FINAL REPORT

Project Title: Comparative Study of Vector Competence of the Simulium damnosum Complex in the Rain Forest of Liberia and the Savanna Region of Sierra Leone.

Project No.: DPE-5542-G-SS-1047-00.

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Duration of The Project: November 1, 1981 - October 31, 1984.

Reporting Period: Project Closing Report.

This is a closing report of a three year investigation of the problem of systematics, distribution, vectorial capacity and dynamics of transmission of onchocerciasis by black fly species of the Simulium damnosum complex in Liberia and to some extent in Sierra Leone.

During this 3 year period the following people have participated on this project:

Name	Capacity
1. Dr. Milan Trpis	Project Director
2. Dr. Aloysius P. Hanson	Co-investigator - Administrative
3. Jonathan Davis	Graduate (Ph.D.) Student
4. David Fryauff	Graduate (Ph.D.) Student
5. Fatroma Bolay	Research Associate, Ministry of Health, Liberia
6. Larry Gee	Laboratory / Field Technician
7. Rosevelt Smith	Driver / Field Technician
8. James Vanhannes	Laboratory / Field Technician
9. Dakermue Yakorlie	Laboratory / Field Technician

RESULTS

Systematics and Distribution of Black Flies.

Within the rain forest biotope of Liberia and Sierra Leone two species of the Simulium damnosum complex, Simulium yahense and Simulium sanctipauli occur in larger rivers and permanent creeks. Our anthropophily and zoophily studies showed that all tested populations of S. yahense were highly anthropophilic, however, some zoophily occurred in almost all populations. On the other hand S. sanctipauli is highly zoophilic and only small percentage of the tested populations showed some anthropophilic tendencies. Since the pattern of distribution of these two species in Liberia and Sierra Leone was similar we decided to concentrate on study of vectorial capacity of these two species in one well defined are. Firestone Rubber Plantation at Harbel was chosen as our study site for ecological and behavioral investigations related to transmission of human onchocerciasis.

Correlation Between Morphological Characters and Enzyme Patterns.

Man biting adults and late instars of S. yahense and S. sanctipauli from 4 ecologically different breeding sites in the Firestone Rubber Plantation were identified morphologically and the monthly species composition of each site was recorded. Samples of the predominant species found in each site were assayd electrophoretically for species-specific variants of phosphoglucomutase (PGM) and trehalase (TRE). Enzyme identifications of flies and larvae were compared with morphological identifications to determine the accuracy of field identifications relying on morphological characters. Enzyme identifications confirmed the accuracy of over 98 % of the adult female identification. Simulium yahense was found to be the predominant man biting species at each site over the 10 month of sampling period. Simulium sanctipauli comprised a small percentage of the biting fly population. Species specific larval enzymes confirmed the accuracy of more than 96 % of the larval identifications. S. yahense was the predominant larval species found in smaller, more shaded, cooler waters, while S. sanctipauli predominated in the single large watercourse - Farmington River. Biting activity of S. sanctipauli was found to

be greatest during wet season months. A more detailed analysis of this subject is given in our manuscript entitled "Identification of Larval and Adult Simulium yahense and Simulium sanctipauli Based on Species Specific Enzyme Markers and their Distribution at Different Breeding Habitats in Central Liberia". This manuscript has been accepted for publication in the American Journal of Tropical Medicine and Hygiene. It is listed at the end of this report as publication No.5.

Distribution of Different Black Fly Species in the Firestone Rubber Plantation.

The distribution of the vector species in the Firestone Rubber Plantation (FRP) has been determined. S. yahense proved to be the most abundant and wide spread vector species.Virtually all fluvial habitats supported development of S. yahense. However the Du River, fast small rivers and fast small creeks demonstrated significant habitat preference. S. sanctipauli was limited in its breeding only in the Farmington River. A manuscript is in preparation and is listed here as No.9.

Duration of Larval Development in Nature.

Under natural conditions, the duration of S. yahense larval development has been determined for the first time. Eclosion began approximately 1.5 days after oviposition. Second and third instar larvae were first observed on day 2-4 after eclosion, and the fourth stage larvae were observed on 6 days after eclosion. The fifth and sixth larval instars appeared on day 11. A manuscript is in preparation and is listed here as No. 10.

Quantitative Studies of Onchocerciasis Transmission by Simulium yahense and Simulium sanctipauli in the Firestone Rubber Plantation at Harbel, Liberia.

Two black fly vectors of onchocerciasis, S. yahense and S. sanctipauli were surveyed in three ecologically different habitats in the (FRP) by men-biting collections at weekly intervals for over 13 months. S. yahense was found to be the predominant man biting species at each site during both wet and dry season. Greatest population of this species occurred during wet season, but its impact on transmission of the disease was most profound during the dry season, when parity, infection, and

infectivity were high. S. sanctipauli was the only other vector species captured, but biting populations of this species were small, and during wet season confined primarily to the vicinity of its breeding site in the Farmington River. Wet season populations of S. sanctipauli were greater than dry season populations at all sites, but similar to S. yahense, dry season populations of S. sanctipauli were characterized by higher rates of parity , infection, and infectivity. Dry season populations of this vector were found to be greater at collection site further inland from its Farmington River breeding site, and may indicate that dry season conditions induce a broader range dispersal. Monthly transmission potentials at each site were attributed primarily to S. yahense, with peak monthly transmission occurring during the dry season months of January - April. Against the WHO standard of 100 as a "tolerable" annual level of onchocerciasis transmission, annual transmission potentials for the three sampling sites were 94, 1877, and 4900, with highest values being calculated for S. yahense breeding site. Although similar seasonal patterns of density, parity,infection, and infectivity were apparent at each type of breeding site, the human risk of onchocerciasis infection by S. yahense was found to differ greatly between sites.

The Prevalence of Onchocerciasis on the Firestone Rubber Plantation.

A survey for the prevalence of onchocerciasis was conducted on divisions 19, 22, 23, and 36. Biopsy of the calf, iliac crest and shoulder was done on a total of 583 individuals. No lateral difference in microfilarial density was observed. A prevalence of 80.8 % was found. Prevalence appeared to be equal between the sexes, and to plateau above 35 years of age. Of those presenting positive skin biopsies, 20.2 % had palpable onchocercomata. Onchocerciasis is considered hyperendemic in the Firestone Rubber Plantation. Published in Am. J. Trop. Med. Hyg. Listed here as No. 1.

The Engorgement Ratio of Simulium yahense in the Firestone Rubber Plantation.

The purpose of this study was to determine what proportion of S. yahense attracted to man successfully feed to repletion. The data indicate that the S. yahense population at FRP is relatively inefficient in obtaining a blood meal. Only 25 % of

the flies attracted to man successfully fed. Preliminary data from Togo indicate that S. soubrense, S. damnosum, and S. squamosum engorgement ratio was 75%, 45%, and 44%, respectively. Published in Am. J. Trop. Med. Hyg. Listed here as No. 4.

A Field Methodology for Procurement of Infective Larvae of Onchocerca volvulus.

Natural vectors of onchocerciasis (S. yahense) were collected in the FRP and 98 individuals were intrathoracically inoculated with 30 fresh obtained skin-dwelling microfilariae. Of the 82 flies disected, 56 were infected and 28 harbored infective larvae. A total of 193 infective O. volvulus larvae were obtained. The mean infective worm burden was 6.9. Published in the Am. J. Trop. Med. Hyg. Listed herte as No.3.

Diel Periodicity in Biting Activity of Black Flies.

Activity of the infected flies peaked (16.6%) at 1100 hours. Over half of the infected females came to the human host before 1100 hours. A secondary peak of infected fly activity occurred at 1700 hours. At this time 10.3% of infected flies were captured. Nevertheless, by this time of day, more than 95.6% infected fly activity had occurred. The hourly average of infected fly activity was 0.35 flies per person per hour which represents 7.7% of the daily means. The simulids which harbored infective larvae of O. volvulus were most likely encountered during the morning hours. The peak activity occurred at 1000 hours representing 15.7% of the total number Infective biting flies captured. Cumulatively, 55.5% of all infected flies were collected by this hour. The number of infective larvae found in flies ranged from 1 to 30. Manuscript in preparation. Listed here as No. 11.

Resting Behavior and Radial Dispersal of Black Flies

Of the 99 S. yahense collected 72.7% were nulliparous. Parous flies comprised 27.3% of the number captured. A total of 10 infected resting flies were observed. Thirty four percent of parous flies were recovered. Significantly more resting flies were observed in the dry season than in the rainy season. For the study of radial dispersal of the vector, a total of 3642 flies were captured on human hosts. Simulium yahense comprised 96.1% of the total while the remaining 3.9% was attracted to S.

sanctipauli. The percent disected was 97.4 for S. yahense and 99.3 for S. sanctipauli. Cumulatively, 73.7% (2,449 collected per person) of all S. yahense and 78.9 S. sanctipauli were captured at distance of 500 m. As distance increased from the rapids the number of infective S. yahense coming to the host declined. However, the proportion of infective flies to number flies disected remained fairly stable. The range of proportion of flies infected was from 1.0% at the rapids to 2.2% at a distance of 1000 m. For the distance of 500 m, 1,500 m and 2,000 m the proportion infective flies equaled 1.6, 1.3, and 1.5 % respectively. In preparation for publication. Listed as No. 13.

SIGNIFICANCE OF THE PROJECT RESULTS TO AID OBJECTIVES.

Because of this project initiation of training two West Africans, one from Liberia (Fatorma K. Bolay) and one from Sierra Leone (Haruna R. Sesay) in Medical Entomology at the Johns Hopkins University took place. Both of these candidates are financially supported by the WHO/TDR special program. They have completed their course work at JHU and are working on their Ph.D. thesis in their native countries.

This project led to a new, special project on biological control of black fly vectors in the Firestone Rubber Plantation, which now in progress. Thus the AID's aim to concrol onchocerciasis in Africa will be achieved here as well. The completed research project contributed to the US help to solve tropical disease problem in developing countries.

MANUSCRIPTS PUBLISHED, IN PRESSS OR IN PREPARATION FOR PUBLICATION, AND TWO DOCTORAL THESIS COMPLETED.

1) Barbiero,V.K. and Trpis, M.: The Prevalence of Onchocerciasis on Selected Divisions of the Firestone Rubber Plantation, Harbel, Liberia; Am. J. Trop. Med. Hyg. 33: 403-409, 1984.

2) Barbiero, V.K. and Trpis, M.: Transmission of Onchocerciasis by Local Black Flies on the Firestone Rubber Plantation, Harbel, Liberia. Am. J. Trop. Med. Hyg. 33:586-594,1984.

3) Barbiero, V.K. and Trpis, M.: A Field Method for the Procurement of Infective Larvae of Onchocerca volvulus. Am. J. Trop. Med.Hyg. 34:731-734,1985.

4) Barbiero, V.K. and Trpis, M.: The Engorgement Ratio of Simulium yahense (Diptera:Culicidae) at the Firestone Rubber Plantation, Harbel, Liberia. J. Med. Entomol. 23:309-312, 1986.

5) Fryauff, D.J. and Trpis, M.: Identification of Larval and Adult Simulium yahense and Simulium sanctipauli Based on Species-Specific Enzyme Markers and their Distribution at Different Breeding Habitats in Central Liberia. (In press, Am. J. Trop. Med. Hyg. 1986).

6) Fryauff, D.J. and Trpis, M.: Quantitative Studies of Onchocerciasis Transmission by Simulium yahense and Simulium sanctipauli in the Firestone Rubber Plantation at Harbel, Liberia. (Submitted for Publication to: Am. J. Trop. Med.Hyg. 1986).

7. Fryauff, D.J. and Trpis, M.: Population Genetics of Onchocerciasis Vectors in the Firestone Rubber Plantation at Harbel, Liberia. (In Preparation).

8) Fryauff, D.J. and Trpis. M.: Age Structure of Vector Black Fly Populations Based upon Onchocerca volvulus Infections. (In Preparation)

9) Davis, J. R. and Trpis, M.: Species Diversity of the Simulium damnosum Complex in Liberia. (In Preparation).

10) Davis, J.R. an Trpis, M.: Larval Development of Simulium yahense under Natural Conditions. (In Preparation).

11) Davis, J.R. and Trpis, M.: Diurnal Pattern of Vector-Host Contact for Members of the Simulium damnosum Complex in Harbel, Liberia. (In Preparation).

12) Davis, J.R. and Trpis, M.: Resting Behavior of the Simuliidae in the Firestone Rubber Plantation at Harbel, Liberia. (In Preparation).

13) Davis, J.R. and Trpis, M.: Radial Dispersal of the Vectors of Onchocerciasis in the Firestone Rubber Plantation at Harbel, Liberia. (In Preparation).

14) Davis, J.R. and Trpis, M.: Quantitative Studies on the Vectors and Transmission of Onchocerciasis in the Firestone Rubber Plantation at Harbel, Liberia. (In Preparation).

15) Trpis, M.: Construction of an Inexpensive Closed Water System for Rearing of Black Flies in the Laboratory. (In Preparation).

16. Fryauff, D.J.: Ecology of Transmission and Population Genetics of Onchocerciasis in the Firestone Rubber Plantation at Harbel, Liberia. (Ph.D. Dissertation). 1986.

17. Davis, J.R.: Distribution and Ecology of the Simulium damnosum Complex of Vectors of Onchocerciasis in the Firestone Rubber Plantation at Harbel, Liberia. (Ph.D. Dissertation). 1986.