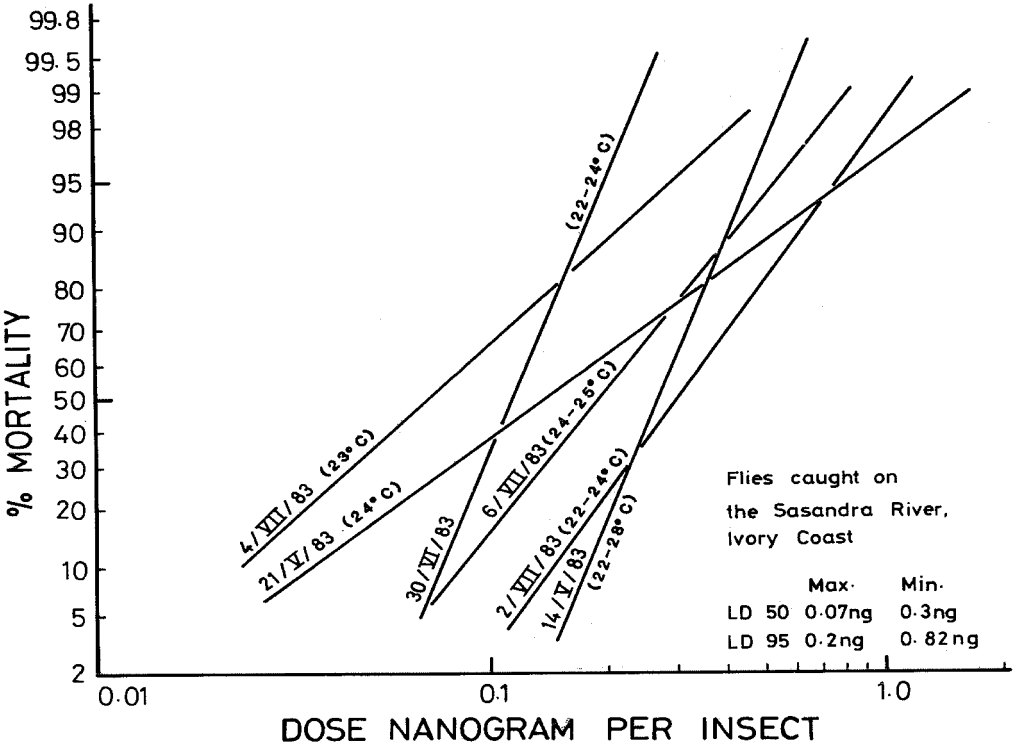

Fig. 5. Dose – mortality curves for adult female *Simulium damnosum* s.l. (= forest cytospecies *S. soubrense/sanctipauli*) exposed to chlorphoxim. Individual curves for 6 replicates with flies from an area with susceptible larvae (20 flies per dose per replicate). 24 hr observation. Parous and nulliparous flies together. Temperature during observation period in parentheses.

populations is reflected in the adults. The LC_{50} and LC_{95} are significantly[4] higher for the "resistant" group for both products. For temephos, the slope of at least part of the dose-mortality curve is also significantly[5] different. The results also established the following diagnostic doses for resistance:

Chlorphoxim: 0.75 nanogram/insect.
Temephos : 50 nanogram/insect.

The precision of this dose is limited by the precision of the application method as described above, which is probably of the order of ± 20%. In the near future, this work will be repeated with a Burkard microapplicator equipped with a microliter syringe. This will allow a more precise determination of the dose. In any case, the diagnostic dose is 1.5 times the observed LC_{100} (susceptible) for chlorphoxim and 2 times the observed LC_{100} (susceptible) for temephos.

The various parameters of the test may be discussed as follows:

[4] Two LC50 or LC95 values were judged to be significantly different when there was no overlapping of their 95% confidence intervals. (P therefore ≤ 0.05).

[5] Two slopes were compared by computing a z value based on the variances of the slopes by the equation:

$$Z = \frac{b_1 - b_2}{\sqrt{s.e^2(b_1) + s.e^2(b_2)}}$$

where b_1 and b_2 = the slopes and $s.e.b_1$ and $s.e.b_2$ = their standard errors respectively. Differences were considered significant when

$z \geq 1.96$ or $z \leq -1.96$ ($p \leq 0.025$).

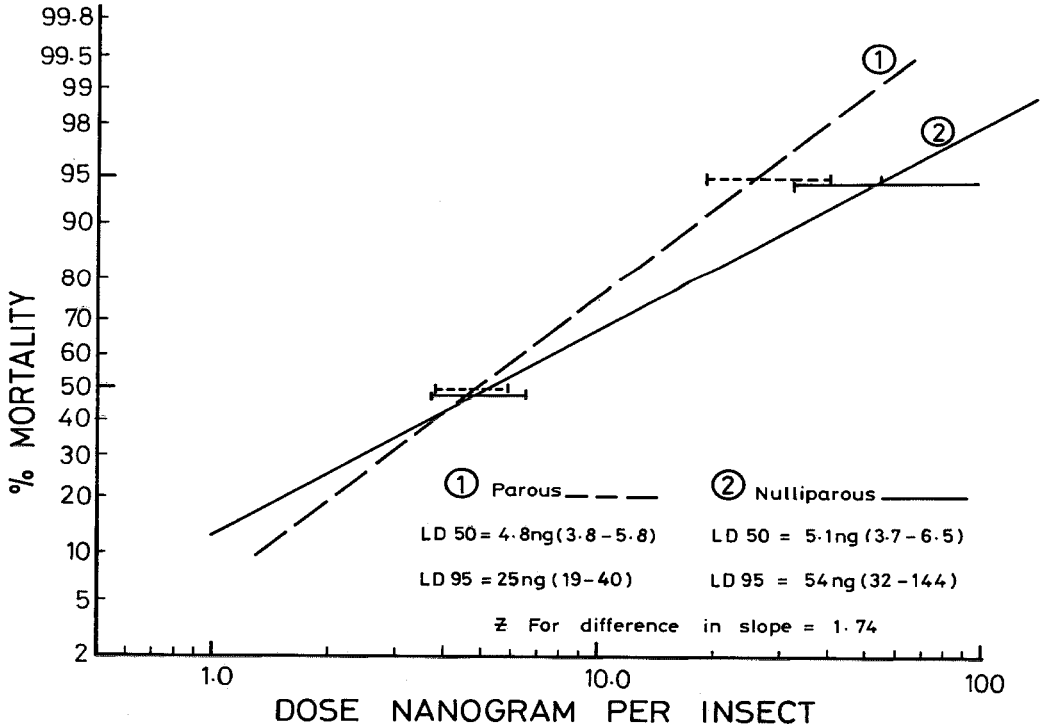

Fig. 6. Comparison of mortality in parous and nulliparous *S. soubrense/sanctipauli* exposed to temephos. Susceptible larval population. 24 hr. observation. Composite of 6 replicates.

NUMBER OF REPLICATES. To determine the baseline LC_{50} and L_{95} with an acceptably narrow confidence interval, it was necessary to perform at least 4 replicates with 6 concentrations and 20 flies per concentration per replicate. It may be possible to use more flies per replicate with mechanical application equipment. For the diagnosis of resistance, 2 replicates with 50 flies each at the diagnostic dose are probably sufficient.

PAROUS VS. NULLIPAROUS FLIES. Although there is an impression that nulliparous flies are somewhat less susceptible and survive better in the control groups, this was not apparent at the level of the LC_{50} and LC_{95} where no significant differences were found. Still, it is prudent to dissect the test flies and record the data separately for parallel analysis if possible.

TIME OF OBSERVATION. For chlorphoxim, there was little increase in mortality after 6 hours, and 3–6 hours was sufficient. For temephos, mortality increased steadily and 24 hours gave lower values and a slightly steeper slope. Thus a uniform 24-hour observation period is recommended.

APPLICATION OF THE TEST IN TREATMENT STRATEGY. Figure 10 schematically presents a typical operational problem facing OCP treatment and evaluation personnel. In the beginning of 1983, the Sassandra River in Ivory Coast was being treated with temephos which killed only the susceptible "savannah" cytospecies of the *S. damnosum* complex. This was followed by an intentional suspension for experimental work followed by treatment with *B.t.* H-14 formulation which was effective against all cytospecies. However, the river was rising rapidly and soon surpassed the limit (50 m^3/sec) for the use of *B.t.* H-14. Beyond this, the volume of *B.t.* H-14 to apply (roughly 1 liter/m^3/sec of discharge) is too great to be practical. At this point (early July) the river was put under chlorphoxim treatment. Resistance to chlorphoxim had occurred in the "forest" cytospecies in this

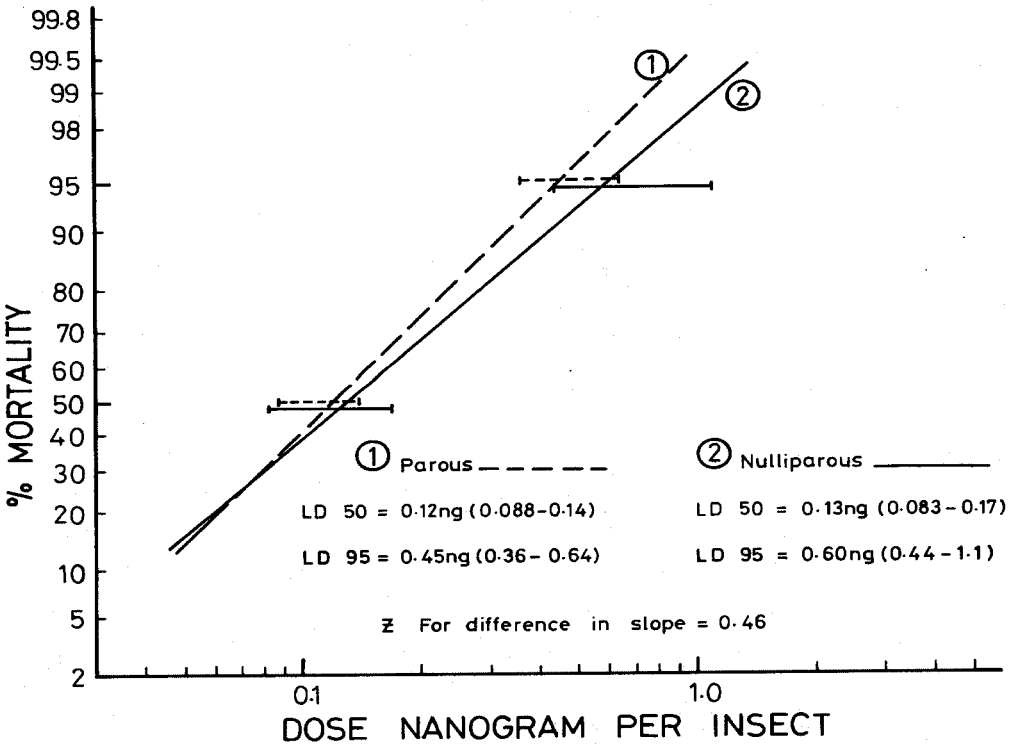

Fig. 7. Comparison of mortality in parous and nulliparous *S. soubrense/sanctipauli* exposed to chlorphoxim. Susceptible larval population. 24 hr. observation. Composite of 6 replicates.

river in 1981, but had reverted in 1982. It was certain, however, that resistance would return after an indeterminate time. Previous experiments had indicated this would be of the order of 4 months, and that the presence of surviving larvae at 0.125 mg/liter in the susceptibility test would coincide with treatment failure.

Chlorphoxim treatments continued quite successfully from early July to mid-September. In mid-September the adult fly catch started to go up. At the same time, floods prevented careful checks of the breeding sites. The fly count began to decline. As the floods receded, a few larvae were found after treatment in early October. However, when a susceptibility test team reached the area, no more larvae could be found even with an extensive search by helicopter. This was probably due to a particularly abrupt drop in water level which disturbed breeding conditions. Adult flies were being caught and on October 25, an adult test showed resistance to chlorphoxim and the decision was taken to return to *B.t.* H-14. This was not confirmed with larval tests until mid-November. Unfortunately, logistical problems prevented implementing *B.t.* H-14 treatments until early December. Nevertheless, the adult susceptibility test had amply fulfilled its role in the decision-making process.

ACKNOWLEDGMENTS

In a program like OCP, any published result is the fruit of the collaboration of a large number of personnel at all levels, from the entomologists in charge of aerial treatments and evaluation, the pilots who carry out prospection and treatment missions, to the drivers and vector collectors. We thank them all heartily without attempting to mention anyone by name.

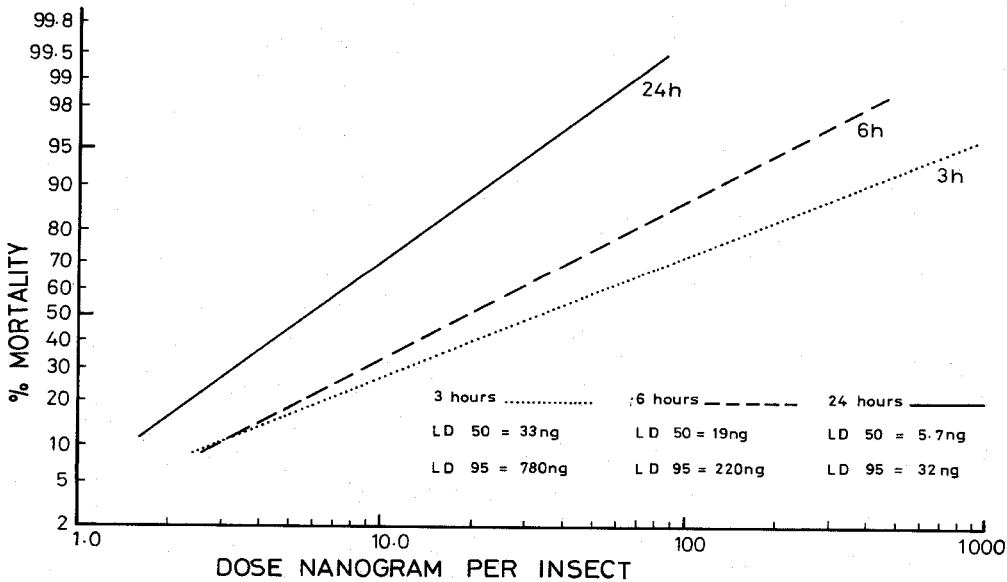

Fig. 8. Comparison of mortality in adult female *S. soubrense/sanctipauli* 3, 6, and 24 hr after treatment with temephos. Susceptible larval population. Parous and nulliparous flies together. Composite of 6 replicates.

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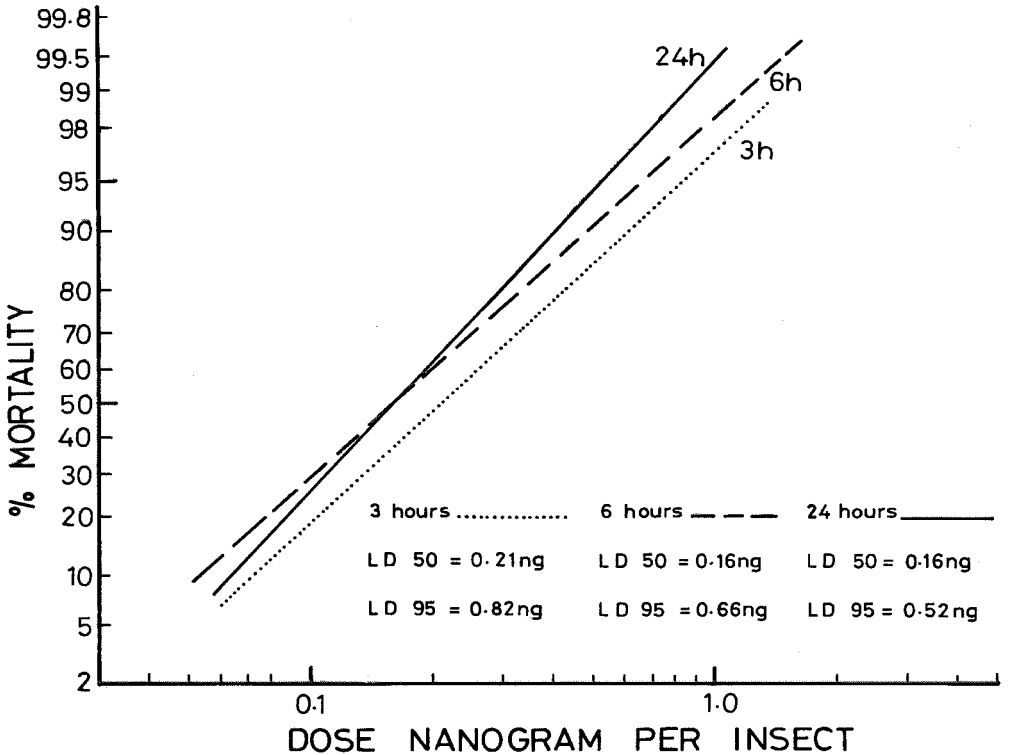

Fig. 9. Comparison of mortality in adult female *S. soubrense/sanctipauli* 3, 6, and 24 hr after treatment with chlorphoxim. Susceptible larval population. Parous and nulliparous flies together. Composite of 6 replicates.

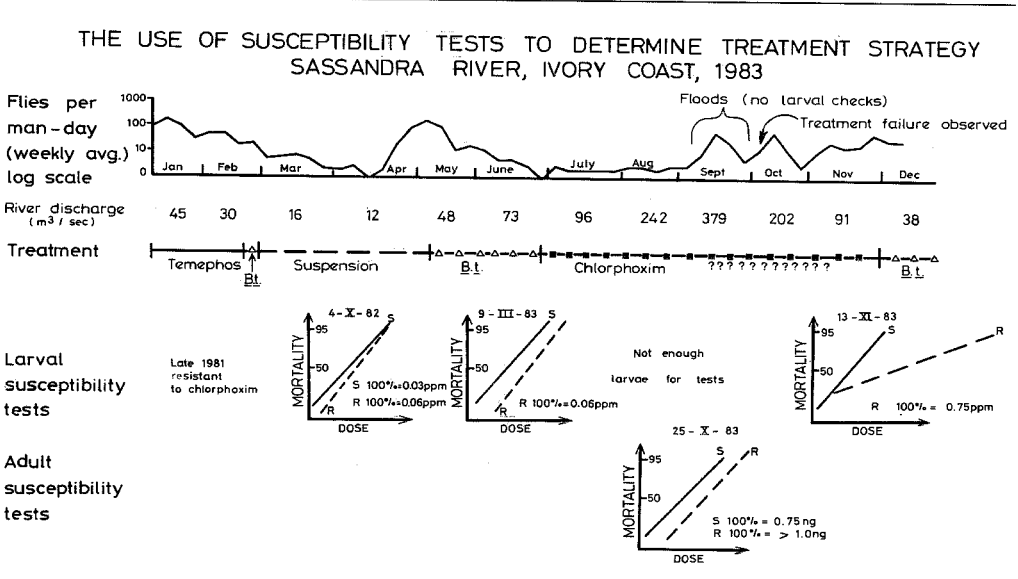

Fig. 10. Example of the use of susceptibility testing in determining strategy of insecticide use.