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ASSESSMENT OF *BACILLUS THURINGIENSIS* SEROTYPE 14 AND *STEINERNEMA FELTIAE* [*NEMATODA : STEINERNEMATIDAE*] FOR CONTROL OF THE *SIMULIUM* VECTORS OF ONCHOCERCIASIS IN MEXICO

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Field evaluations of *Bacillus thuringiensis* Berliner serotype 14 and the nematode *Steinernema feltiae* Filipjev 1934 (= *Neoaplectana carpocapsae*) were conducted against *Simulium ochraceum* Walker and other simuliid vectors of onchocerciasis in Mexico. *B. thuringiensis* was highly toxic, causing up to 100 % larval mortality, but only for short distances downstream. Increased dosage did not enhance downstream carry of *B. thuringiensis*. Highest rates of mortality were recorded against early instar larvae. Stream treatment with *S. feltiae* did not result in significant larval mortality because the infective-stage nematodes were either not ingested or were injured during ingestion by the larval mouthparts.

Current efforts to control human onchocerciasis (river blindness) have centered on elimination of the black fly vectors, thus interrupting the transmission cycle between the vector and the causative agent, *Onchocerca volvulus* Lenck. This approach has been the subject of a major project in West Africa since 1971 : the World Health Organization's Onchocerciasis Control Programme (**Davies** *et al.*, 1978). A large scale vector control program for the other major foci, in Central America, has not been initiated. In contrast to West Africa, where vector black fly populations inhabit large river systems, Central American vectors breed in very small streams under dense vegetative cover in difficult mountainous terrain. Consequently, the applicability of the African vector control approach to conditions in the American onchocerciasis foci is limited at best.

Control efforts in West Africa against the vector complex, *Simulium damnosum* Theobald, have used the organophosphate temephos (Abate ®). In Central America, where the principal vector of onchocerciasis is *S. ochraceum* Walker (although *S. callidum* Dyar & Shannon and *S. metallicum* Bellardi have also been implicated), vector control efforts have been largely experimental but have also relied on chemical larvicides. DDT was used against larvae of vector species as early as 1944 (**Fairchild & Barreda**, 1945). More recently, a pilot study conducted in Guatemala using temephos provided an 84 % reduction of black flies in isolated areas dominated by perennial streams. Insufficient control, however, was obtained in areas with numerous temporary streams (**Takaoka** *et al.*, 1981).

The adoption of nonchemical agents for *Simulium* control has been a recent development. Extensively tested and highly effective (see review by **Gaugler & Finney**, 1982) the entomopathogenic bacterium *Bacillus thuringiensis* (serotype 14) has emerged as the black fly larvicide of choice in areas where chemical control agents are unsuitable. Its field efficacy has been demonstrated in numerous trials in the United States and Canada, as well as in Africa against *S. damnosum*. **Lacey** *et al.* (1982), for example, reported that a single treatment completely removed *S. damnosum* for a distance of 19 km in one African river. Consequently, the bacterium is replacing temephos in areas within the Onchocerciasis Control Programme where resistance is a problem. In the single reported field trial against American vectors of *O. volvulus*, *B. thuringiensis* proved to be highly toxic to *S. ochraceum* in Guatemala, although only for short distances (**Undeen** *et al.*, 1981). **Briggs** *et al.* (1981) demonstrated the sensitivity of Mexican vectors of onchocerciasis to the bacterium in laboratory tests. *Steinernema feltiae* Filipjev 1934 (= *Neoaplectana carpocapsae*), a nematode which parasitizes numerous terrestrial insects (**Gaugler**, 1981), is also lethal to black flies and has been field tested in New York State against *S. vittatum* (**Gaugler & Molloy**, 1981b). We report the results of field trials with these two biological control agents against vectors of *O. volvulus* in Mexico.

MATERIALS AND METHODS

An aqueous concentrate of the Sandoz, Inc. (San Diego, CA) "Teknar WDC" formulation (Potency : 600 I.T.U./mg ; lot n° 21501) was used for *B. thuringiensis* applications. The Mexican strain of *S. feltiae* produced in crickets by BR Supply Company (Exeter, CA) was used as the nematode inoculum. The infective-stage nematodes were hand carried in an ice chest from California to Mexico under continuous aeration to ensure high viability.

Stream trials were conducted during the dry season, December 1981, near the village of Morelos within the Soconusco onchocerciasis focus. The largest (6800 km^2) of the 3 zones in Mexico where onchocerciasis is endemic, the Soconusco focus, is in the State of Chiapas and borders on Guatemala.

Three streams from the Centro de Investigaciones Ecologicas del Sureste (CIES) onchocerciasis study area were selected for treatment : Southwest I (SWI), Northwest III (NWIII) and Southwest II (SWII). All 3 streams were turbulent and rocky, and located on steep mountain slopes. Most black fly larvae inhabiting these streams were attached to fallen leaves as there was minimal trailing vegetation. Pupation was not noted during the test period.

The SWI stream averaged 62 cm in width, 7.2 cm in depth, and had a discharge rate of 1020 liters/min. Stream temperature was 19.4 °C for the SWI stream at the time of treatment. Species composition prior to treatment was 50 % *S. ochraceum*, 38 % *S. metallicum* and 12 % *S. callidum*. Pretreatment counts of early, mid and late instar larvae (differentiated by relative size) were 41, 29.5 and 29.5 %, respectively. This stream was treated with the Teknar formulation at a concentration of 45 ppm (9.6 x 10^5 spores/ml) for 0.5 min.

The stream identified as NWIII averaged 57 cm wide and 5.8 cm deep. The discharge rate was 1080 liters/min and the water temperature 18.5 °C at the time of treatment. The pretreatment larval population was made up of 66 % *S. ochraceum*, 23 % *S. metallicum* and 11 % *S. callidum*. This population was composed of 56 % early, 19 % mid, and 25 % late instar larvae. The bacterium was introduced into this stream to create a concentration of 7.5 ppm (1.6 x 10^5 spores/ml) over a 1 min exposure period.

The SWII stream averaged 38.8 cm in width, 6.3 cm in depth, had a discharge of 228 liters/min and a water temperature of 20 °C at time of treatment. Species composition prior to

treatment was 5.7 % *S. ochraceum,* 76.8 % *S. metallicum* and 17.5 % *S. callidum* ; instar composition was 32.4 % early, 44.9 % mid and 22.7 % late instars. Infective-stage juveniles of *S. feltiae* (92.5 % viability) were introduced into the stream over a 30 min period to produce a 43.8 nematodes/ml treatment concentration.

Posttreatment larval mortality was assessed using artificial substrates made of white polyvinyl chloride (PVC) plastic (8 x 10 cm x 1.5 mm). These rigid substrates were firmly secured to the stream bed with nails, and positioned to allow a smooth laminar flow of water over the substrates while permitting easy counting of attached larvae without disturbance. Substrate placement is vital to prevent covering by sand or debris, but this technique is well suited for use with small streams in remote locations. Substrates were placed in each stream 24 h before treatment and the number and instar of colonizing larvae were determined just before treatment. Counts of surviving larvae were made on the same substrates 24 h after exposure to measure changes in black fly populations caused by treatment. Substrates upstream from the point of application served as controls. Data were corrected for downstream larval drift and egg hatch by placing a substrate in each sampling site immediately after treatment and counting colonizing larvae 24 h later.

RESULTS

Stream treatment with *B. thuringiensis* resulted in sharp reductions of simuliid larvae, although only for short distances (table 1). In the SWI stream, a 100 % larval reduction was realized for the initial 10 m downstream from the application point, but treatment effectiveness dropped precipitously between the 40 and 100 m sampling sites (89 and 29 % mortality, respectively). No change was detected in larval populations upstream from the point of application. Analogous results were obtained in the NWIII stream where black flies were also totally eliminated from the nearest downstream sampling site (25 m) and 77 % mortality was recorded at 50 m downstream. Effectiveness again dropped off quickly further downstream : only 24 % of the vector population was removed from artificial substrates at the 100 m site. In contrast to these declines, larval numbers increased at the upstream control site.

Comparison of the pretreatment to the posttreatment instar composition of each stream indicated that early instars were more susceptible to the bacterium than late instar larvae. Early instars made up 34 % of all larvae collected before treatment at the 40 m site of the SWI stream, but only 4 % following treatment : a reduction of nearly 90 %. Late instars were reduced by 35 % at this site, but because of the sharp decrease in early instars, they made up 66 % of the total posttreatment count compared to 36 % of the pretreatment population. The change in instar composition was not as severe at the 100 m sampling site where overall mortality was 29 % (table 1) ; still the ratio of early to late instars changed from 3 :1 to 1 :1 following treatment, indicating that early instars had sustained a disproportionate population reduction. Similar results were recorded from the NWIII stream where early instars declined by 95 % at the 50 m site, while late instars suffered a more modest 41 % decline in numbers. Differences in susceptibility were discernable only at the median sampling sites because there were insufficient survivors at the nearer sites to permit meaningful analysis, and at the furthest sites insufficient population stress (mortality) occurred to make differences apparent.

TABLE 1

Effect of stream treatment with Bacillus thuringiensis *(serotype 14) on* Simulium *spp. populations attached to artificial stream substrates*

Ave. distance from treatment site (m)	Number of substrates	Number of larvae		Percent change
		Pretreatment	Posttreatment	
Stream : SWI (a)				
control (c)	4	38	38	0.0
10	5	64	0	− 100.0
40	5	102	11	− 89.2
100	3	41	29	− 29.3
Stream : NWIII (b)				
control (c)	6	191	234	+ 22.5
25	9	200	0	− 100.0
50	6	90	21	− 76.7
100	6	135	102	− 24.4

(a) Discharge rate 1020 liters/min, temperature 19.4 °C, dosage rate 45 ppm (9.6×10^5 spores/ml) for 0.5 min.

(b) Discharge rate 1080 liters/min, temperature 18.5 °C, dosage rate 7.5 ppm (1.6×10^5 spores/ml) for 1 min.

(c) Control site located upstream from the treatment site.

In contrast to the sharp population reductions caused by *B. thuringiensis*, treatment of the SWII stream with infective-stage juveniles of *S. feltiae* did not result in appreciable black fly mortality. Only 15 % (3/20) of late instar larvae collected 1-10 m downstream from the treatment site were found to be infected 24 h after treatment (15 % mortality could thus be projected, since infection invariably results in host death – **Molloy** *et al.*, 1980). Dissection of larvae collected 11-20 and 35-45 m downstream showed infection rates of 5 % (1/20) and 0 % (0/20), respectively. One infected *S. ochraceum* larva was detected at the furthest downstream sampling site (70 m). Each infected larva had been penetrated by a single *S. feltiae* juvenile. Examination of larvae collected 30 min after treatment established that mid and late instar larvae had usually ingested nematodes, but few of these nematodes had survived ingestion. Most nematodes showed conspicuous signs of injury caused by larval mouthparts.

DISCUSSION

Despite its obvious toxicity to the Mexican vectors of *O. volvulus*, the poor downstream carry (the point where mortality falls below 80 % – **Undeen & Lacey**, 1982) of *B. thuringiensis* in the small streams preferred by the vector, seriously limits the bacterium's potential. **Undeen & Lacey** (1982) have clearly identified stream size (discharge) as the principal factor governing carry of *B. thuringiensis* preparations, concluding that the high substrate area to volume ratio in small streams precludes acceptable carry, a phenomenon observed for all simuliid larvicides. Thus, **Lacey** *et al.* (1982) obtained carry for at least 34 km in a large West African river (discharge = 2.7×10^7 liters/min), while in Guatemala **Undeen** *et al.* (1982) reported carry of only 14, 30 and 50 m in *S. ochraceum* streams having discharge rates of 10, 156 and 210 liters/min, respectively. Based on these findings greater carry might have been expected in the present

study, since our discharge rates ranged to 1080 liters/min or 20-100 times the discharges recorded from the Guatemalan streams. Accurate comparisons between the 3 studies cannot be made because of unknown differences in instar and species compositions as well as stream morphology ; however, it is apparent that discharge alone is a crude estimator of carry. While we are in agreement with **Undeen & Lacey** (1982) that carry is primarily a function of discharge, the interaction of discharge with other stream parameters is poorly understood. For example, stream depth has not been emphasized as a limiting factor, yet the probability of a suspended particle rapidly contacting and adhering to a substrate would be greatest in a shallow stream. This and other factors (i.e., turbulence, turbidity, pools, substrate, larval density) should be the subject of future studies if accurate predictions of stream carry are to be realized.

The similarities between the SWI and NWIII streams in discharge, temperature, morphology, and instar and species structure provided a nearly ideal arena in which to test the conclusion of **Undeen** *et al.* (1981) that there is no correlation between *B. thuringiensis* dosage and downstream carry in small streams. Our finding of virtually no difference in estimated carry between these 2 streams (SWI = 49 m ; NWIII = 47 m), despite a three-fold difference in dosage, lends support to their position.

Although previously unreported for Central American black flies, differences in susceptibility to *B. thuringiensis* because of larval size (instar) are well documented for other black fly species (**Gaugler & Finney**, 1982).

The ineffectiveness of *S. feltiae* against Central American black flies was unexpected, since an earlier field trial in New York State had produced 50 % mortality of late instar *S. vittatum* larvae at a stream temperature of 9-12 °C (**Gaugler & Molloy**, 1981b). It had been anticipated that the warm water temperatures in Mexico would significantly enhance nematode efficacy. Given the favorable temperature (20 °C), our unsatisfactory results in the present study are almost certainly a function of the small size of the vector black flies in Central America. Laboratory exposures have shown that only last instar larvae of *S. vittatum*, with postgenal lengths >481 μm, are large enough to ingest *S. feltiae* infectives without consistently causing nematode injury from the mouthparts (**Gaugler & Molloy**, 1981a). The postgenal lengths of last instar larvae of *S. ochraceum* (n = 50), *S. metallicum* (n = 50) and *S. callidum* (n = 50) averaged 364, 452 and 461 μm, respectively.

Further investigation into the use of *Steinernema* spp. against black flies and other aquatic insects cannot be recommended. These nematodes are not adapted to the aquatic environment as demonstrated by this and other (**Finney & Harding**, 1981) studies. As a result they are unable to seek out hosts and to initiate active infections, but must rely on passive ingestion for host contact. Future work on the use of nematodes for black fly control should concentrate on black fly mermithids, since these natural black fly parasites are ecologically adapted to the host habitat. Inoculative releases in remote breeding sites where mermithids might provide permanent partial control following a single application deserves special attention. This approach seems especially appealing for Central America, because it is believed that due to the low transmission rate of *O. volvulus* here, partial black fly control could sharply reduce disease transmission (**Hamon** *et al.*, 1974).

Conventional black fly control techniques are not entirely practical for use in the Mexican foci, where up to 15,000 small streams (**Davies**, 1974) in extremely rugged and remote terrain may require treatment. Our present inability to overcome the short carry of *B. thuringiensis* in these streams even at large doses, suggests that applications at intervals of 50-100 m would be needed to achieve control. **Davies** (1974) suggested that chemical larvicides would require application at somewhat more favorable intervals of 500 m, although **Mallen** (1974) referred to DDT being applied at intervals of 250 m. However, the rapid development of resistance to temephos

and chlorophoxim in parts of the West African Onchocerciasis Control Programme (**Guillet** *et al.*, 1980 ; **Kurtak** *et al.*, 1982), and their subsequent replacement with *B. thuringiensis*, suggest that the bacterium is ultimately likely to play a key role in any vector control approach. Even so, limitations of carry for both chemical and biological insecticides compel the development of new control approaches. Areas worthy of study include the development of slow release techniques that dispense larvicides over periods of weeks or months, and control agents that can become established and recycle in the vector habitat. Regardless of the final approach taken, an onchocerciasis eradication project should be implemented before the Mexican disease foci expand.

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RÉSUMÉ

Evaluation de *Bacillus thuringiensis* sérotype 14 et de *Steinernema feltiae* [*Nematoda : Steinernematidae*] pour la lutte contre les simulies vectrices de l'onchocercose au Mexique

Des expérimentations dans la nature avec *Bacillus thuringiensis* Berliner, serotype 14, et le nématode *Steinernema feltiae* Filipjev (= *Neoaplectana feltiae*) ont été réalisées contre *Simulium ochraceum* Walker et d'autres simulies vectrices de l'onchocercose au Mexique. *B. thuringiensis* a été fortement toxique et a provoqué jusqu'à 100 % de mortalité mais seulement sur de courtes distances dans le courant. L'augmentation des doses n'a pas entraîné d'amélioration. Les taux de mortalité les plus élevés furent obtenus chez les larves des jeunes stades. Le traitement du cours d'eau avec *S. feltiae* n'a pas abouti à une mortalité significative parce que les stades infestants du nématode étaient soit non ingérés, soit lésés lors de l'ingestion par les pièces buccales des larves.

REFERENCES

Briggs, J.D., Alvarado, C., Aguirre, A. – 1981. Susceptibilidad de vectores de malaria y onchocerciasis a *Bacillus thuringiensis*. – *Proc. IX Reunion Nacional de Control Biologico*, Oaxaca, Mexico, 389-397.

Davies, J.B. – 1974. Problems facing the control of *Simulium* species in the onchocerciasis zones of Mexico. In : Research and Control of Onchocerciasis in the Western Hemisphere. – *Pan. Am. Health Organ. Sci. Publ.* No. 298, 96-99.

Davies, J.B., LeBerre, R., Walsh, J.F. & Cliff, B. – 1978. Onchocerciasis and *Simulium* control in the Volta River Basin. – *Mosq. News*, 38, 466-472.

Fairchild, G.M. & Barreda, E.A. – 1945. DDT as a larvicide against *Simulium*. – *J. Econ. Entomol.*, 38, 694-699.

Finney, J.R. & Harding, J.B. – 1981. Some factors affecting the use of *Neoaplectana* sp. for mosquito control. – *Mosq. News,* 41, 798-799.

Gaugler, R. – 1981. The biological control potential of neoaplectanid nematodes. – *J. Nematol.,* 13, 241-249.

Gaugler, R. & Finney, J.R. – 1982. A review of *Bacillus thuringiensis* var. *israelensis* (serotype 14) as a biological control agent of black flies [*Simuliidae*]. – *Misc. Publ. Entomol. Soc. Am.,* 12, 1-17.

Gaugler, R. & Molloy, D. – 1981a. Instar susceptibility of *Simulium vittatum* [*Diptera : Simuliidae*] to the entomogenous nematode *Neoaplectana carpocapsae.* – *J. Nematol.,* 13, 1-5.

Gaugler, R. & Molloy, D. – 1981b. Field evaluation of the entomogenous nematode, *Neoaplectana carpocapsae*, as a biological control agent of black flies [*Diptera : Simuliidae*]. – *Mosq., News,* 41, 459-464.

Guillet, P., Escaffre., H., Ouedraogo, M. & Quillévéré, D. – 1980. Mise en évidence d'une résistance au temephos dans le complexe *Simulium damnosum (S. sanctipauli* et *S. soubrense)* en Côte d'Ivoire. – *Cah O.R.S.T.O.M., Entomol. Med. Parasitol.,* 18, 291-299.

Hamon, J., Quelennec, G., Villars, R., Marr., J.D. M., Stiles, A.R., Gratz, N.G., Parker, J.D. & Arata, A.A. – 1974. Onchocerciasis vectors in the Western Hemisphere. B. Vector control aspects. In : Research and Control of Onchocerciasis in the Western Hemisphere. – *Pan. Am. Health Organ. Sci. Publ.* No. 298, 69-84.

Kurtak, D., Ouedraogo, M., Ocran, M., Télé, B. & Guillet, P. – 1982. Preliminary note on the appearance in Ivory Coast of resistance to chlorphoxim in *Simulium soubrense/sanctipauli* larvae already resistant to temephos (Abate®). – *W.H.O. Doc. VBC*/82.8S0, 12 p.

Lacey, L.A., Escaffre, H., Philippon, B., Seketeli, A. & Guillet, P. – 1982. Large river treatment with *Bacillus thuringiensis* var. *israelensis* H-14) for the control of *Simulium damnosum* s. 1. in the Onchocerciasis Control Programme. – *Tropenmed. Parasitol.,* 33, 97-101.

Mallen, M.R. – 1974. Onchocerciasis in Mexico. In : Research and Control of Onchocerciasis in the Western Hemisphere. – *Pan. Am. Health Organ. Sci. Publ.* No. 298, 112-115.

Molloy, D., Gaugler, R. & Jamnback, H. – 1980. The pathogenicity of *Neoaplectana carpocapsae* to black fly larvae. – *J. Invertebr. Pathol.,* 36, 302-306.

Takaoka, H., Ochoa, J.O., Takahasi, M. & Takahaski, H. – 1981. Evaluation of temephos as a larvicide against *Simulium ochraceum* [*Diptera : Simuliidae*] in Guatemala. – *J. Med. Entomol.,* 18, 145-152.

Undeen, A.H. & Lacey, L.A. – 1981. Field procedures for the evaluation of *Bacillus thuringiensis* var. *israelensis* (Serotype 14) against black flies [*Simuliidae*] and nontarget organisms in streams. – *Misc. Publ. Entomol. Soc. Am.* 12, 25-30.

Undeen, A.H., Takaoka, H. & Hansen, K. – 1981. A test of *Bacillus thuringiensis* var. *israelensis* de Barjac as a larvicide of *Simulium ochraceum*, the Central American vector of onchocerciasis. – *Mosq. News,* 41, 37-40.