SIMULIUM EXIGUUM AND SIMULIUM METALLICUM AS POTENTIAL VECTORS OF ONCHOCERCA GUTTUROSA IN EL VALLE, COLOMBIA

A DISSERTATION

SUBMITTED ON THE 26th DAY OF NOVEMBER 1980

TO THE DEPARTMENT OF TROPICAL MEDICINE

OF THE SCHOOL OF PUBLIC HEALTH AND TROPICAL MEDICINE

OF THE TULANE UNIVERSITY

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF SCIENCE

BY

Kirby O. Kloter

KIRBY ORRIN KLOTER

Approved:

Thomas C. Orihel

Thomas C. Orihel, Ph.D., Chairman

Thomas G. Akers

Thomas G. Akers, Ph.D.

Barnett L Cline

Barnett L. Cline, M.D., Ph.D.

Jack H. Esslinger

Jack H. Esslinger, Ph.D.

Maurice D. Little

Maurice D. Little, Ph.D.

Robert C. Lowrie, Jr.

Robert C. Lowrie, Sc.D.

Harold Trapido

Harold Trapido, Ph.D.

SIMULIUM EXIGUUM AND SIMULIUM METALLICUM AS POTENTIAL VECTORS OF ONCHOCERCA GUTTUROSA IN EL VALLE, COLOMBIA

AN ABSTRACT

SUBMITTED ON THE 26th DAY OF NOVEMBER 1980

TO THE DEPARTMENT OF TROPICAL MEDICINE

OF THE SCHOOL OF PUBLIC HEALTH AND TROPICAL MEDICINE

OF THE TULANE UNIVERSITY

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF SCIENCE

BY

Kirby O. Kloter

KIRBY ORRIN KLOTER

Approved:

Thomas C. Orihel, Ph.D., Chairman

Thomas G. Akers, Ph.D.

Barnett L. Cline, M.D., Ph.D.

Jack H. Esslinger, Ph.D.

Maurice D. Little, Ph.D.

Robert C. Lowrie, Sc.D.

Harold Trapido, Ph.D.

ABSTRACT

Epizootiological investigations revealed that Onchocerca gutturosa, a filarial parasite of cattle, is enzootic in the Department of Valle, Colombia. Based on examination of circumumbilical skin snips, about 6% of the cattle surveyed were infected with the filaria. On the other hand, the prevalence of infection was much higher in animals brought to Valle from neighboring areas for slaughter in the local abattoir. In these instances prevalence rates reached 21% and 93% based on circumumbilical and suboccipital skin snips, respectively. It was concluded that the prevalence of infection in Valle cattle was probably underestimated since prevalence rate was based on examination of circumumbilical snips alone.

It was demonstrated that microfilariae of O. gutturosa tend to concentrate in the skin about the head and less frequently in the ventral body skin. Further, microfilarial concentrations in excess of 4,000 mf/g were required to infect Simulium exiguum and S. metallicum. Natural infections in wild-caught blackflies were rare and larval development of the parasite was often aborted or delayed. Third-stage larvae were found only in S. exiguum. Data indicated that there was a potential for natural infection of only 5 parous S. exiguum per 1,000.

Studies of ovarian development of both S. exiguum and S. metallicum in the laboratory showed that both species were anautogenous and oogenesis took less than 60 hours. Marking-release-recapture studies demonstrated that the gonotrophic cycle of both species required approximately 3 days. The period required for O. gutturosa to reach the infective stage was estimated to be from 5 to 8 days indicating that infected flies must

survive 2 gonotrophic periods to transmit the parasite. However, calculations based upon mortality studies of wild-caught flies suggested that few flies from populations of either species survived the mortality risks over that time period. In addition, the marking-release-recapture studies indicated that S. exiguum had the greatest potential for dispersing the parasite because its oviposition sites were located farthest from cattle pastures.

Behavior studies disclosed that S. exiguum and S. metallicum had significantly different feeding patterns. About 20% and 13% of the landing populations of S. metallicum and S. exiguum, respectively, were interrupted feeders as determined by finding landing flies with ovaries advanced beyond Christophers' Stage II or with blood in their midguts. Bloodmeal identification studies showed that of the interrupted feeding populations returning to feed, about 2/3 and 1/3, respectively, had fed upon other cattle or horses. Interrupted feeding probably contributed to the low feeding efficiency of these blackfly species, especially S. metallicum. Field studies showed that only 27% of landing S. metallicum fed per hour while 51% of S. exiguum fed. Furthermore, interrupted feeding was considered to have reduced the potential for both species to become infected with O. gutturosa microfilariae and, in addition, probably contributed to the loss of infective larvae during feeding upon hosts other than cattle.

Field studies revealed that S. exiguum was always more abundant than S. metallicum at the study site. Peak abundance of both species occurred during the dry season.

Potentially infective, parous populations of S. exiguum and S. metallicum varied seasonally from 15 to 110 and 10 to 40 landing flies per hour, respectively. Observed fluctuations were often dramatic and

population decline was most often associated with increased rainfall. Such fluctuation was probably connected with larval washout or a high rate of adult population turnover due to a short, average lifespan of these species. Fly activity was greatest during the early morning hours for both species. S. exiguum and S. metallicum parous flies were most active in the morning and afternoon, respectively. Based upon the proposed rate of natural infection in S. exiguum, each cow received only 1.7 infective bites per day. While parous fly activity was high, this rate was low as compared to other Onchocerca transmission rates and was considered insufficient to maintain a cycle of infection in nature.

ACKNOWLEDGEMENT

The investigator wishes to express his gratitude and appreciation to Dr. Thomas C. Orihel for his guidance, encouragement and continued support under which this study was carried out. Special gratitude is extended to Drs. T. G. Akers, B. L. Cline, J. H. Esslinger, M. D. Little, R. C. Lowrie, and Dr. H. Trapido for serving as members of the dissertation committee and for their valuable editorial and critical advice during the preparation of this manuscript. The suggestions and evaluation which were offered during the course of the research in Colombia by research staff of the International Center for Medical Research by Drs. A. D'Alessandro, W. J. Kozek, N. G. Saravia, Dr. and Mrs. M. A. Tidwell, and Dr. J. K. Werner are gratefully acknowledged. Appreciation is expressed to ICMR non-technical staff especially Ms. D. Amort, M. Izquierdo, J. Latham and G. Bolanos for the excellent logistical support offered between New Orleans and Cali. Thanks goes to Peace Corps Volunteers including M. Bonato, J. Brooks and M. Wietzel who willingly gave of much spare time to help with extensive field work. Gratitude is expressed to Drs. M. Q. Bong and E. A. Malek as well as D. D. Bowman, F. B. Cogswell, J. L. Smith and J. A. Yates for their friendship and encouragement during long hours of writing. Finally, warmest appreciation is extended to my family and to Ann G. Storms for their kindness and personal support during the tenure of work on this study.

This study was supported by the Tulane University International Center for Medical Research, grant AI-10050 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Onchocerca gutturosa (Neumann, 1910) is a common, filarial, nematode parasite of cattle which is transmitted by biting flies. The skin-dwelling microfilariae of the parasite are ingested by the arthropod intermediate host and develop to the 3rd-stage in the thoracic flight muscles. O. gutturosa has a cosmopolitan distribution in that the parasite has been found in cattle in Europe, Africa, Asia and India (Eichler and Nelson, 1971). More recent reports from these regions expanded even further the known distribution of the parasite. O. gutturosa has also been found in cattle throughout the eastern U.S. (Rabalais et al. 1973), in Colombia, South America (Eberhard and Orihel, 1978) and in Australia (Ottley and Moorhouse, 1978).

Simulium ornatum has been incriminated as the principal vector of O. gutturosa in the Palearctic Region as demonstrated and documented by Eichler and Nelson (1971) and Eichler (1973a). Bain (1979) suggested that biting gnats were potential vectors after observing complete intermediate development of the parasite in laboratory infected Culicoides nubeculosus. There are no reports of development of O. gutturosa in any arthropods of the Ethiopian, Nearctic or Neotropical Regions. Species of the Simulium damnosum complex in Africa have been suspected as possible vectors because of their formidable potential for transmission of human onchocerciasis. Although Service (1977) reported that members of the complex readily fed upon cattle, Denke and Bain (1978) failed to obtain development of O. gutturosa in S. damnosum from Togo.

Zoophilic black flies in other regions may also be involved in the transmission of O. gutturosa because of their strong host-preference for cattle. For example, in the Nearctic Region, Addelnur (1968), Jones and Richey (1956) and James and Harwood (1972), respectively, observed that Simulium jenningsi, S. venustum and S. vittatum are important pests of cattle. In Central America, Dalmat (1955) and Lewis and Aldecoa (1962) reported that Simulium exiguum and Simulium metallicum fed upon cattle. After finding larval filariae in wild-caught S. exiguum and S. metallicum, Gibson and Dalmat (1952) suggested that the larvae were those of O. gutturosa since those black fly species were feeding primarily upon cattle. The zoophilic biting habits of Colombian S. exiguum and S. metallicum, emphasized by their bovine host-preference (Guttman, 1972; San Martin et al., 1973; Tidwell and Tidwell, 1979), circumstantially suggests these species as possible vectors of O. gutturosa in Colombia. Despite the documented relationship of black flies and cattle, studies of the vector potential of Simulium species for O. gutturosa in the tropics are lacking.

Some observations of the development of O. gutturosa in S. ornatum have been described by Eichler and Nelson (1971) and Eichler (1973a). They observed that about 25% of microfilariae ingested by a fly were able to migrate from the bloodmeal and through the midgut wall prior to the formation of the peritrophic membrane. Microfilariae entered the hemocoel of a fly within 60 minutes after ingestion and then moved anteriorly entering the thoracic muscles after 6 hours. This migratory route is similar to that reported for Onchocerca volvulus in S. damnosum by Duke and Lewis (1964) and Laurence (1966). Eichler and Nelson (1971) found infective O. gutturosa larvae in the head of S. ornatum 13 to 15 days after infection, reconfirming the long development period of the parasite first reported by Steward (1937).

Eichler and Nelson (1971) and Eichler (1973a) demonstrated that O. gutturosa microfilariae exhibited behavioral characteristics that enhanced their potential for uptake by the vector by concentrating in the skin of the umbilical area where S. ornatum usually fed. They also noted that the microfilariae showed seasonal periodic concentration in the superficial layers of the umbilical skin when biting populations of S. ornatum were most abundant. Although these findings appear to be biologically sound, evidence is accumulating to indicate that the O. gutturosa life-cycle studies up to and including the work by Kolstrup (1975) actually were dealing with a related parasite described as Onchocerca lienalis.

Identification of O. gutturosa from the nuchal ligament and gastrosplenic omentum of cattle by Webber et al. (1975), Eichler and Nelson (1971) and Kolstrup (1975) showed the parasite did not have a single anatomical site preference in the host. However, Eberhard (1976), Denke and Bain (1978) and Bain et al. (1978) frequently found mixed infections of O. gutturosa and O. lienalis in cows which were skin snipped and later necropsied. The adults of O. gutturosa were found exclusively in the area of the nuchal ligament while those of O. lienalis were found around the gastrosplenic omentum. These observations on the site-specific locations of these parasites are in agreement with those of numerous investigators including Hussein et al. (1975), Scholtens et al. (1977), Chauhan and Pande (1978) and Ottley and Moorhouse (1978). Morphological studies by Eberhard (1976, 1979), which distinguished O. gutturosa microfilariae and adults from those of O. lienalis, indicate that Eichler and Nelson (1971) incorrectly synonomized O. gutturosa with O. lienalis. It is possible, therefore, that Eichler and Nelson (1971) and other O. gutturosa life-cycle researchers, unaware of the existence of O. lienalis,

were actually dealing with cattle parasitized by both bovine Onchocerca species or only O. lienalis when infecting S. ornatum.

O. gutturosa life-cycle studies are similarly confounded by lack of firm knowledge relating to the typical spacial distribution pattern of its skin-dwelling microfilariae. Microfilarial skin concentration patterns are an important consideration in Onchocerca transmission studies because it is generally considered that the microfilarial distribution of a parasite corresponds with the biting habits of vectors (Mazzotti, 1951; Kershaw et al., 1954). It may be that such behavior is endogenously regulated, perhaps by microfilariae directing themselves by temperature gradients in the skin (Eichler and Nelson, 1971; Mellor, 1973; Rabalais, 1974), but may also be the product of exogenous forces of selection due to vector-feeding at preferred sites over evolutionary time. Another view holds that skin-dwelling microfilariae concentrate in the vicinity of the adult worm (Venkataratnam and Kershaw, 1960; 1961). In any case, Eichler and Nelson (1971) were the first to demonstrate that O. gutturosa microfilariae were most concentrated in the circumumbilical skin of infected cows. These findings have been confirmed by Voltava and Thomson (1973), Kolstrup (1975) and Scholtens (1977). However, conflicting evidence is presented by Bain et al. (1977) and Hussein (1978), who showed that O. gutturosa microfilariae were concentrated in the skin around the head of infected animals. Bain et al. (1979) suggest that those who found O. gutturosa microfilariae most concentrated in the umbilical region were probably sampling O. lienalis infections thought to be O. gutturosa or mixed infections of both parasites.

O. gutturosa life-cycle work done before and including that of

Kolstrup (1975) is very difficult to interpret in regard to which parasite's development was actually described. Where the parasite has been positively identified after the taxonomic work of Eberhard (1976, 1979), workers have not been able to obtain intermediate development of O. gutturosa in tropical species of black flies (Denke and Bain, 1978). On the other hand as mentioned earlier, Bain (1979) has observed its development in biting gnats. The biology of O. gutturosa in the neotropics remains virtually unstudied and it is not known if Simulium species are good vectors of the parasite. Consequently, the present research was conducted to study the epizootiology of O. gutturosa in Colombia, to determine if S. exiguum and S. metallicum served as intermediate hosts of the parasite, and to define the vector potentials of these black fly species for the transmission of bovine onchocerciasis.

STATEMENT OF PROBLEM

In addition to human disease produced by Onchocerca volvulus, there are several related species of Onchocerca which parasitize a wide range of domestic and wild ungulates, worldwide. Zoonotic infections with various species of Onchocerca including O. gutturosa, have been found on several occasions in humans living in areas where animal infections were prevalent. Infection of both cattle and humans with O. gutturosa justifies the need to learn more about the biology of the parasite in terms of vector requirements, behavior and other host-parasite interactions. Studies of Simulium biology are needed to provide related information about their potential as vectors of O. gutturosa and other bovine or human diseases that they may biologically or mechanically transmit. Such studies are particularly significant in Colombia where a focus of O. volvulus is maintained by black fly transmission and where livestock production is often hampered by other Simulium-borne diseases.

The host-parasite-vector relationship of O. gutturosa have been studied adequately only for European strains of the parasite. Very little is known about the vectors of bovine onchocerciasis in the tropics. Therefore, in-depth studies are needed to define the potential of Simulium species for transmitting O. gutturosa in Colombia. The present study was conducted toward this end.

The objectives of this study are: (1) describe the development of O. gutturosa in Simulium exiguum and Simulium metallicum from the Cauca Valley of Colombia, (2) describe the spacial distribution of microfilariae

of O. gutturosa in the skin of the definitive hosts and evaluate these findings as a measurement of vector potential particularly in relation to feeding behavior of selected Simulium species and (3) determine the vector potential of S. exiguum and S. metallicum for transmission of O. gutturosa in terms of the following parameters:

a. rates of natural infection with O. gutturosa larvae,

b. fluctuations of daily and seasonal black fly populations and parity,

c. rates of black fly feeding efficiency including the amount of interrupted feeding occurring in populations of S. exiguum and S. metallicum,

d. analysis of host-preferences of S. exiguum and S. metallicum,

e. length of the gonotrophic cycles of S. exiguum and S. metallicum in relation to the incubation period of the parasite.

MATERIALS AND METHODS

I. Development of Onchocerca gutturosa in Simulium exiguum and Simulium metallicum

A systematic search for cattle infected with O. gutturosa was carried out in the western foothills of the central Andean mountain range on farms located near Palmira City, Department of Valle, Colombia, and extended south to farms in the Department of Cauca and into the eastern foothills of the Pacific Andean range near Pance Village in Valle (Figure 1). Cows were skin snipped in the early morning at sunrise. The animals selected for snipping were milking cows older than 3 years which favored potential exposure to O. gutturosa. Skin snips were taken from the circumumbilical region of the cow, cut into 1 mm strips and submerged in 3 ml of Dulbecco's phosphate buffered saline (PBS). Stoppered tubes containing the snips were transported to the laboratory in an insulated chest. After incubation at 34 C for several hours, 3 ml of 5% formalin were added to each tube to fix the contents. The snips were removed and the contents of the tubes were centrifuged at 2,500 RPM for 5 minutes. The supernate was decanted and all of the sediment was examined for O. gutturosa microfilariae at 100 magnifications under a compound microscope. The microfilariae present were counted and measured. Each snip of skin, which had been placed in a glycerol solution, was removed, pressed between filter paper and weighed. The number of microfilariae per gram of skin was calculated for each snip. Sediment containing microfilariae was dried on each slide, fixed in 70% alcohol at 60 C, stained with Delafields' hematoxylin and

permanently coverslipped. Microfilariae from each positive cow were re-measured to assure that they were O. gutturosa

A Holstein-Clavel cow and a Zebu cow were selected as donor animals because their microfilarial skin concentrations were relatively high, ranging between 2,500 to 5,000 mf/g. On collection days a cow was tethered during the morning flight period (7:30 to 8:30 a.m.) of S. exiguum and S. metallicum. Engorged flies which fed off the teats, udder or umbilicus region were aspirated into one-half pint, carton, collection cups fitted with a bobbinet top and a plastic petri dish bottom. Moist paper toweling was placed on the bobbinet and this was covered with a plastic petri top to retain high humidity. The cartons were transported to the laboratory in an insulated chest.

In the laboratory, flies were individually sorted, identified and each fly was transferred to an individual holding tube (Figuroa et al., 1977). Filter paper pledgetts in the ends of tubes were moistened with a 20% boiled sugarwater solution. The tubes were placed in groups of 6 upon a water-moistened filter paper and stored in a covered square plastic petri dish. Ten dishes were stacked in a plastic-lined, wooden box with a sponge, which maintained humidity between 90 to 95%. The flies were held at ambient temperatures between 73-79 F (23-26 C). Flies were checked every 12 hours and each moribund or freshly dead fly was dissected on a glass slide under a dissecting microscope. The head and thorax of each fly was macerated in PBS, evenly spread under a 18 mm^2 glass coverslip and examined for developing filarial larvae at 200 magnifications under a compound microscope. Larvae which were found were immobilized when necessary with a drop of 10% dimethyl sulfoxide (Lowrie and Eberhard, 1980), photographed. measured and fixed with one drop of 5% gluteraldehyde. The coverslip was

sealed around the sides with nail polish, and when dry, semipermanent slides were prepared by overmounting with a 22 mm^2 coverslip.

Flies were collected on several occasions in order to determine the numbers of microfilariae ingested by freshly engorged S. exiguum and S. metallicum. Field dissections and examinations were done 5-10 minutes after engorgement. Engorged flies were captured and killed, the abdomen was removed from the thorax and the latter was crushed to express any developing filarial larvae which were present. The bloodmeal was removed from the abdomen, macerated in PBS, evenly spread on a glass slide under a 22 mm^2 glass coverslip and screened for microfilariae and advanced larval stages at 100 magnifications with a compound microscope.

II. Behavior of Microfilariae

A. Seasonal variation in the concentration of microfilariae in the circumumbilical (CU) skin: Skin concentrations of microfilariae were studied in CU biopsies taken from a cow each month for a year. One infected cow was used as the study subject from May 1979 to April 1980. Each snip was taken from the cow in the early morning as described earlier with the following exception: A superficial wedge of skin, no deeper than to the bases of the hair follicles, was removed from the large skin snip. The skin snips were individually placed in tubes with 3 ml of PBS, transported to the laboratory and processed to isolate and count microfilariae per gram of skin as described above.

B. Concentration of microfilariae in the skin at the utmost dorsal and ventral aspects of cattle: A comparison of the number of microfilariae in CU and suboccipital triangle (SOT) skin snips was made from slaughtered cattle known to be infected with O. gutturosa adult worms. Large slips of CU and SOT skin were cut from infected cattle immediately after slaughter

and transported to the laboratory within 3 hours, where the slips were paired, ordered according to number and washed. A 3 cm^2 area was shaved on each slip and biopsied by removing a slice of skin with a depth of approximately 2 mm and weighing between 100 and 500 mg. Biopsies were individually placed in conical centrifuged tubes containing 3 ml of PBS and incubated for 4 hours at 34 C. Samples were processed to isolate microfilariae and their numbers were calculated per gram of skin for each skin snip as described above.

III. Vector Studies

A. Seasonal and daily activity of black flies: A site was selected near Lomitas village, Valle (Figure 1, Site No. 4) in order to study the seasonal and daily activity of nulliparous and parous S. exiguum and S. metallicum in relation to changes in temperature, humidity and rainfall. The site was in an open pasture in a wooded, subtropical ecological zone (Holdridge, 1967) at an altitude of 1,175 m. Two bull-calves, 9 to 15 months old were used as bait-animals during the tenure of the study. Bi-weekly fly collections began in February 1979 and continued for more than a year. On a given collection day, a calf was tethered and 2 collectors aspirated all landing black flies during the course of an hour. There were 5 one-hour collection periods per day defined as follows: Period I: early morning (7:30-8:30 a.m.), Period II: midmorning (9:30-10:30 a.m.), Period III: midday (11:30 a.m.-12:30 p.m.), Period IV: midafternoon (2:00-3:00 p.m.), and Period V: late afternoon (4:00-5:00 p.m.). Flies which were aspirated were transferred to a common collecting cup as described earlier. After each collection period, the cup with flies was provided with a humidifying pad moistened with water and stored in a cooler at temperatures between 50-65 F (10-18 C). Measurements of wind speed, temperature and humidity were recorded as representative of existing

weather conditions for the previous collection hour. At the end of the day, collections were transported to the laboratory and stored overnight at temperatures between 45-60 F (7-15 C). Each collection of flies was processed approximately 24 hours after it was made. Flies were killed by a short exposure to chloroform vapor and were sorted, identified and counted. The parous rates of S. exiguum and S. metallicum were determined by dissecting and age-grading 10% of each capture per period. Flies of each species were randomly selected, the abdomen of each was placed in 1 drop of PBS, the gut removed, color of the blood recorded and the thorax was crushed to express any filarial larvae which were present. The quantity of fat body was also noted. The ovaries of each specimen were extracted from the abdomen, each was spread on the periphery of the PBS drop and examined at 200 magnifications under a compound microscope. The condition of at least 5 ovarioles from each fly was evaluated for scarring, presence of follicular relics and the stage of ovarian development as defined by Detinova (1962) and Collins and Cupp (1978).

Seasonal expressions of fly activity and weather data were defined as follows: The average number of flies active per hour for each collection was calculated by dividing the daily sum of captures by 5. The average number of parous flies active per hour was determined by extrapolating the parous total per day from the proportion of flies determined to be parous in the subset of dissections obtained from the 5 capture periods. The percentages of parous flies per hour was calculated by dividing the average number of parous flies active per hour by the average number of total flies active per hour. Seasonal temperatures and humidities were calculated by averaging the 5 temperature or humidity readings after each of the collection periods on a capture day. Seasonal rainfall for the Lomitas area was estimated, based upon the averages of daily rainfall

readings recorded by the Corporacion Autonomica del Valle at three stations near the site. Points of accumulated rainfall were computed by adding rainfall data over 28 days prior to each of the capture days.

Daily expressions of fly activity and weather data were defined as follows: The average number of flies active per hour was calculated by totaling the number of flies collected in a sampling period and dividing by 26, the number of sampling periods over the course of the study. The hourly averages for parous fly activity were calculated per period by summing extrapolated percentages of flies active per hour and dividing by 26. The percentages of parous flies active per hour were determined by dividing the average number of parous flies active per hour by the average total number of flies active per hour. The weighted averages of the precentages of flies active for each period were calculated by summing the percentages active per period and dividing by 26. Daily weather data were compiled by summing the temperatures or humidities for each period and dividing by 26.

For analyzing activity as it related to temperature and humidity, the percentages of fly activity within all capture hours were graphically compared with their respective temperature and humidity readings at capture. Curves of activity relative to temperature and humidity were estimated by averaging the activity percentages falling between 10 humidity percentage points or 5 F (2.8 C) increments on the graphs.

B. Natural filarial infections in wild-caught black flies: Flies which were collected for the seasonal/daily activity studies, but not dissected, were processed by a Baermann technique (Ash and Schacher, 1971; Wilton and Collins, 1977). Individual dissections of wild-caught flies were also made to determine if natural infections with filarial larvae occurred.

C. Interrupted feeding studies: For both S. exiguum and S. metallicum, midguts and pairs of ovaries were examined from 10% of flies collected in each of 14 biweekly collections furnishing data for gonotrophic profiles on interrupted feeders as determined by finding flies with ovarioles developed beyond Christophers' Stage II or blood in their midguts. In order to determine the age of blood in such flies, color changes in the bloodmeals of engorged flies, which were collected after feeding and held in the laboratory, were observed at intervals up to 72 hours postfeeding.

The identification of black fly bloodmeals with serotypes other than that of the bait-animal from which flies were collected was done using the double immunodiffusion test described by Saravia (1973). All antisera used for bloodmeal analysis were provided by Dr. N. G. Saravia. Briefly, bloodmeals were dissected from flies, absorbed onto filter paper strips, dried and later eluted with PBS, and elutions were tested for precipitin reactions between wells filled with various antisera in agar plates.

Since it was not known if the test system was sensitive enough to be used for identification of very small bloodmeals, the average bloodmeal sizes of S. exiguum and S. metallicum were determined to discover if small quantities of sera ingested by individual flies were identifiable. Fully engorged S. exiguum or S. metallicum were collected at random from a tethered cow and individually killed with chloroform as needed. Each fly was placed under a dissecting microscope at 15 magnifications and its midgut was punctured with a minuten pin. Slight pressure was applied to the distended abdomen and the complete bloodmeal was drawn into a 20 mm^3, microcapillary pipet which was coated with a dried 5% heparin solution. The pipets were plugged and stored at 70-80 F (21-27 C) for transport. In the laboratory, the tubes were centrifuged at 2,500 RPM for 5 minutes to

separate serum from blood. The standard length of the capillary tubes up to the 20 mm^3 volume line was determined to be 79 mm. After centrifugation, the volumes of whole-blood or serum within a tube were determined by measuring the length of the tube occupied by each, computing the percentage of volume for each (length-occupied/79 mm) and multiplying this percentage by 20 mm^3. The means and standard deviations for the bloodmeals of each species were calculated for the volumetric quantities. These means were taken to represent the average maximum bloodmeal and average maximum serum meal for S. exiguum and S. metallicum. Once the averages for each species were established, the sensitivity of the test system in relation to these quantities was determined as follows: A quantity of pure bovine serum, equal to the maximum serum meal of S. metallicum, was treated as an actual serum meal and diluted with 0.02 ml of PBS. This was the same amount of PBS that was used to elute dried bloodmeals. This dilution and its serial 2-fold dilutions were dispensed into wells of a test plate and reacted against anticow serum to determine the minimum serum meal size of S. metallicum that was detectable with the test system.

D. Landing and feeding rates: Two mature Holstein cows, each with white belly and no pigmentation on udder or teats, were tethered 4 m apart at a shaded collection site. Two collection trials of one-hour were made per day, the first from 7:30-8:30 a.m. and the second from 4:00-5:00 p.m. The cows were designated as No. 1 and No. 2. At the commencement of a trial, a collector was stationed on each side of No. 1 to aspirate all landing flies and a single collector was stationed with No. 2 to aspirate all flies which had landed and were engaged in feeding. Switching

occurred every 15 minutes over the 4 periods during a one-hour trial. Flies were aspirated into collection cups, transported to the laboratory, killed, and landing and feeding collections were counted. Parity rates were determined by methods previously described. Feeding efficiency rates for both species were established by plotting landing rates/hour versus feeding rates/hour as points on a linear graph which were regressed using the least squares formula. The established slope for rates of both species was taken to represent the feeding efficiency for S. exiguum and S. metallicum.

E. Gonotrophic cycle studies: Several methods were used to study the gonotrophic cycle of S. exiguum and S. metallicum. Headings include the parameters used to measure each component and, in parenthesis, the component evaluated by the study.

Postecdysis ovariole development (anautogeny): In order to study if S. exiguum and S. metallicum were anautogenous, their pupae were collected from streams for study. The vegetation containing pupae was trimmed to 5 to 15 cm lengths, placed in 1 quart jars with creek water and transported to the laboratory. The jars were capped with one-pint carton cylinders covered at one end with fine mesh bobbinet and air hoses were passed through the cylinder and fixed with aeration stones which hung at the bottom of each jar. The water was aerated for 120 hours after collection at 70-75 (21-24 C) to elicit adult emergence. Emerged flies were collected, males and females were identified to species, sorted and counted and females were dissected in order to determine their ovarian state of development.

Postfeeding/preoviposition mortality (survivorship potential through oogenesis): Engorged S. exiguum and S. metallicum were collected from

tethered cows, aspirated into collection cups and transported to the laboratory where they were transferred to individual holding tubes. Parity was determined by dissecting groups of flies from each species. Test flies were checked every 24 hours for mortality during the period of oogenesis. In order to more accurately compare mortality rates of these species during oogenesis, the S. metallicum rate was adjusted by taking its lower parity into consideration.

Maturation time of eggs (oogenesis period): The sequence of complete egg development including the periods of postecdysis and postfeeding egg development were studied. In order to follow postecdysis ovarian development, pupae were collected and flies harvested as mentioned above. Emerged flies were maintained individually and groups were killed and their ovaries examined at 6-hour intervals from immediately after emergence up until 54 hours. The lengths of the primary and secondary oocytes of well spread ovarioles were measured and the Christophers' stage of each was determined. Flies used for the study of postfeeding egg development were collected after engorging on tethered cows. They were individually maintained in tubes and groups of either species were sequentially dissected for ovariole examination. The first group was dissected one-hour after capture and subsequent groups were examined thereafter at 6 hour intervals postfeeding through 60 hours. The ranges of length established for primary and secondary oocytes were based upon measurements of at least 30 ovarioles from 10 different flies exhibiting a developmental Christophers' stage as defined by Detinova (1962) and one final state denoting oviposition readiness.

Time to oviposition (oogenesis timing): The time from engorgement to induced oviposition was determined for S. exiguum and S. metallicum in the laboratory. The following method to induce oviposition was developed:

Groups of engorged S. exiguum and S. metallicum were collected from tethered cows, transported to the laboratory and transferred to holding tubes. After a period of egg development, flies were subjected to oviposition trials. Stacks of oviposition trays, each consisting of 6 glass culture tubes (13 x 100 mm) containing 1 ml of distilled water and placed within a 90 mm^2 plastic petri dish at a 10° slant were prepared to receive gravid flies. Each fly was transferred to an individual oviposition tube and contained with a cork stopper. Oviposition trays with flies in tubes were placed in a strong light with the end containing the water directed at the light source. S. exiguum trials were conducted beginning at 36, 42, 48, 72 and 96 hours postfeeding and S. metallicum trials occurred beginning at 42, 48, 96 and 120 hours postfeeding. A fly was held in an oviposition tube for no more than 1.5 hours during a trial. After each trial, gravid flies were aspirated back into holding tubes until the next trial. Counts were made of the number of flies that escaped in placement, drowned in oviposition tubes, survived through the oviposition trials and that oviposited per trial. The eggs which were oviposited by each fly were also counted on the inside of each tube. In addition reductive changes in ovariole scars and granular relics were observed in flies after oviposition. Reductive ovarian scar changes were studied from flies dissected one-hour post oviposition and at 6-hour intervals after oviposition up to 72 hours. Parous flies of each species were dissected after each of the aforementioned time intervals from groups of flies that were recontained in tubes after oviposition. The condition of the ovarian scars was recorded and typical examples of states of reduction of scar and relic formation for flies in each time interval were photographed.

Postoviposition mortality (survivorship potential after oviposition): Engorged S. exiguum and S. metallicum were collected, transported to the laboratory and oviposition trials were made after 3 days of egg development. Rates of mortality were based upon the survivorship of oviposited flies compared with gravid controls. A tally of dead flies was made at 24-hour intervals after oviposition to determine mortality in relation with time. The resulting mortality data were subjected to statistical analysis between intraspecific mortality rates of flies with retained eggs versus oviposited flies as well as interspecific rates between oviposited S. exiguum and S. metallicum using a Chi-Square Test.

F. Capture, marking, release and recapture studies: The feasibility of using fluorescein powders for marking black flies was tested in the laboratory. The marking compound consisted of orange or blue fluorescein powder mixed 1 to 1 with gum arabic powder to make a sticky pigment-gum compound. Engorged S. exiguum and S. metallicum were aspirated into collection cups and some of each species were killed and age-graded to determine parity rates. The remaining collections were transferred in equal numbers into 2 marking cups of the same description as collection cups, but with carton caps over the petri covers on both ends. A flit-gun duster with the nozzle pinched to a slit size of 1 mm was used to dust the flies in the marking test groups. After misting the confined flies with water, the selected marking powder was applied. The nozzle of the duster was fitted into the side vent of the marking cup and pumped until traces of powder escaped through the bobbinet around the edge of the cup. Two groups of either S. exiguum or S. metallicum were separately marked with 2 different colored compounds. Control flies were transferred to a marking cup and atomized with water, but were not marked.

Immediately after dusting or water atomization, each fly was transferred to an individual holding tube. After transfer, each dusted fly was examined in darkness with a dissecting microscope at 15 manifications under a blacklight (Westinghouse No. F15T8/BLB) to confirm that the dusting compound was adhering. The tubed flies were stored and checked every 24 hours for mortality. Dead flies were counted to determine rates of mortality for control and marked groups of S. exiguum and S. metallicum. Dead flies in marked groups also were examined with the blacklight to verify that they had retained their marks throughout their survival in the laboratory.

The length of time of the gonotrophic cycles of S. exiguum and S. metallicum was measured under natural conditions using a capture, release and recapture technique of flies marked with fluorescent compounds. Studies were conducted for both species during June and July 1979. For each species a 9-day trial consisted of 2 consecutive days of collection and marking, a day of rest, followed by 6 consecutive days of attempting to recapture marked flies. On marking days, 3 cows were tethered within 15 m of each other and collectors aspirated fully engorged flies from them during the early morning flight period. When a marking cup contained 200 flies, they were dusted with a marking compound. Orange and blue compounds were used to dust collections on days 1 and 2, respectively. After marking, flies were released over a large sheet of filter paper and permitted to fly off at will. During the morning flight periods on days 4 to 9 of each marking trial, 3 cows were tethered and collectors aspirated all incoming flies into clean collection cups. Flies were returned to the laboratory, killed, sorted, identified and examined with a dissecting microscope at 15 magnifications under a blacklight for

fluorescence with either orange or blue compound. Flies which showed fluorescent marks were immediately dissected to determine if they were newly parous. The numbers of parous S. exiguum and S. metallicum which were clearly marked were used to determine a frequency distribution for the lengths of time required by each species to pass through a gonotrophic cycle in nature.

RESULTS

I. Parasite Studies

A. Distribution and prevalence of the parasite: Cattle were skin snipped on 13 farms in the Departments of Cauca and Valle in order to determine in what area O. gutturosa was common and what was the frequency of infection (Figure 1). The farms visited, number of cattle snipped, and the density of microfilariae in circumumbilical snips of infected animals are summarized in Table 1. In all, 105 cattle were examined and of these 6 (5.7%) were positive. Positive animals were found on 3 of 13 farms in the Cauca Valley. The density of microfilariae in infected animals ranged from approximately 60 to 2,400 mf/g. Of the 6 cows infected with O. gutturosa, 2 originated from the Department of Cordoba and 4 were born and raised in the Cauca Valley. Consequently, there were at least two foci of infections in the study area. One focus comprised native-born cattle from Cajibio, Cauca which were infected on their home range. The other focus consisted of cattle which were imported to Lomitas, Valle and were previously infected on a farm in the Department of Cordoba before arrival in Lomitas.

B. Behavior of microfilariae

Seasonal variation: Concentrations of microfilariae in skin biopsied each month from a cow over a year were determined in order to see if fluctuations occurred in syncrony with changes in black fly population abundance. Microfilarial concentrations from circumumbilical snips showed considerable fluctuation (Table 2). Counts varied from a high of 2,380 mf/g in May to a low of 0 mf/g in January 1980. Densities tended

to diminish after the first snip in May, reaching a low of 32 mf/g in September, rising again in October to 689 mf/g and dropping, thereafter, to very low levels between 0 and 100 mf/g. Counts per gram of superficial skin were negligible except in July and February when there were 279 and 645 mf/g of skin, respectively. Seasonal changes in the microfilarial concentrations of the skin were not consistent with changes in black fly population abundance.

The average count from deep snips was about 588 mf/g as compared with an average of 92 mf/g for superficial snips, resulting in a ratio of 6.3:1. There was poor correlation between skin snip size and the number of microfilariae present in circumumbilical skin, but generally as skin snip thickness increased, less microfilariae were harvested per gram of skin.

Anatomical distribution: Biopsies were taken from skin on the head and belly of slaughtered cattle infected with O. gutturosa in order to determine where microfilariae were most concentrated. It was observed that more than 92% of biopsies from the suboccipital (SOT) region were positive whereas only 21.4% of the circumumbilical (CU) biopsies from the same animals harbored microfilariae (Table 3). The highest concentration found in a SOT biopsy was 3,076 mf/g while the highest from a CU snip was 203 mf/g. The average count from a SOT snip was about 501 mf/g as compared with an average of 33 mf/g from CU snips, producing a ratio of 15:1. The majority of these infected animals were imported from the Department of Caqueta where, as indicated by the rates of infection, O. gutturosa transmission was intense.

II. Vector Studies

A. Development of the parasite in black flies: Experimental studies were undertaken to determine the course of O. gutturosa infection in

naturally fed, laboratory-maintained S. exiguum and S. metallicum. Of 163 S. exiguum and 73 S. metallicum which were allowed to fully engorge upon a cow with a circumumbilical skin density of between 0 and 2,500 mf/g during the course of the study, no microfilariae were found upon dissection of their bloodmeals immediately postfeeding (Table 4). When 668 S. exiguum were examined at various intervals after feeding upon another cow with circumumbilical counts between 4,000 and 4,000 mf/g, 6 (0.9%) contained filarial larvae in various stages of disintegration or development. Only 1 out of 264 (0.4%) S. metallicum dissected at intervals after feeding upon the same cow contained a filarial larva.

Figures 2 and 3 show disintegrating 1st-stage larvae which were found after 5 days of incubation in S. exiguum and S. metallicum, respectively. Larvae which were found on days 5 and 7 indicated that development of the filariae was both abnormal and retarded (Figures 4 and 5). The larva in Figure 4 was in late 1st-stage with an anal plug, but invaginations in the cuticle along with some possible melanization indicate its development was irregular. The larva in Figure 5 was in the early 2nd-stage, invaginations were pronounced producing an abnormal bulbous protrusion at the caudal end, no anal plug was present and the anterior portion of the formative esophagus was melanized indicating that development was irregular and terminating. Figure 6 shows the most advanced filaria that was found in a black fly demonstrating a normally developing 3rd-stage larva that was dissected from S. exiguum 3 days after the fly bloodfed. Development of this larva was more advanced than expected for 3 days of incubation, suggesting that it was the product of infection at a natural feeding prior to the experimental feeding. However, the presence of a 3rd-stage larva in S. exiguum

indicated that complete intermediate development of O. gutturosa was possible in this species of black fly.

B. Filariae in wild-caught black flies: An effort was made to determine to what extent S. exiguum and S. metallicum were naturally infected with filariae, especially O. gutturosa. A total of 1,472 S. exiguum and 1,121 S. metallicum collected in Pance and Lomitas were dissected, and none contained developing filariae. Furthermore, from a total of 15,807 S. exiguum and 7,093 S. metallicum were collected in Lomitas at 2-week intervals over a year, pooled by species and "baermannized," and no filarial larvae were found.

C. Interrupted feeding: A plausable explanation to account for the inability to experimentally infect S. exiguum and S. metallicum as well as for the negligible natural infection rates in wild-caught flies was that interrupted feeding was limiting the opportunity for the ingestion of microfilariae. The amount of interrupted feeding was determined in S. exiguum and S. metallicum populations by age and gonotrophic state. A gonotrophic profile for S. exiguum is illustrated in Figure 7. Approximately 49% of S. exiguum were nulliparous. The total population of nulliparous in CH-II or less composed 43% of the population while nulliparous in CH-I made up only 1% of the population. There was always an abundance of S. exiguum in CH-II, some of which were interrupted feeders. However, there were more nulliparous as interrupted feeders in advanced stages of Christophers' development, but consecutively fewer flies were found as interrupted feeders in a higher state of gonotrophic development. About 51% of the population was parous, but 48% were in CH-II or less. CH-I, parous S. exiguum composed 0.6% of the total population. In general, parous CH-II, S. exiguum were the most abundant among the parous population,

but more parous interrupted feeders were found in advanced Christophers' stages. There was a noticeable scarcity of parous CH-IV and V interrupted feeders. Overall, 31% of the interrupted feeding of S. exiguum resulted in ovaries advancing beyond CH-III. Of the total S. exiguum population, 13.4% were interrupted feeders.

A gonotrophic profile for S. metallicum is shown in Figure 8. About 60% of S. metallicum were nulliparous. The total population of nulliparous in CH-II or less composed about 49% of the general population while only 2% of nulliparous were in CH-I stage. There was an abundance of nulliparous in CH-II and although a small percentage were interrupted feeders the greatest proportion of such flies were in CH-III. About 40% of the population was parous, and 37% of the general population were parous CH-II or less. CH-I parous flies composed only 1.3% of the total population. Parous flies in CH-II were most abundant and a few were interrupted feeders. However, most parous flies detected as interrupted feeders were in CH-III while there was an absence of such flies found in CH-IV and V. For the whole population, 15% of the interrupted feeding resulted in advancing the gonotrophic cycle in flies beyond CH-III. About 19.8% of S. metallicum were interrupted feeders.

Tables 5 and 6 compare the relative percentages of interrupted feeding populations with those of the general populations of S. exiguum and S. metallicum, respectively. For both species, a higher percentage of nulliparous flies and a lower percentage of parous flies had interrupted meals relative to the comparative percentages of flies in these categories in the general populations.

Interrupted feeding was confirmed by the identification of bloodmeals of a different serotype than that of the bait-animal around which the blooded flies were collected. Figure 9 shows a strong positive

reaction of an unknown bloodmeal to anticow serum (AC) using the double immunodiffusion test. This reaction was typical of the type of precipitin line that was considered as acceptable for a positive bloodmeal identification. Table 7 shows the comprehensive results of analysis of 46 S. exiguum and 34 S. metallicum bloodmeals that were harvested after the flies were collected from a bull-calf bait. Of the S. exiguum bloodmeals, 23.9% were positively identified and of these 63.3%, 27.3% and 9.1% were shown to be blood of cow, horse and human origin, respectively. S. exiguum bloodmeals which produced colored eluate were always identifiable, whereas only 18.6% of bloodmeals without colored eluate were identified. Nine of 36 unknown S. metallicum bloodmeals were identified producing an identification success rate of 26.5%. Two-thirds of these meals were identified as of cow origin and one-third were of horse origin. About 66.7% of the bloodmeals producing colored eluate were identified while only 17.8% of bloodmeals without colored eluate were identified. For S. exiguum, 36.4%, and for S. metallicum, 33.3% of the identifiable bloodmeals were of a different serotype from that of the bull-calf bait-animal.

D. Bloodmeal size and processes of extraction, digestion, and excretion: The low success rate of bloodmeal identification suggested that the test system was not sensitive enough to identify small bloodmeals. Similarly, equivocal results were often obtained when trying to identify known bloodmeals of freshly engorged S. exiguum and S. metallicum. Figure 10 shows the results of an attempted identification of bloodmeals of freshly engorged S. exiguum and S. metallicum. Although these bloodmeals were of similar age and quantity, only three of them were positively identified. Therefore, an attempt was made to determine the size of

S. exiguum and S. metallicum blood and serum meals and to find out what proportion of these meals were identifiable by double immunodiffusion methods.

The results of the determination of bloodmeal volumes and serum-meal volumes for fully engorged S. exiguum and S. metallicum are given in Table 8. The mean amount of whole blood ingested by S. exiguum was 0.667 ± 0.150 mm^3 (range 0.430-0.937 mm^3) and the mean amount of serum ingested was 0.248 ± 0.086 mm^3 (range 0.127-0.379 mm^3). The S. exiguum whole-blood-to-serum-ratio was 2.7:1 (range 1.15:1-4.9:1). The mean amount of whole blood ingested by S. metallicum was 1.098 ± 0.149 mm^3 (range 0.810-1.342) and the mean amount of serum ingested was 0.490 ± 0.239 mm^3 (range 0.189-0.759 mm^3). The S. metallicum whole-blood-to-serum-ratio was 2.2:1 (range 1.3:1-6.4:1). On the average, S. metallicum whole blood and serum meals were 2 times larger than those of S. exiguum.

Figure 11 shows the reaction of various dilutions of a mock maximum-serum-meal of S. metallicum to hyperimmune cow antiserum. This test indicates that when serial on-half dilutions of 1/5th of a maximum meal of S. metallicum were tested for reaction with cow antiserum (AC), reaction was positive up to 1:6,400 of a serum dilution. This dilution was equivalent to 1/80th of the quantity of serum that was ingested by the average, engorged S. metallicum and was theoretically the minimum identifiable serum meal of this species. When serial one-half dilutions of 1/5th of the maximum-serum-meal of S. exiguum were tested for reaction with cow antiserum, as expected, reactions were positive up to 1:6,400 of a serum dilution. However, this dilution was 1/40th of the quantity of serum that was ingested by the average S. exiguum and was theoretically the minimum-serum-meal which was identifiable for this species.

Given that the test appeared to be very sensitive, but that serum meals were highly variable in volume, this led to the conclusion that some bloodmeals were not identifiable because the blood ingested by flies contained serum as well as serous fluid or that serum was often digested and excreted from bloodmeals prior to analysis. The former possibility was supported by the contention that mechanisms related to blood extraction during interrupted feeding were preventing flies from obtaining a blood meal with a normal volume of pure serum. The possibility that serum was being digested by flies was evaluated by following the course of bloodmeal digestion to see as a bloodmeal aged if it tended to produce colorless eluate. It was found that bloodmeals which were over 48 hours old always produced colorless eluate. These eluates were identifiable less than 20% of the time with the gel-diffusion test. The possibility that excretion of serum by flies was reducing the potential for bloodmeal identification was confirmed by the observation that both S. exiguum and S. metallicum excreted fluids during digestion up until 72 hours postfeeding. Since bloodmeals were never processed until 24 hours after flies were captured, digestion and excretion of serum occurred for at least this period of time prior to the preparation of bloodmeals for analysis by gel-diffusion methods.

E. Feeding efficiency: Since interrupted feeding was an indicator of inefficient feeding behavior, a study was undertaken to determine if the rates of feeding among landing populations of S. exiguum and S. metallicum differed and if these rates increased when fly populations contained higher proportions of nulliparous flies. The comparative numbers and parous rates of landing versus feeding populations of S. exiguum during 2 flight periods on 5 collection days are shown in Table 9. The

capture totals for early morning flights indicate that about 44% of the population was capable of feeding, while during afternoon flights nearly 50% fed. During the morning, feeding and landing flies had the same parous rates of 72%. In the afternoon, feeding flies has a parous rate of 52% and the rate for landing flies was 60%. Comparison of the morning and afternoon flights shows that the morning population of flies had a higher cumulative parous rate of 72% while the afternoon population had a cumulative rate of 56%. Overall, the parity of feeding flies was lower than landing flies, indicating that the feeding populations were composed of more nulliparous flies.

Table 10 shows the comparative numbers and parous rates of landing vs. feeding populations of S. metallicum. The capture totals for the early morning flights show that 36% of the population was capable of feeding and the same percent had successful feeding in the afternoon. During the morning and afternoon flights feeding flies had lower parous rates than landing flies. The afternoon population of flies had a higher cumulative parous rate of 77% than the morning population with its rate of 38%. Overall, the parity rates of feeding flies was lower than landing flies and so feeding populations were composed of more nulliparous flies.

Figure 12 shows each landing rate plotted against feeding rate for both S. exiguum and S. metallicum. In general, as landing rates of each species increased, the number of feeding individuals increased proportionately. However, the feeding efficiency for landing S. metallicum was lower than for landing S. exiguum. When the feeding versus landing rates were regressed for S. exiguum a single line was constructed which best fit the feeding rates as they increased in proportion to the landing rates. The slope of the regression line indicates the proportion of feeding

versus landing flies when S. exiguum populations varied from low to high abundance. The regression of points for landing versus feeding S. exiguum showed that slightly more than 50% (Slope = 51.2/100) of the landing population of S. exiguum was capable of feeding when flies were landing at rates between 50 and 300 flies/hour. For S. metallicum, regression points plotted for landing versus feeding flies showed that slightly less than 30% (Slope = 27.7/100) of the landing population was capable of feeding when landing rates were between 100 to 1,200 flies/hour. Therefore, S. exiguum had a rate of feeding efficiency about 2 times greater than that of S. metallicum.

F. Gonotrophic period - laboratory studies: Since black flies were required to live through the period of intermediate development of O. gutturosa before eventual transmission of the parasite occurred, the number of gonotrophic cycles through which the average S. exiguum and S. metallicum survived during that time was an important measure of potential transmission capability. Therefore, studies were done to define the length of gonotrophic cycles and survivorship potentials of populations of S. exiguum and S. metallicum exposed to mortality risks which were related to different stages in the life-cycle during a gonotrophic period.

Anautogeny: Postemergence ovarian development was studied in S. exiguum and S. metallicum in order to see if both species exhibited gonotrophic concordance, i.e. complete egg development stimulated and nourished by a bloodmeal. Furthermore, throughout this study more females than males were produced from pupae. This raises the question whether a genetic balance gave rise to the production of more females as a guard against losses due to the risks associated with oviposition.

The dissection results of wild-caught, laboratory-emerged S. exiguum in regard to their state of ovarian development soon after ecdysis are shown in Table 11. Nearly 73% of the flies had ovarioles in CH-I stage. About 24% had ovarioles in CH-N stage, while only 2.8% had advanced to the ovarian diapause stage of CH-II. There were no cases of ovaries advancing beyond CH-II. Twice as many female flies emerged as males. The dissection results of laboratory-emerged S. metallicum are shown in Table 12. About 88% of the S. metallicum contained ovaries in CH-I, while only 7.9% of the flies had ovaries in CH-N. Approximately 4.4% of the flies contained ovarioles in the diapause stage CH-II. There was no evidence of ovarian development beyond diapause and about 1.5-times-more females S. metallicum emerged than males. Both species were anautogenous.

Oogenesis timing: The duration of egg development including the postemergence and postfeeding periods was studied in order to ascertain the amount of time oogenesis contributed to the gonotrophic period and to define morphological changes in eggs of S. exiguum and S. metallicum that signaled oviposition readiness. Table 14 shows the sequential steps in the ovariole development of S. exiguum and S. metallicum in the post-ecdysis phase followed by the postfeeding phase. Ovariole sizes of the more "petit" S. exiguum were smaller throughout these stages of development. The CH-N and CH-I stages of S. exiguum most closely approximated the primary and secondary oocyte length of S. metallicum, but the length of the ovarioles in higher Christophers' stages for S. metallicum were always greater. The various stages of development in S. metallicum are shown in Figures 13 to 24. Figure 13 shows an ovariole in CH-N stage dissected from a fly soon after emergence. The ovariole is very small and barely shows any differentiation of primordial nurse cells. Figure 21

shows a secondary follicle that was a good example of a CH-I ovariole. The nurse cells are more visible. Figure 14 shows a CH-IIA stage ovariole in which the primary follicle has visible nurse cells, but an empty yolk vacuole. Figure 15 shows a CH-IIB primary follicle ovariole in which the yolk vacuole has filled with yolk material. Figures 16 to 19 show sequential development of primary follicles from CH-IIIA to CH-IVB in which the length of the egg increased nearly 3 times. Yolk deposition has crowded the nurse cells and nucleus into the distal end of the egg in Figure 19. The maximum egg size was first obtained as eggs developed to Christophers' VA stage as shown in Figure 20. Table 13 indicates that the maximum egg sizes were generally obtained between 30 and 54 hours postfeeding for S. metallicum and slightly earlier for S. exiguum. Figure 20 shows a good example of complete development in which the fully developed egg has opposing flat and convex sides. Figure 21 shows the ultimate stage of development (CH-VB) in which the mature oocyte was in a state of oviposition readiness. The tunica was easily stretched and the chorion was sloughing around the mature egg. The state of oviposition readiness was reached as early as 48 hours postfeeding for S. metallicum and earlier at 42 hours for S. exiguum. Figure 22 shows a newly oviposited S. metallicum egg with expanded chorion and Figure 23 shows the tunica containing follicular debris from whence the egg was delivered leaving a scar. Figure 24 shows a rather rare event in which the primary follicle has degenerated, the fly took more blood and the secondary follicle advanced to a late CH-IIB stage.

Time to oviposition: The frequency of induced oviposition at various intervals postfeeding was studied in the laboratory in order to confirm the timing of actual oviposition readiness. Table 14 shows the fate of

wild-caught, bloodfed S. exiguum held in the laboratory and subjected to oviposition trials. Flies oviposited as early as 42 hours postfeeding, but the majority of flies oviposited within 48 hours. After 48 hours survivorship was very low, thus decreasing the potential for oviposition, but the percentage of ovipositing survivors was very high at 81%. Table 15 shows the fate of wild-caught, bloodfed S. metallicum held in the laboratory and subjected to oviposition trials. This species oviposited as early as 48 hours postfeeding with a majority ovipositing within 3 days of bloodfeeding. The most dramatic mortality was experienced between day 3 and 4, but this did not decrease markedly the oviposition potential which was never very high. The percentage of ovipositing survivors increased fairly steadily at each successive trial.

Figure 25 shows the frequency of oviposition expressed as a percent of the total oviposited flies for S. exiguum and S. metallicum. S. exiguum oviposited earlier with far more frequency on day 2 than did S. metallicum. The majority of S. exiguum were ready to oviposite within 48 hours of feeding while S. metallicum were most oviposition-ready at 72 hours postfeeding.

Postfeeding/preoviposition mortality: Survival through the postfeeding/preoviposition period was essential for flies in order to successfully complete oogenesis prior to seeking an oviposition site. Since this period is the first stage in the gonotrophic cycle, survival during this time in wild-caught populations was studied in the laboratory to determine when the major mortality increment occurred for S. exiguum and S. metallicum as a measure of the probable time before which oviposition was required. The percentages of mortality and the adjacent cumulative percentage of mortality of S. exiguum and S. metallicum are

shown in Table 16. The S. exiguum population experienced its highest mortality at 108 hour postfeeding and there were no survivors after 156 hours. S. metallicum also experienced its highest mortality at 108 hours, but this mortality was 10% less than for S. exiguum during the same interval and the cumulative percentage of death up until that time was more than 15% less than for S. exiguum. There were no survivors in the S. metallicum population after 180 hours. Chi-square analysis indicated that there was a significant difference between the mortality rates of these species. When the mortality rate of S. metallicum was increased by adjustment to account for its lower parity rate, there was only a probability of 10 to 25% that the rates of the two species were the same. For both species the major increment of mortality occurred on day 4 indicating that survival prior to this time enhanced the possibility of oviposition.

Postoviposition mortality: Survival through the postoviposition period was essential for flies in order to complete a gonotrophic cycle by successfully obtaining a subsequent bloodmeal. Since this period was the final stage a gonotrophic cycle, postoviposition survival was studied in wild-caught fly populations maintained in the laboratory to determine when the major mortality increment occurred for S. exiguum and S. metallicum as a measure of the probable time before which flies needed to obtain bloodmeals. The percentages of mortality of gravid and postoviposited S. exiguum and S. metallicum under labotatory maintenance are shown in Table 17. A comparison of the rates for the gravid populations of both species shows there was very little difference in mortalities and in both cases survivorship was extended. The greatest difference in the postoviposition death rates occurred 24 hours after oviposition when S.

exiguum had twice the mortality rate of S. metallicum. After the first day, the death rates of S. metallicum were slightly higher than those for S. exiguum. There was a statistically significant difference in the mortality rates for the two species. Figure 26 compares the cumulative percentages of mortality for both species and shows that the higher progressive rate of mortality of S. exiguum was due to its vastly higher first-day increment of mortality. There were no survivors in the S. metallicum study population after day 6 and none in the S. exiguum population after day 5. The major increment of mortality occurred on day 1 for S. exiguum and on day 3 for S. metallicum indicating that survival prior to these times enhanced the possibility of successful subsequent bloodfeeding. However, because of earlier expected mortality, S. exiguum populations were required to obtain blood sooner.

Given that black flies were required to survive more than one gonotrophic cycle before O. gutturosa transmission occurred, calculations were made to determine the possible mortality rates of S. exiguum and S. metallicum populations passing through several gonotrophic cycles. Based upon observed postfeeding and postoviposition mortalities, survivorship rates through 1 gonotrophic cycle were generated for S. exiguum and S. metallicum. Table 18 and 19, respectively, show these results along with expected mortalities for populations of S. exiguum and S. metallicum experiencing the risks of passing through 3 gonotrophic cycles. The results of the theoretical calculations for survivorship showed that from populations of 10,000 nulliparous only 3 S. exiguum and 210 S. metallicum were likely to live through two gonotrophic cycles.

G. Gonotrophic periods in nature: Using a capture, release and recapture technique for black flies marked with fluorescein compounds,

attempts were made to establish the lengths of the gonotrophic periods of S. exiguum and S. metallicum in nature.

Feasibility: Since it was not known if the fluorescein compounds used for marking were deleterious to black flies, their effects upon survival of wild-caught, laboratory-maintained S. exiguum and S. metallicum were studied. Table 20 illustrates the cumulative rates of mortality of wild-caught, bloodfed S. exiguum, either marked or unmarked, and maintained in the laboratory. Unmarked S. exiguum had the best survivorship in comparison with marked test groups of flies. Those marked with orange compound had about 25% mortality before the midday minimum oviposition period. Over 50% of the orange-marked, S. exiguum study population was dead by the period of maximum oviposition deadline. In contrast, S. exiguum marked with blue compound experienced about 50% mortality before the minimum oviposition period and 65% were dead before the period of maximum oviposition. S. exiguum marked with blue compound were more subject to mortality than those marked with orange during the 3 day period of ovarian development.

Table 21 compares the rates of mortality of wild-caught, bloodfed S. metallicum groups of marked and unmarked flies that were maintained in the laboratory. Unmarked S. metallicum of Test I and II survived better than the groups of marked flies. In Test I, S. exiguum marked with orange compound, there was only 8% mortality prior to the minimum period for oviposition, while those marked with blue compound showed a mortality of 40% before the minimum period. Test I S. metallicum marked with orange compound and those marked with blue compound showed respective mortalities of 25% and 57% prior to the maximum oviposition period. The mortalities up to this point were considerably less for

S. metallicum marked with either compound. However, S. metallicum marked with blue compound were also more subject to mortality during the 3-day period for oogenesis. S. metallicum mortalities for Test I and Test II were similar although those marked with blue survived better in Test II. The group in Test II marked with a one to one mixture of orange and blue compounds showed a cumulative mortality that was between the rates observed for S. metallicum marked with either orange or blue compound. Overall, S. metallicum was less affected by marking with with either color of compound than S. exiguum.

Reductive changes in ovarian scars: Accurate identification of ovarian scars was required in order to document that marked flies had actually oviposited. Therefore, reductive changes in the follicular relics of S. exiguum and S. metallicum induced to oviposite in the laboratory were studied over time. At 18 hours postoviposition, S. exiguum had very visible scars which consisted of consolidated relic material inside of the ovarian tunica sheath. In contrast. S. metallicum quite often retained highly visible scars up to 80 hours postoviposition. Some degree of scarring, usually in the form of refractile, greenish-yellow granules, was evident in both species up to 150 hours postoviposition. As either species aged after oviposition, fewer ovarioles in an ovarian cluster were found to have visible scars. Scars of both species always were identifiable up to 48 hours postoviposition. Figures 27 to 30 depict a sequence of ovarian scar reduction observed in S. metallicum at 1, 18, 36 and 48 hours postoviposition, respectively. Figure 27 shows a large amount of follicular debris contained within the tunica sheath. Figures 28 and 29 are typical examples of scars with relics or granules which were typically encountered when dissecting

wild-caught flies in order to evaluate parity. A fly was called parous only if it was in a state of reduction at least as visible as shown in Figure 30 and if several ovarioles of a specimen were observed in a similar or more visible state of scarring. Although Figure 30 demonstrates very little follicular granulation, the condition at this particular state of reduction shows the minimum of granulation that was encountered at 48 hours postoviposition.

Field trials: Flies were marked with different colored compounds on consecutive days in order to judge the effectiveness of each compound as a marker and to provide a means of delineating the time interval from marking to return. Tables 22 and 23 summarize the recapture results for S. exiguum and S. metallicum, respectively. About 0.5% of S. exiguum returned while almost 1.8% of S. metallicum returned after marking. Eight of the S. exiguum returnees were marked with blue compound and one was marked with orange compound. Of the S. metallicum returnees, 8 were marked with blue compound whereas 4 were marked with orange compound. S. metallicum had a recapture rate about 3 times greater than S. exiguum and in the case of both species the return rates for blue-marked flies was significantly higher than those for orange-marked flies.

Figure 31 shows the times at which S. exiguum and S. metallicum marked with orange or blue compounds were recaptured. With both species, marked specimens which had oviposited returned for a subsequent bloodmeal most frequently 72 hours later. S. exiguum returnees showed a less pronounced clustering around the 72-hour interval than S. metallicum returnees. On the average, the gonotrophic period for both species was 3 days in nature.

H. Daily activity and seasonal abundance of black flies: Activity and abundance of parous and nulliparous populations of black flies were studied in order to determine environmental and life-cycle factors which were influencing fluctuation and to ascertain when O. gutturosa transmission was most likely to occur.

Daily rates of activity: The daily activity of landing populations of S. exiguum compared with changes in temperature and humidity is illustrated in Figure 32. The daily activity of S. exiguum was most pronounced during the early morning, averaging about 180 flies/hour. This activity declined to a low for the model day during the midday period to 100 flies/hour and then increased after noon to around 120 flies/hour. The activity of potentially infected parous flies decreased markedly after the first period and only increased again slightly during the midafternoon period. About 75 parous flies/hour were active in the early morning and this decreased to about 50 flies/hour for sampling periods during the rest of the day. Proportionally more parous flies made up the composition of the total population of active flies in the mid and late afternoon samples. Generally, the percentage of parous flies was lower in connection with low temperatures and high humidity, and higher with high temperatures and low humidity. This indicated that parous flies were more likely to be active under unfavorable conditions. The weighted average of all active flies per period accurately coincided with the total activity averages and confirmed that high landing activity was correlated with low temperature and high humidity.

Figure 33 illustrates the temperature and humidities at which various estimated proportions of a model S. exiguum population were likely to be active. Activity for the general population was lowest when humidity was extremely low and temperature was extremely high. Population were most active when humidity was between 80 to 90% and temperatures were

between 70 and 80 F (21 to 23 C). Very high humidity, typical during or after rainfall or very atypically cool mornings, depressed population activity.

The daily landing activity of populations of S. metallicum compared with changes in temperature and humidity is illustrated in Figure 34. The daily activity of S. metallicum was most pronounced in the early morning and late afternoon capture periods. The lowest activities were during the midday and midafternoon periods when about 35 flies landed per hour. Total landing fly averages were perfectly related to variations in temperature and humidity; lower temperatures and high humidity being correlated with high activity and just the opposite. The average number of potentially infected parous S. metallicum landing populations paralleled the landing activity of the total population. An average of 30 parous flies were active during the early morning period which decreased to 10 flies per hour during midday and midafternoon and then increased to a high of 50 parous flies per hour in late afternoon. The proportion of parous flies within the total population was constant between 25 to 30% for the early morning through midafternoon. This percent increased dramatically in the late afternoon to about 55%. There was no connection of parous activity with changes in temperature and humidity. On the other hand, the weighted average of all active flies per period coincided with the total rates for S. metallicum landing per hour and confirmed that high activity was correlated with moderately low temperatures and moderately higher humidities.

Figure 35 illustrates the temperature and humidities at which various estimated proportions of a model S. metallicum population were likely to be active. Activity for the general population was lowest when humidity was extremely low and temperature was extremely high. Landing populations

were highest when humidity was around 80% and temperatures were between 70 and 75 F (20 to 22 C). Population activity was depressed at very high humidity and very low temperatures.

Seasonal rates of abundance: The seasonal abundance of landing populations of S. exiguum compared with changes in temperature, humidity and rainfall are illustrated in Figure 36. In February through March landing rates of the total population were moderately low at around 100 flies/hour. This rate dropped to less than 25 landing flies/hour throughout April and May as rainfall accumulations and humidity increased and daily temperature decreased. In mid-June rates began to climb and reached a high in late July of 400 flies/hour after several weeks of little rainfall, low humidity and high temperature. Activity dropped to 200 flies/hour with the onset of rains in late August when there was a marked rise in humidity and a drop in temperature. Rainfall continued to accumulate through the months of September, October and November, sustaining high humidity. Through that time landing rates gradually declined to a very low level of 25 flies/hour. When rainfall ceased for a brief interlude in the early weeks of December, landing rates rebounded and remained steady at around 100 flies/hour until the end of the study in March. The seasonal activity of landing populations of potentially infected parous flies followed the pattern for the total population. Their lowest activity was in April at around 15 flies/hour and the highest rate was in late July when 110 parous flies were active per hour. This was followed by another decline to around 20 parous landing flies per hour in early December. Over time the parous populations composed 17 to 85% of the total landing population. Four major peaks of relative parous activity occurred during May, August, October and February when, in every case, rainfall accumulations were high and increasing.

The seasonal abundance of landing populations of S. metallicum compared with changes in temperature, humidity and rainfall are illustrated in Figure 37. In February through March the landing rates of the total S. metallicum population were high between 60 to 80 flies/hour. As rainfall accumulations increased through April along with increases in humidity, rates began to drop to a low in early May of 30 flies/hour. Despite occasional heavy rainfall accumulations in Late May and early June, populations began to increase. Landing rates were highest at around 100 flies/hour from late June through mid-August when rainfall had stopped. A precipitous drop in landing rates occurred in mid-August when a brief series of heavy rains fell and humidity rose sharply. Populations rebounded in late September while there was a moderate accumulation of rainfall and fairly high temperature and humidity. As more rainfall accumulated in October and November, landing rates declined to a low in early December of 30 flies/hour. A slight surge in landing rates occurred in late December during a dry period, but thereafter, rates declined and remained low at between 20 to 30 flies/hour as rainfall accumulations increased at the end of the study. Landing rates of potentially infected parous flies closely followed the pattern for total population activity throughout the study. A low parous landing rate of 10 to 15 flies/hour was observed in April through May climbing to a high rate of 40 flies/hour in mid-June. This was followed by another low in mid-August after which rates rose slightly until November and then declined again to the lowest parous landing rate of approximately 5 flies/hour in February and March. During the course of the study parous flies composed between 23 to 66% of the total landing population. Six well defined peaks of relative parous activity occurred during the course of the study when, in every case, rainfall accumulations and humidity were high.

DISCUSSION

Skin snip surveys in the Departments of Valle and Cauca demonstrated that O. gutturosa was present in cattle areas where black flies were frequently feeding on cows. Although it was difficult to determine generally if infected cattle were born and reared in Valle or Cauca, it was confirmed that at least 2 infected animals from Cauca farms had been in the valley all their lives and developed onchocerciasis. The majority of other infected cattle had been transported into the valley from the northern Pacific Coast. Beef cattle were frequently brought to farms in the Cauca Valley for fattening prior to slaughter. The high prevalence of onchocerciasis (90%) in cattle slaughtered in the Cali abattoir suggested that the majority of cows transported to the valley from the eastern plains (Llanos) were also infected with O. gutturosa. These are important epizootiological considerations, since in all likelihood these cattle served as the major reservoir of infection in the valley. These considerations may be of even greater consequence and concern since other more serious arthropod-borne diseases of cattle such as trypanosomiasis and anaplasmosis may be similarly transported into Valle and through available local arthropod vectors become established in endemic or epidemic form.

A few of the cows found positive for O. gutturosa had high circumumbilical (CU) skin densities indicating that possibly they were good donors for infecting black flies. Eichler and Nelson (1971) and Eichler (1971) observed that O. gutturosa microfilarial concentrations were highest in anatomical sites where vectors fed and varied in accordance with the seasonal abundance of potential vectors. The present study of

seasonal concentrations of microfilariae from CU skin snips indicated that there was considerable variation in microfilarial skin densities of the cow. Changes were not coincident with seasonal fluctuations in black fly populations, but these observations on one animal may not have been typical of the population of infected animals. Furthermore, the cow was often treated with Negubon^{R} and this systemic insecticide may have destroyed microfilariae and biased sampling. The parasiticidal effects of Negubon^{R} on nematode larvae are well documented by Schoop and Lamina (1959) and Khamis et al. (1973).

Maximum microfilarial concentrations were found most frequently in skin snips from the suboccipital (SOT) area of slaughtered animals. These findings are in agreement with Bain et al. (1977) and Elbihari and Hussein (1978), but contrary to the findings of Eichler and Nelson (1971). Since the primary site of microfilarial concentration was around the head, it may be concluded that the prevalence rate based upon taking CU snips was inaccurate. CU snips from slaughter house samples were much larger than CU snips taken in the general survey. Therefore, it was probable that only the highest microfilarial concentrations were detectable in the general survey while even light concentrations were found in slaughtered animals. Given these considerations it was likely that many infections were missed in the general survey and that the actual prevalence of infection in animals on local farms in the valley was much higher. Nevertheless, the prevalence rate of about 6% in the valley, which was based upon small CU snips, was probably the best indicator of animals which were serving as potential reservoirs, since using a technique of low sensitivity tended to uncover infections with more pronounced concentrations.

It was obvious that hosts with exceptionally high microfilarial skin densities were the best donors. Eichler (1973a) found microfilariae in

bloodmeals of Simulium ornatum which fed upon a cow with a CU density as low as 250 mf/g, but was best able to infect flies when densities were between 1,000 and 13,000 mf/g. The present study showed that CU concentrations of 2,500 mf/g were insufficient to infect flies whereas when CU concentrations were above 4,000 mf/g, both S. exiguum and S. metallicum became infected. However, infections were rare. These findings indicate that a very high CU skin density was probably required to infect S. exiguum and S. metallicum on a regular basis. This conclusion is reasonable since it has been observed with many black fly vector studies dealing with onchocerciasis that the best microfilarial donors had exceptionally high microfilarial skin densities. For example, Fuglsang et al. (1976) reported that moderately to heavily infected O. volvulus patients had skin densities in excess of 30,000 mf/g. Duke (1962) found that the average microfilarial intakes of O. volvulus by Simulium damnosum increased proportionally with densities available in the skin until they reached 10 mf/fly when densities were around 150,000 mf/g per average skin snip. Studies by Collins et al. (1977) showed that when 60,000 mf/g of skin were available, Simulium ochraceum intakes were as high as 100 mf/g with subsequent development of 2 infective larvae per fly. In other Latin American foci, W. Kozek (personal communication) and Tidwell and Tidwell (1979), respectively, observed that S. ochraceum in Guatemala and S. exiguum in Colombia were more likely to become infected if patients infected with O. volvulus had skin densities of 4,000 mf/g. The present study did not demonstrate the density threshold of O. gutturosa microfilariae that were needed to consistently infect S. exiguum and S. metallicum, but levels probably had to greatly exceed 5,000 mf/g.

The following observations helped to define the significance of O. gutturosa microfilarial behavior in relation to the role of Simulium

species or other biting flies in serving as potential vectors of the parasite in Colombia. First, microfilariae were found 4 times more frequently in SOT snips as compared with CU snips. Furthermore, SOT snips had about 15 times greater average microfilarial densities than CU snips. This indicated that a potential vector typically feeding around the head was more likely to ingest microfilariae with a bloodmeal. Although there were no cases of Simulium species of Valle feeding around the head, it is possible that a species like Simulium sanguineum, abundant in the Colombian plains, may feed around the head of cattle. Ear-feeding black flies had been observed elsewhere for S. ornatum (Supperer, 1957) and for Simulium equinum (Kolstrup, 1975). Second, the average microfilarial concentrations for SOT snips was closer to the apparent threshold needed to infect S. exiguum and S. metallicum. However, since these species did not feed around the head, this finding has little bearing on the dynamics of transmission by S. exiguum and S. metallicum. On the other hand, this microfilarial behavior points to the possibility that a biting fly other than a black fly or another Simulium species outside of Valle was associated with O. gutturosa transmission. Finally, O. gutturosa microfilariae did, in a few cases, concentrate in sufficient numbers in the skin around the belly to serve as a suitable reservoir for infection of S. exiguum and S. metallicum. However, this type of microfilarial distribution was rare and so the probabilities dictated that with few suitable donors available, not many Simulium were likely to become infected.

The present study did not indicate that it was possible to readily infect S. metallicum when fed upon a cow with a CU density in excess of 4,000 mf/g. One fly contained a larva after feeding, but it was disintegrating in the thoracic muscles. Although S. metallicum was capable

of ingesting 2 times more blood than S. exiguum, flies frequently did not engorge to repletion on a single host. Furthermore, such short feedings were often interrupted when flies moved position to begin feeding again on the same host. Since the belly was the chosen landing site for S. exiguum, open feeding positions without hair were often scarce and apparently feeding pools were difficult to scarify in the coarse surface of the belly skin. Therefore, while nervous feeding behavior was common, its occurrence was probably enhanced by the greater difficulty attached to feeding on the thick belly skin along the midline of cattle.

Several possibilities, all related to feeding behavior and the mechanisms of feeding may explain the low infection rate of S. metallicum. First, microfilarial concentrations along the midline and the CU region were often variable. Therefore, the majority of experimental flies may have fed upon areas with exceptionally low microfilarial concentrations while only a few fed by chance on areas with high enough concentrations to assure infection. Second, low infection rates in S. metallicum were observed possibly because microfilariae were being caught in the cibarium of the buccopharyngeal apparatus. Teeth were seen on the cibarium of S. metallicum, but they were not well developed confirming the observations of Omar and Garms (1975). Nevertheless these teeth may have served to a small degree to prevent clear passage of microfilariae to the midgut of S. metallicum. Finally, short duration and interrupted feeding may have prevented flies from penetrating to depths of the papillary layer of the skin where O. gutturosa microfilariae have been found to be concentrated (Ivanov, 1964; Eichler, 1973a) and the capillary system is more diffuse (Bloom and Fawcett, 1968). S. metallicum blood-

meals had highly variable amounts of serum accounting for as much as 75% of the volume of a bloodmeal. This was not possible unless the serum volume contained some other fraction such as serous fluid. Therefore, it is contended that S. metallicum bloodmeals often contained additional volumes of serous fluid which was ingested because flies did not penetrate the thick skin to the capillary bed. Furthermore, poor penetration also resulted in the ingestion of few microfilariae.

S. exiguum appeared to be a more competent intermediate host of O. gutturosa. However, it was only possible to infect flies when CU densities were above 4,000 mf/g. The experimental infection rate in S. exiguum was more than twice that found for S. metallicum, but development in some flies was abortive as indicated by melanization and disintegration of larvae. Melanization as a product of host-parasite incompatibility has been observed in various studies using biting flies as intermediate hosts that were not the natural vectors of filarial parasites (Kartman, 1953; Oohuman et al., 1974; Loc et al., 1980). Other O. gutturosa larvae found in S. exiguum were developing normally, but in some cases development was delayed which probably indicated a degree of incompatibility.

The best evidence for implicating S. exiguum as a competent intermediate host was that a healthy, 3rd-stage larva was found developing in this species. Given the advanced state of development of this larva it was likely that the specimen was a product of natural infection prior to the experimental feeding, and so, it was possible to generate a rate of natural infection for S. exiguum. The parous rate for 505 dissected flies was 36.5% indicating that 184 flies were liable to be infected or that there was an infection rate of about 5 parous flies per 1,000 (0.5%).

This was a very low infection rate to maintain a cycle of transmission when, for example, rates of infection for Simulium species with O. volvulus larvae in holoendemic zones have been shown to consistently exceed 10% (Duke, 1968a, 1968c; Garms, 1975). However, lower rates between 0.32 and 1.2% have been occasionally reported for black flies transmitting O. volvulus in hypoendemic zones in Guatemala (Deleon, 1957; Gibson, 1965; and Collins, 1979).

The feeding site preference of S. exiguum probably affected its potential for infection. Although it was not a fastidious feeder, interrupted feeding occurred with this species because cows were able to dislodge feeding flies from their udders and teats with their tails. Furthermore, as has been previously observed by Eichler and Nelson (1971), microfilarial concentrations may not have been very high in the teats and udders of donor animals. In contrast, good evidence to indicate that this species was a more efficient feeder than S. metallicum was that S. exiguum bloodmeals contained proportionally less serum per volume of blood intake, probably due to less ingestion of serous fluid. Since S. exiguum fed long at one site, it had a greater opportunity to penetrate to the capillary bed to extract a bloodmeal as well as microfilariae from deeper tissues. Because the bloodmeal size of S. exiguum was comparatively small, mechanisms related to its technique of pool feeding may explain its somewhat higher rate of infection. Perhaps S. exiguum had some capability for concentrating microfilariae as has been demonstrated for Simulium sanguineum by Shelley et al. (1979). After observing that S. exiguum ingested higher concentrations of microfilariae from onchocerciasis patients than other black fly species under study, Duke (1970) suggested that this species had a salivary substance that attracted microfilariae to the biting site.

Furthermore, it is certain that microfilariae were not obstructed upon passage to the midgut by the cibarium of S. exiguum. Examinations of the buccopharyngeal apparatus of S. exiguum supported the observations of Dalmat (1955) that the apparatus of this species is hyaline and smooth.

Given that the 3rd-stage larva was a natural infection in S. exiguum, calculations suggest that the larva had reached its advanced state in approximately 5 days and required 2 to 3 additional days to reach infectivity. Therefore, it can be assumed that intermediate development of O. gutturosa required about 7 to 8 days to reach the 3rd (infective)-stage. Interestingly, Bain (1979) reported that O. gutturosa required approximately the same time period for development in Culicoides nubeculosis. However, this period is far different than the 15 days which Eichler (1973a) found necessary for comparable development in S. ornatum. Although no mention was made as to what temperature flies were held at during this earlier study, low temperature may have extended the length of the cycle of development.

Variation in gonotrophic cycles: The period of incubation proposed in this study conforms best with prior observations of Onchocerca development, however, its calculation was most subject to change if large variation in the gonotrophic period occurred for S. exiguum. The speed at which a black fly passes through each gonotrophic cycle is influenced by the physiological state of a fly as it arrives to gain its bloodmeal. A fly species with an autogenous gonotrophic cycle may have an extended secondary cycle because energy reserves have been depleted while generating eggs without a bloodmeal. Furthermore, autogenous species have diminished vector potential because a large portion of their lives is expended before taking a potentially infective bloodmeal. Neither species was autogenous,

but S. metallicum appeared to have a more rapid advancement of ovaries to the CH-II, diapause stage (Tables 11 and 12). For both species the full extent of their lives prior to feeding was spent in seeking a bloodmeal which favored gaining a potential infection.

The period of oogenesis was variable for both species (Table 13) which undoubtedly contributed to variability in the gonotrophic periods. The sequence of ovarian development of S. exiguum and S. metallicum (Figures 13 to 21) was similar to that described for S. damnosum (Wanson, 1950; Lewis, 1957), S. metallicum (Ramirez-Perez et al., 1976), S. ochraceum (Cupp and Collins, 1979) and S. ornatum (Davies, 1957). The upper limit for ovarian development of 60 hours observed in the present study for S. exiguum and S. metallicum was the same for those aforementioned species with the exception of S. ornatum which needed 4 days for complete oogenesis (Davies, 1957). On the other hand, S. exiguum and S. metallicum often completed oogenesis as early as 42 and 48 hours, respectively. Consequently, oogenesis in these Colombian species was frequently much shorter than previously reported for any other black fly species.

Oviposition which was induced in the laboratory confirmed that oogenesis was often completed two days after S. exiguum and S. metallicum had bloodfed. This timing also confirmed that sloughing of the chorion and stretching of the tunica around ovarioles was a good indicator of oviposition readiness. Nevertheless, the time from feeding to actual oviposition for both species was variable and often extended, suggesting again that the lengths of their gonotrophic periods were subject to this variation.

The variation observed in pre- and postfeeding oogenesis and in the timing of oviposition was probably influenced by the amounts of fat

body contained in flies at emergence or just prior to feeding and by the size of bloodmeals which flies ingested. It was possible that flies with high energy reserves or flies that had fed to repletion developed eggs more rapidly. In any case, oogenesis always occurred within 60 hours and oviposition was successfully induced after 3 days on the average.

Variability in the length of the gonotrophic cycle under natural conditions was well illustrated by the different return times for flies which were marked, released and recaptured (Figure 31, Tables 22 and 23). Such variability was to be expected based upon the observed variation in oogenesis, however, other circumstances may have contributed to extending these cycles in nature. Long distance flight to oviposition sites may have consumed bloodmeal constituents which would have otherwise been applied to egg generation. Clements (1963) points out that mosquitoes use blood sugars and proteins for flight and it is reasonable to assume that black flies do the same. The utilization of this quick energy resource for flight implies that a more extended period of digestion would be needed to break down remaining bloodmeal constituents which are applied to oogenesis. Delayed access to these resources may therefore increase oogenesis time. Furthermore, the time required for successfully obtaining a bloodmeal after oviposition in order to complete a gonotrophic cycle may be highly variable. The cycle may have been extended depending upon the amount of time expended for resting after oviposition, for nectar seeking as it was required, and for flight if oviposition sites were not near cattle pastures.

The overall return rates for S. exiguum and S. metallicum were 0.51% and 1.78%, respectively. They were in the range that might be expected for a black fly recapture study. Using aniline dyes, Dalmat (1952)

recaptured 0.19, 0.33 and 0.28% of marked S. metallicum, S. ochraceum and S. callidum, respectively. Thompson (1976) reported recapture rates between 0.12 and 3.98% for S. damnosum marked with oil paint. In the present study flies marked with blue compound returned more frequently than those marked with orange indicating, despite laboratory observations, that blue compound increased mortality, it was more adherent. In general, a lower rate of mortality as well as a greater tolerance to marking compounds favored the probability of more frequent return of S. metallicum. However, its ability to relocate marking sites was probably due to the fact that this species was released in closer proximity to oviposition sites. S. metallicum immatures were found in abundance in smaller streams in the foothills where the recapture site was located and most cattle were pastured. S. exiguum larvae were most frequently found in larger water courses in the valley at some distance from cattle areas. Therefore, the probability of recapture of S. exiguum was reduced because this species had to fly greater distances to oviposit and return to the marking site. Added flight time undoubtedly contributed to extending the length of time of the gonotrophic cycle of S. exiguum as observed in the marking-recapture study, since marked specimens were required to fly to more distant oviposition sites. On the other hand, the pattern of feeding in one place and ovipositing at a more distant place favored the dispersal of O. gutturosa by potentially infected S. exiguum.

Survivorship: The average length of the gonotrophic cycle in nature for S. exiguum and S. metallicum was about 80 and 74 hours, respectively. This indicated that, when feeding in the morning or afternoon, both species were generally prepared to take another bloodmeal 3 days later.

Given that a three-day cycle was typical and that the incubation period of O. gutturosa was around 8 days, the greatest potential for transmission was probably 9 days after flies were infected (Figure 38). However, observations of laboratory mortality of S. exiguum and S. metallicum indicated that survival of flies which had not even undergone the rigors of repeated oogenesis, was poor for the period of time equivalent to 3 gonotrophic cycles. Calculations based upon expected mortality for both species passing through 2 gonotrophic cycles indicated that the survival rate of S. metallicum was higher. However, these observations also indicated that it was unlikely that either S. exiguum or S. metallicum would survive 3 gonotrophic periods and feed for a 4th time. These laboratory observations may provide an accurate estimation of natural mortality for these black fly species. For example, Duke (1968b) showed that one day after the completion of 2 gonotrophic cycles a population of potentially infective, parous S. damnosum decreased 60% and following the third oviposition, only 10% of the population survived. In other terms, 130 of an original nulliparous population survived 2 gonotrophic cycles while only 1 of 1000 flies survived 3 gonotrophic cycles.

Interrupted feeding: Successful feeding probably most influenced the survival rate of S. exiguum and S. metallicum in nature. Both species exhibited a certain amount of interrupted feeding. The interrupted feeding populations were composed of disproportionally high numbers of nulliparous flies when compared to the proportion of nulliparous in the general populations (Table 5 and 6). This supports the contention that nulliparous fed less persistently because their fat body reserves were ample enough not to require a sustained feeding drive. Since blood was required to advance ovaries beyond CH-II, this explained the relatively high proportions of blooded nulliparous and parous flies

with advanced stage ovaries among the populations of interrupted feeders as compared with their counterparts in the general population. On the other hand, because flies did not digest all blood before egg development advanced, proportionally less unblooded, advanced-stage interrupted feeders were captured as compared with the high proportion of such captured in the general populations. Since blood was found still undigested in a high proportion of interrupted feeders, this indicated that such flies were returning soon after each interrupted feeding. The persistency of both S. exiguum parous and nulliparous flies to return to feed while still blooded was illustrated by the very high proportion of such returnees. The low fraction of parous, unblooded captures for both species was indicative of the persistent feeding of older flies but suggested that few parous flies with advanced stage ovaries returned for additional bloodmeals after an interrupted meal was digested. Relative to the proportion of parous flies in the general populations, S. exiguum parous flies were encountered slightly less frequently as interrupted feeders. In addition, interrupted bloodmeals of S. exiguum were more ample, providing for more advanced ovarian development per interrupted feeding. Both of these findings suggested that S. exiguum parous individuals were more persistent feeders. Furthermore, when considering the overall lower rate of S. exiguum interrupted feeding, it was obvious that this species was more persistent in its feeding activity.

In terms of energy costs to the bionomics of both species, interrupted feeding was very inefficient since blood nutrient taken with insufficient meals was probably sacrificed during host-seeking to complete those meals. Such losses were undoubtedly at the expense of egg

building. Thus, it was not surprising that much higher percentages of advanced-stage flies were found in CH-III rather than higher Christophers' stages, especially so with S. metallicum (Figures 7 and 8). Interspecific competition for feeding space may have accounted for the higher rate of interrupted feeding, but this was unlikely because absolute numbers of feeding individuals were never very high during the course of the study. S. exiguum had greater absolute numbers, but less interrupted feeding, suggesting that even under crowded conditions this species persistently fed. However, S. metallicum was probably confronted with more difficult feeding conditions. Most S. exiguum fed on the thin, hairless skin of the teats and udder while the preferred feeding site of S. metallicum was the thick belly-skin along the midline of the cow.

The study of landing and feeding rates of S. exiguum and S. metallicum supports the conclusions drawn about the interrupted feeding behavior of these species. The feeding efficiency for landing S. exiguum was 20% higher than the rate for S. metallicum (Figure 12). The difference during this study may have been due to the fact that S. metallicum landing rates were often much higher than those of S. exiguum. Greater competition for feeding space probably had the effect of decreasing S. metallicum feeding efficiency even more. The feeding efficiency rates were more a measure of the success of feeding over time than of interrupted feeding. However, interrupted feeding decreased such a success and, therefore, its influence was incorporated within the rates. The observation that higher parity rates increased feeding efficiency was supported by finding that the S. exiguum overall parous rate was 64% as compared with 57% for S. metallicum. The majority of feeding flies were parous flies as indicated by parous rates of feeding

S. exiguum and S. metallicum of 62% and 55%, respectively (Tables 10 and 11). However, the feeding populations of both S. metallicum and S. exiguum contained more nulliparous than did the landing populations. Therefore, while parous flies were the majority of persistent feeders, at least a portion of the nulliparous population was equally persistent. The absence of fat body in parous flies undoubtedly motivated persistent feeding. However, many of the feeding nulliparous also had low fat body reserves suggesting that they were part of an older population of nulliparous that had depleted their energy reserves during unsuccessful quests for blood, or younger flies that emerged with little fat body.

A breakdown of feeding efficiency data for S. exiguum demonstrated around 50% of the morning and evening flights fed when parous rates were 72% and 52%, respectively. The highest parity was in the morning but connected to a feeding rate equivalent to that of the afternoon when parity was lower. It was likely that young parous flies, just beginning host-seeking, composed the majority of flies which fed in the morning. Their feeding efficiency was undoubtedly high because few flies in this group had fat body reserve. In the afternoon, when the parous rate dropped, the feeding rate was probably sustained by an increment of successfully feeding, morning-emerged nulliparous with low energy reserves. The same data breakdown for S. metallicum indicated that about 36% of the morning or afternoon landing flies fed when parous rates were 36% and 74%, respectively. In this case the highest parity was observed in the afternoon, but was connected to a feeding rate equivalent to that of the morning when parity was lower. The same dynamics applied, except that young parous S. metallicum, which had probably oviposited later in the day, arrived to successfully feed in the afternoon when their energy reserves were declining. Thus, there was a comparatively high rate of

parity among the afternoon feeders. In the morning, when the parous rate was observed to be low, the feeding rate was sustained by nulliparous. This group probably emerged late on the previous day, and consumed energy reserves while surviving through the night, which motivated them to successfully feed during the morning flight period.

The final proof that interrupted feeding existed was demonstrated when landing flies with bloodmeals of a different serotype were captured upon a bull-calf bait-animal (Table 7). The success rates of identifying bloodmeals of S. metallicum and S. exiguum were about the same. Around 25% of the bloodmeals of these species were identifiable. The low success rate was not explained by the possibility that the bloodmeals which were randomly selected were too small, since 1/80th and 1/40th of the average maximum serum meals of S. metallicum and S. exiguum, respectively, were identifiable (Figure 11, Table 8). Natural bloodmeals of these species probably contained quantities of serous fluid which lacked the immunoglobulin necessary to elicit a precipitin reaction. It was also possible that serum was excreted by flies from bloodmeals prior to testing. S. exiguum and S. metallicum were observed to excrete fluids throughout the 48 to 72 hour period of digestion. Clements (1963) noted that such excretion from mosquitoes largely consisted of nitrogenous wastes, but 10% of this material was protein. If S. exiguum and S. metallicum excreted serum protein, then these losses reduced the quantities of bloodmeals reactants identifiable with the precipitin test. With mosquitoes, blood proteins were partially digested within 3 hours of a bloodfeeding, serum globulins being broken down first (Clements, 1963). Therefore, serum was probably lost during digestion in S. exiguum and S. metallicum. Some serum digestion was certain, since bloodmeals were not dissected from specimens until 24

hours after flies were captured. Furthermore, the potential for identifying a bloodmeal was diminished if its eluate was clear. Older bloodmeals, as indicated by darkening of hemoglobin due to digestion, less often produced colored eluates. Therefore, colored eluates were probably from bloodmeals that had undergone less digestion. It was also possible that serum was lost or inactivated during the process involved in preparing eluate for testing.

Of the bloodmeals that were positively identified from both species, cow feedings were found to have occurred 2 times more frequently than horse feedings, and there was only one case of an identified human bloodmeal. This suggested that S. exiguum and S. metallicum were more prone to feed upon cattle than horses and even less likely to feed upon humans. However, these findings probably reflected the availability of these animals to host-seeking flies. Cattle were the predominant large mammal in the collection zone followed by horses and then humans. In addition, the study showed that these black fly species did not feed upon birds. Whether these black flies fed upon small mammals was not determined in this study. However, no flies were observed to feed upon small domestic animals and the occupation of land within the collection zone by small, wild mammals was limited because of natural habitat restrictions imposed by widespread landuse by crop and cattle growing concerns. This study supports the findings of Guttman (1972) that S. exiguum and S. metallicum have a host preference for large farm animals, particularly cows and horses.

The study further demonstrated that it was common for either black fly species to begin feeding upon one host and pass to another one to complete a bloodmeal. Other observations indicated that it was common for a fly to make several short duration feedings on the same host.

Therefore, cases of interrupted feeding occurred on one host, in between hosts of a kind and in between hosts of different species. As short duration feeding may have limited the chance for infecting S. exiguum and S. metallicum, interrupted feeding on one host and intraspecific, interrupted feeding probably reduced the potential for the transmission of infective larvae. Although unspecified, a certain quantity of O. gutturosa third-stage larvae was undoubtedly needed to infect a host. Therefore, each passage of infective larvae during an interrupted feed which was below such a threshold, was wasted. With interspecific feeding, any passage of larvae to the wrong host was a wasted infective feed. Therefore, if these black fly species were competent intermediate hosts of O. gutturosa in Valle, the actual transmission of the parasite probably was frequently interrupted by wastefeeding. These findings are in agreement with the proposal of Trapido et al. (1973) that wastefeeding of Colombian Simulium species outside of the human onchocerciasis zone reduced the potential for Onchocerca volvulus transmission.

The fastidious feeding behavior of S. metallicum has been noted by Jamnback et al. (1971) and Crosskey (1973). Observations by Dalmat (1955) showed that S. metallicum often took much longer to engorge than S. exiguum. Crosskey (1962) proposed that prolonged feeding time of black flies on hosts infected with Onchocerca enabled more microfilariae to be released into the feeding pool, thus increasing the potential for ingestion of more microfilariae. This supposition, however, did not apply to black fly species for which engorgement depended upon several interrupted bloodfeedings. Therefore, as Crosskey (1973) maintains, vector potential of a Simulium species will be lower if interrupted feeding is common. In this regard, the present study showed that because of interrupted

feeding, the potential of S. metallicum for transmitting O. gutturosa was lower than that of S. exiguum.

Daily activity: Feeding behavior of S. exiguum and S. metallicum was modified in response to the physiological state of flies as it related to hunger drive. Valle black flies also responded to mating and oviposition drives as well as to environmental stimuli. Therefore, daily population activity was an integrated pattern of responses of individuals in the populations, each of which was acting in accordance with these drives and stimuli.

The model for daily activity of S. exiguum illustrates that both nulliparous and parous flies were more active during the morning flight period (Figure 32). At that time, temperature and humidity were more favorable to flight and host-seeking activity Conversely, mid-day temperatures and humidities were less suitable for flight activity and so host-seeking activity was diminished. S. metallicum host-seeking activity more accurately conformed with changes in temperature and humidity, since afternoon host-seeking was nearly as pronounced as the morning activity peak (Figure 34). Activity of both species was stimulated by defined thresholds of temperature and humidity (Figures 33 and 35). In general, bloodsucking flies do not possess efficient regulatory mechanisms to prevent water loss. Studies have shown that high temperature together with low humidity caused dessication and death of mosquitoes (Clements, 1963). The present study demonstrated that S. exiguum and S. metallicum have adapted host-seeking behavior to coincide with times when temperature and humidity were not deleterious.

The percentages of parous fly activity for each species illustrate fluctuations which occurred due to more subtle changes in population composition (Figures 32 and 34). These changes were relative to

changes in nulliparous activity, but occurred because fly behavior was subject to variations caused by life cycle activities in which subsets of the parous and nulliparous populations participated. The gradual rise in relative activity of S. exiguum percent parous began after mid-morning. This activity was undoubtedly sustained by increments of parous flies that had oviposited begining at mid-morning and joined the host-seeking population with greater frequency after that time. The more dramatic increase, later in the day, of the relative activity of the parous S. metallicum suggest that the gravid population of this species began its oviposition activity later than S. exiguum. S. metallicum oviposition probably began in early afternoon. Therefore, additional increments of new parous flies substantially increased the relative proportion of host-seeking activity in late afternoon. Similar patterns of percent parous daily activity were observed when studying feeding efficiency.

The principal feeding times of these species together with their subsequent periods of oogenesis probably set the timing for oviposition. Both species more actively fed in the early morning, but S. exiguum had a shorter oogenesis period. Therefore, more S. exiguum were probably ready to oviposit early on the 3rd day after feeding and did so with greater frequency. Oviposition later in the afternoon by S. metallicum may have occurred because of a more extended oogenesis period after the principal time of feeding. Laboratory trials indicated that S. exiguum was more easily induced to oviposit in the morning. It was more difficult to elicit morning oviposition of S. metallicum. While there is no documentation of the oviposition timing of S. exiguum in nature, Dalmat (1955) observed that S. metallicum most frequently oviposited in the afternoon.

The principal period of host-seeking activity, as indicated by the weighted averages for percentage of active flies, was in the early to mid-morning for both species (Tables 32 and 34). Dalmat (1955), Jamnback et al. (1971) and Guttman (1972) reported a similar timing for the biting peak of S. metallicum. Guttman (1972) found that the biting peak was coincident with lower temperatures and high humidity. Guttman (1972) and Tidwell et al. (1980) reported that S. exiguum daily activity peaked during the morning hours coincident with lower temperatures and high humidities.

Parous fly host-seeking was most pronounced in the morning for S. exiguum and in the afternoon for S. metallicum. Therefore, it would be expected that most transmission of O. gutturosa by potentially infective parous flies occurred during these biting peaks. No daily rate of transmission may be calculated for S. metallicum since there was no evidence of natural infection in this species. However, if the rate of 5 infected parous flies per 1000 is applied to S. exiguum, a potential daily rate of transmission may be calculated. In Lomitas, parous flies landed at an overall rate of 53 flies/cow/hour. Based upon the feeding efficiency of S. exiguum, 54% fed, giving a rate of 29 feeding parous flies/cow/hour. There were 12 host-seeking hours during the day and so about 343 parous flies fed on each cow per day. Therefore, at the rate of 5 infected parous flies per 1000 there was an average of 1.7 potential transmissions by S. exiguum per day to each cow. The only similar data that are available for comparison are for endemic areas where S. damnosum transmitted O. volvulus. Duke (1968a) found that about 100 infective S. damnosum were biting each man per day in the holoendemic village of Bolo, Cameroon. In four other Cameroon villages with moderate seasonal O. volvulus

transmission, Duke et al. (1957) established that S. damnosum biting rates were between 0.82 to 14.79 infective flies/man/day. The rate of potential transmission for O. gutturosa of 1.7 S. exiguum/cow/day is at the bottom of this comparative range. Therefore, it was questionable that such a rate maintained a cycle of transmission for O. gutturosa in Valle.

Seasonal population fluctuations: Seasonal changes in S. exiguum and S. metallicum biting density often occurred and, therefore, the potential for O. gutturosa transmission changed in accordance with seasonal fly population fluctuation. The influence of rainfall on the population fluctuation of both species was most remarkable, but especially for S. exiguum. As rainfall accumulations increased from March throughout May, populations of both species declined. With the cessation of rain and the onset of the dry season in June, populations of both species increased dramatically. Larval populations, which preceded adult populations and served as their resource, were probably most influenced by rainfall. The ill effects of rainfall on larval populations were related to the following considerations: 1) Increased rainfall probably resulted in washout and death of larvae because of increased stream velocities. This phenomenon has been documented for black flies by Williams (1962) and Carlson (1967). 2) Heavy rainfall probably caused an increase in runoff containing chemical pesticides used excessively for the control of crop pests in the area. This runoff may have increased mortality of black fly larvae. 3) During the rainy season, smaller irrigation ditches were often closed because crops were sufficiently watered by rainfall. Since these ditches served as a major site of colonization of S. metallicum larvae, its populations were most diminished by this practice. Rainfall

related population decline with Simulium species has been described by Lewis and Aldeocoa (1962), Ramirez-Perez et al. (1977) and Wilton and Collins (1978) for S. metallicum, and by Vargas (1945), Dalmat (1955b) and Guttman (1972) for both S. exiguum and S. metallicum.

S. exiguum demonstrated its highest seasonal nulliparous activity of 260 flies/hour coinciding with the parous peak of 140 flies/hour in late July. However, on collection days a month prior or just after this time, parous activity was nearly as high, but nulliparous rates were much lower. During these times (June-September), rainfall had stopped, humidity was diminishing and temperatures remained slightly above average. Evidently such conditions were favorable to S. exiguum parous survivorship and host-seeking activity. Also, feeding efficiency of nulliparous flies may have been higher during this period resulting in more bloodmeals, oviposition and parous fly production. Feeding conditions related to competition for feeding sites, were probably less favorable during periods when fly density was highest (June-September) and more favorable during periods of low population density (April-May; October-November). Thus the percent parous tended to be lower during peak density and was highest during low fly density periods.

S. metallicum populations experienced their highest nulliparous peak of 70 flies/hour coincident with a moderately high parous rate of 30 flies/hour in late July. These observations were made about 6 weeks after the parous population reached its high of 37 flies/hour and when a rather low nulliparous rate of 23 flies/hour was observed. The more important trend, however, was that parous rates, although slightly lower than the observed peak, remained moderately high from June to November. It appeared that environmental conditions had less effect on the survivorship and activity of parous S. metallicum. Only in August did the parous and

nulliparous populations drop dramatically, probably responding to the precipitous drop in temperature. In general the parous population did not fluctuate as dramatically as that of S. exiguum. This stability was probably due to longer survivorship. On the other hand, changes in the percentages of active parous flies in the population appeared to be density dependent. Rates of parous activity dropped off when high numbers of nulliparous dominated the population. This occurred during March and April and again in October and November. Conversely, lower nulliparous host-seeking densities were often connected with higher densities of parous flies especially during the month of June. At this time nulliparous feeding success due to less competition, probably resulted in the production of more parous flies from the available nulliparous population.

Three studies show precedent for establishing parous rate for black flies on a seasonal basis. Duke (1968a) showed that rates of parity for 3 S. damnosum transmission seasons only varied from 37 to 39%. In a 2 year study, Garms (1973) found rates of parity for S. damnosum varied more widely from 4 to 35%. In Guatemala, Garms (1975) observed that rates of parity for S. metallicum varied from 18 to 29% over a three-month period. In the present study relative changes in seasonal rates of parous activity were frequently extreme, varying from 17 to 85% and 23 to 66% for S. exiguum and S. metallicum, respectively. Such variation suggested that populations of both species were somewhat unstable. Changes in the environment undoubtedly contributed to this instability by shortening the average life span of flies which resulted in a high turnover rate for both S. exiguum and S. metallicum populations.

SUMMARY AND CONCLUSIONS

Infection of cattle with Onchocerca gutturosa in the Department of Valle was common. It was determined that most infected animals were brought into the area whereas only a small proportion of infections were autochthonous. However, transmission did occur in the Cauca Valley and local black flies, particularly Simulium exiguum, appeared to be capable of serving as vectors.

In the course of skin snip surveys, it was noted that microfilariae of O. gutturosa were concentrated in the skin of the head rather than the umbilical area. Microfilariae were found in 93% of the imported cattle which were examined by biopsy from the suboccipital triangle whereas only 25% of circumumbilical biopsies were positive from the same animals. Of cattle surveyed on local farms in Valle, only 6% were infected, but these observations were based upon examinations of small, circumumbilical skin snips. Therefore, future surveys should be based upon suboccipital snips, which will undoubtedly disclose a higher prevalence of onchocerciasis than was observed in the present study.

High concentrations of microfilariae around the head and low concentrations around the umbilical area of infected cattle suggested that biting flies were more likely to become infected if they normally fed around the head. These findings also suggested that a potential vector feeding around the belly was less likely to be a principal vector. Both black fly species under study fed exclusively on the ventral surface of cattle and it was determined that circumumbilical skin densities in excess of 4,000 mf/g were required to infect natural feeding of

S. exiguum and S. metallicum. Normal development of O. gutturosa occurred only in S. exiguum, but even with this black fly species, rates of infection were low.

Studies of the gonotrophic cycles and population dynamics of black fly vectors helped to define their potential for transmission of O. gutturosa. The gonotrophic cycles of both S. exiguum and S. metallicum were approximately 3 days. The incubation period of the parasite was between 5 to 8 days, and so transmission probably occurred after 2 or 3 gonotrophic cycles on the 3rd or 4th feeding of infected flies, respectively. During this time, S. exiguum had the greatest potential for dispersing the parasite because it oviposition sites were farthest from cattle pastures and, therefore, its flight range was greater.

The number of S. exiguum and S. metallicum that were host-seeking per hour were often high as compared with the biting activity of other Onchocerca vectors. Potentially-infected, parous populations of both black fly species always composed a large proportion of the host-seeking populations. S. exiguum and S. metallicum parous flies were most active in the early morning and late afternoon, respectively. Their populations were most abundant during the dry season. However, the average longevity of individuals in populations was judged to be short. Calculations based upon mortality of wild-caught, laboratory-maintained flies indicated that few individuals of either species survived a period of more than 2 gonotrophic cycles. Therefore, since the potential for survival through the incubation period of the parasite was low, this suggested that not many infected S. exiguum and S. metallicum lived long enough to transmit O. gutturosa. This was supported by the fact that the natural infection rate of flies with 3rd-stage larvae was negligible for S. metallicum

and very low for S. exiguum when compared with established rates of Onchocerca transmission in zones of high prevalence.

Both species, but particularly S. metallicum, had the habit of interrupted feeding. This practice may be important in transmission, since fewer microfilariae were likely to be ingested and less infective larvae transferred during a short bloodfeeding. Such feeding behavior possibly accounted for the low rates of natural infection. Furthermore, since these black fly species had a broad host range, infective larvae were potentially lost due to wastefeeding.

In general, entomological studies indicated that high replacement rates, interrupted feeding and a wide host preference limited the vector potential of S. exiguum and S. metallicum for transmitting O. gutturosa. Furthermore, such adaptations undoubtedly severely reduced the efficiency of these black fly species to act as vectors of parasites having biological transmission cycles with long extrinsic incubation periods. On the other hand, S. exiguum and S. metallicum were probably important vectors of bovine and equine diseases which required mechanical transmission by biting flies.

Table 1 - Results of examination of umbilical skin snips for Onchocerca gutturosa microfilariae from cattle on farms in the Departments of Valle and Cauca, Colombia.

Site Number[1]	Date Snipped	Farm Locale	No. Examined Neg.	No. Examined Pos.	Mf. Count per gram	Origin
1	22X78	Buitrera	1	0	--	Valle
2	22X78	Buitrera	3	0	--	Valle
3	27X78	Lomitas	13	2[a]	100, 600	Valle, Cordoba
4	24X78	Lomitas	1	0	--	Valle
5	6IV79	Lomitas	4	0	--	Valle
6	9IV79	Lomitas	3	0	--	Valle, Cauca
7	11IV79	Bolivar	10	0	--	Valle, Cauca
8	NO	Florida	2**	2**	See 3	Cordoba
9	9V79	Caloto	10	0	--	Valle, Cordoba
10	11V79	Cajibio	13	1[b]	80	Cauca, Valle
11	16V79	Cajibio	12	3[c]	100, 200 2100	Cauca, Valle
12	30V79	Cajibio	10	0	--	Valle, Cauca
13	23VI79	Pance	9	0	--	Valle
14	2IV80	Pance	10	0	--	Valle
		Subtotals	99	+ 6 =	105 Total with 5.7% POSITIVE	

[1] Refer to Map, Figure 1.

[a] Positive cows from Cordoba.

[b] Positive cow from Cauca.

[c] 2 positive cows from Cauca and 1 positive cow from Valle

** These cows were found positive on Site Number 3, but came from Site Number 8.

Table 2 - Number of Onchocerca gutturosa microfilariae shed from deep and superficial umbilical skin snips cut monthly over a year from an infected cow.

Snip Number	Date Snipped	Deep Snip[1] No. Mf.	Deep Snip[1] Weight of Snip (mg)	Deep Snip[1] Mf/g	Superficial Snip[2] No. Mf.	Superficial Snip[2] Weight of Snip (mg)	Superficial Snip[2] Mf/g
1	16V79	71	29.83	2380	*	*	*
2	29VI79	29	16.14	1798	*	*	*
3**	27VII79	16	14.17	1129	1	3.58	279
4	29VIII79	13	26.78	485	0	1.81	0
5	28IX79	1	31.00	32	0	5.15	0
6	26X79	12	17.14	689	0	2.14	0
7	26XI79	11	42.56	258	0	2.54	0
8	17XII79	2	39.51	46	0	5.41	0
9	21I80	0	21.41	0	0	3.26	0
10	26II80	2	23.25	96	1	1.55	645
11	31III80	2	19.41	103	0	1.84	0
12	24IV80	2	53.06	38	0	5.86	0
			Average	587.8		Average	92.4
			Ratio	6.3		:	1

[1] Biopsy of fold of skin

[2] Biopsy of skin just to base of hair follicles

* No sample

** Cow became pregnant prior to this snipping date. Furthermore, monthly treatment was begun with Negubon^{R} systemic insecticide for Dermatobia

Table 3 - Comparison of the number of microfilariae shed from deep skin snips around the umbilical button and from the suboccipital triangle of cows with Onchocerca gutturosa adult worms in the nuchal ligament. The cows were slaughtered in La Florista Matadero, Cali, Valle, Colombia on April 19, 1980.

			Umbilical Snips			Neck Snips		
Cow No.	Origin	Sex	No. Mf/ Snip 1	mg Wt./ Snip 2	Mf/g 3	No. Mf/ Snip 4	mg Wt./ Snip 5	Mf/g 6
1	Medellin	M	0	224.9	0	102	303.8	336
2	Medellin	M	16	183.4	87	10	220.7	45
3	Caqueta	M	0	220.8	0	18	223.9	80
4	Caqueta	M	0	193.9	0	0	219.3	0
5	Valle	F	0	275.9	0	0	177.0	0
7	Caqueta	M	0	145.8	0	11	96.5	114
8	Caqueta	M	2	270.2	7	213	113.4	1879
9	Caqueta	M	2	305.6	7	13	353.1	37
10	Caqueta	M	0	171.8	0	422	136.7	3076
12	Caqueta	M	0	144.0	0	10	137.8	72
13	Caqueta	M	0	217.8	0	359	200.9	1784
14	Caqueta	M	0	512.8	0	114	208.6	545
15	Caqueta	M	1	118.9	8	51	147.5	344
17	Caqueta	M	0	311.1	0	204	233.9	875
18	Valle	M	3	199.8	15	12	237.3	51
20	Caqueta	M	0	272.8	0	4	147.5	27
21	Caqueta	M	0	160.1	0	19	159.6	119
29	Caqueta	M	0	96.9	0	5	57.9	86
32	Caqueta	M	0	104.2	0	26	71.1	366
36	Huila	M	0	116.5	0	70	76.7	909
37	Caqueta	M	0	325.1	0	4	228.7	17
38	Caqueta	F	203	264.3	769	184	374.6	489
40	Caqueta	F	0	311.9	0	207	184.4	1124
41	Caqueta	M	4	166.9	24	23	108.9	211
42	Caqueta	F	0	188.3	0	3	109.7	27
45	Caqueta	F	0	164.5	0	21	129.5	163
46	Caqueta	F	0	70.5	0	19	115.9	164
49	Caqueta	F	0	139.7	0	64	58.4	1096
			Ave. No. Mf/g =		33			501
			Ratio =		1		:	15

Table 4 - Dissection results of laboratory-maintained black flies after engorging upon a cow infected with Onchocerca gutturosa.

Day / Hour	Freshly Engorged	1 (12 24)	2 (36 48)	3 (60 72)	4 (84 96)	5 (108 120)	6 (132 144)	7 (156 168)	8 (180 192)	9 (204 216)	Total
		Dissection Time Post-Feeding									
		Simulium exiguum									
Number Dissected	163[a]	1 2[b]	3 5	21 11	18 41	53 62	40 29	48 14	23 22	4 8	668
Number Infected	-	- -	- -	1 -	- -	2 1	- 2	- -	- -	- -	6
		Simulium metallicum									
Number Dissected	73[a]	13[b] 3	3 9	15 3	9 11	10 11	14 10	18 31	4 11	5 11	264
Number Infected	-	- -	- -	- -	- -	- 1	- -	- -	- -	- -	1

[a] Cumulative dissection results of flies captured after fully engorging on Holstein-Clavel on Rosero Farm, Site No. 4 on 27 collecting days

[b] Cumulative dissection results of flies captured after fully engorging on Zebu on Estrada Farm, Site No. 3 on 16 collecting days.

Table 5 - Comparison of a population of interrupted feeders with the general population of Simulium exiguum by percentages of flies categorized according to the state of parity and engorgement. Interrupted feeders were designated as flies in the population with ovaries advanced beyond Christophers' Stage II and/or blood in the midgut at the time of capture.[1]

	Interrupted Feeding Population			General Population		
	Nulliparous	Parous	Total for Parity State	Nulliparous	Parous	Total for Parity State
Without Blood	14.9	1.5	16.4	43.3	45.4	88.8
With Blood	43.3	46.3	83.6	5.8	5.4	11.2
Total for State of Engorgement	58.2	41.8	100[a]	49.2	50.8	100[b]

[1] Data on flies captured over 14 consecutive biweekly collection days from late 1979 and early 1980.

[a] Percentage based upon a total of 67 flies which was 13.4% of the general population

[b] Percentage based upon a population of 500 flies.

Table 6 - Comparison of a population of interrupted feeders with the general population of Simulium metallicum by percentages of flies categorized according to state of parity and engorgement. Interrupted feeders were designated as flies in the population with ovaries advanced beyond Christophers' Stage II and/or blood in the midgut at the time of capture.[1]

	Interrupted Feeding Population			General Population		
	Nulliparous	Parous	Total for Parity State	Nulliparous	Parous	Total for Parity State
Without Blood	31.4	2.8	34.2	53.3	33.7	87.0
With Blood	32.9	32.9	65.8	6.5	6.5	13.0
Total for State of Engorgement	64.3	35.7	100[a]	59.8	40.2	100[b]

[1] Data on flies captured over 14 consequtive, biweekly collection days from late 1979 and early 1980.

[a] Percentage based upon a total of 70 flies, which was 19.8% of the general population.

[b] Percentage based upon a population of 353 flies.

Table 7 - Results of analysis of unknown bloodmeals from partially engorged Simulium exiguum and Simulium metallicum using a gel-diffusion, precipitin test.

Antisera/ (Sensitivity)	Simulium exiguum			Simulium metallicum		
	Eluate Colored[+]		Positive Identification	Eluate Colored[+]		Positive Identification
	No	Yes		No	Yes	
Bird/ (1:16,000)	0	0	0	0	0	0
Cow/ (1:16,000)	5	2	7	3	3	6
Horse/ (1:16,000)	2	1	3	2	1	3
Human/ (1:32,000)	1	0	1	0	0	0
Unidentified	35	0	--	23	2	--
Subtotal	43[a]	3[b]		28[c]	6[d]	
TOTAL	46		11	34		9

[+] Presence of hemoglobin in eluate.

[a] 18.6 percent of bloodmeals of S. exiguum without colored eluate were identified.

[b] 100 percent of bloodmeals of S. exiguum with colored eluate were identified.

[c] 17.8 percent of bloodmeals of S. metallicum without colored eluate were identified.

[d] 66.6 percent of bloodmeals of S. metallicum with colored eluate were identified.

Table 8 - Bloodmeal volumes of fully engorged *Simulium exiguum* and *Simulium metallicum*.

Sample	*Simulium exiguum*		*Simulium metallicum*	
	Whole Blood (mm^3)	Serum Only (mm^3)	Whole Blood (mm^3)	Serum Only (mm^3)
1	0.506	0.127	1.013	0.456
2	0.633	0.253	1.215	0.329
3	0.696	0.329	1.013	0.506
4	0.937	0.253	1.051	0.379
5	0.569	0.379	1.266	0.759
6	0.861	0.253	1.342	1.013
7	0.633	0.127	0.810	0.203
8	0.797	0.354	1.038	0.608
9	0.607	0.253	1.215	0.189
10	0.430	0.151	1.013	0.456
Mean	0.667[a]	0.248[a]	1.098[b]	0.490[b]
Standard Deviation	± 0.150	± 0.086	± 0.149	± 0.239

[a] *S. exiguum* whole blood to serum ratio = 2.7:1

[b] *S. metallicum* whole blood to serum ratio = 2.2:1

Table 9 - Parous rates of landing versus feeding populations of *Simulium exiguum*.

Collection Date	Early Morning Flight					Late Afternoon Flight				
	Landing Flies (X)	Parity	Feeding Flies (Y)	Parity	Percent Feeding	Landing Flies (X)	Parity	Feeding Flies (Y)	Parity	Percent Feeding
25I80	127	8/10	96	8/10	75.5	84	6/10	44	5/10	52.3
7II80	86	9/10	23	9/10	26.7	171	8/10	115	6/10	67.2
12II80	302	7/10	143	9/10	47.4	107	5/10	39	4/10	36.4
7III80	102	10/10	36	8/10	35.3	64	6/10	37	7/10	57.8
20III80	142	2/10	37	2/10	26.1	189	5/10	71	4/10	37.6
Capture Total	759	36/50	335	36/50	44.1	615	30/50	306	26/50	49.8

Parity of Landing Flies	Parity of Feeding Flies
66 Percent	62 Percent

Table 10 - Parous rates of landing versus feeding populations of Simulium metallicum.

	Early Morning Flight					Late Afternoon Flight				
Collection Date	Landing Flies (X)	Parity	Feeding Flies (Y)	Parity	Percent Feeding	Landing Flies (X)	Parity	Feeding Flies (Y)	Parity	Percent Feeding
25I80	352	7/10	226	6/10	64.2	344	9/10	223	7/10	64.8
7II80	137	3/10	67	2/10	48.9	563	9/10	167	8/10	29.6
12II80	1,465	4/10	522	4/10	35.6	399	10/10	115	10/10	28.8
7III80	843	4/10	308	3/10	36.5	544	4/10	242	4/10	44.4
20III80	706	2/10	149	3/10	21.1	1,056	8/10	281	8/10	26.6
Capture Total	3,506	20/50	1,272	18/50	36.3	2,906	40/50	1,038	37/50	37.7

Parity of Landing Flies	Parity of Feeding Flies
60 Percent	55 Percent

Table 11 - Dissection results, 0-18 hours post-ecdysis, demonstrating anautogeny of laboratory-emerged Simulium exiguum.

Date Pupae Collected	Collection Site	Estimated No. Pupae Collected	No. Emerged ♂	No. Emerged ♀	Christophers' Stage of Emerged Females-Percent of Total N	1	2
20 VI 79	Lomitas	60	6	9	11.1	88.9	0.0
28 VII79	Florida	150	12	41	17.0	80.5	2.5
21&81X79	Florida	170	15	23	4.5	95.5	0.0
22 XI79	Florida	60	11	13	30.7	61.5	7.8
13 II80	Florida	80	6	16	12.5	81.3	6.2
18 IV80	Lomitas	120	37	62[a]	67.7	32.3	0.0
	TOTAL	640	87[b]	164[b]	23.9[c]	73.3[c]	2.8[c]

[a] All flies in this sample were dissected within one hour of emergence

[b] Male : Female Ratio = 1 : 1.9

[c] Weighted Averages

Table 12 - Dissection results, 0-18 hours post-ecdysis, demonstrating anautogeny of laboratory-emerged *Simulium metallicum*.

Date Pupae Collected	Collection Site	Estimated No. Pupae Collected	No. Emerged ♂	No. Emerged ♀	Christophers' Stage of Emerged Females- Percent of Total N	1	2
3 V79	Pance	300	3	19	5.2	73.7	21.1
18 VII79	Pance	80	15	17	0.0	100.0	0.0
6&11IX79	Pance	150	23	38	0.0	94.7	5.3
6 XII79	Pance	60	13	12	0.0	100.0	0.0
22 II80	Pance	50	7	9	0.0	100.0	0.0
15 IV80	Pance	50	18	26[a]	42.3	57.7	0.0
	TOTAL	690	79[b]	121[b]	7.9[c]	87.7[c]	4.4[c]

[a] All flies in this sample were dissected within one hour of emergence.

[b] Male : Female Ratio = 1 : 1.5

[c] Weighted Averages

Table 13 - Simulium metallicum and Simulium exiguum ovariole development after ecdysis to just prior to oviposition.

	Christophers Stage	Simulium metallicum[1] Hours Elapsed	Simulium metallicum[1] Ovariole Size[3] Length in micra	Simulium exiguum[2] Hours Elapsed	Simulium exiguum[2] Ovariole Size[3] Length in micra
Postecdysis	Ch- N	0-24	15-27(8)	0-24	11-27(8)
	Ch- I	24-48	27-38(8)	24-48	23-34(8-11)
	Ch- IIA	36-54	38-57(11)	36-54	30-49(11)
Postfeeding	Ch- IIA	1- 6	38-61(11)	1- 6	30-49(11)
	Ch- IIB	1- 6	61-72(11)	1- 6	46-57(11)
	Ch-IIIA	6-12	61-87(11-19)	6-18	53-87(15)
	Ch-IIIB	6-18	87-114(15-19)	12-18	76-106(15-19)
	Ch- IVA	18-30	106-152(19-23)	18-30	95-137(19)
	Ch- IVB	24-48	148-190(23-24)	24-36	122-163(23)
	Ch- VA	30-54	186-209(27-38)	30-48	152-205(23-24)
	Ch- VB	48-60	186-216(30-53)	42-60	152-209(30-42)

[1] Average mature egg production per fly = 198.3 (Range: 41 - 314)

[2] Average mature egg production per fly = 112.6 (Range: 43 - 175)

[3] Range of measurements of at least 30 ovarioles from 10 different flies for each Christophers' Stage: Primary Oocyte Range (Secondary Oocyte Range).

Table 14 - Fate of 180 bloodfed Simulium exiguum subjected to 5 consecutive oviposition trials in the laboratory.

	Hour at Trial (Postfeeding)					
	36[a]	42[a]	48	72	96	TOTAL
Escaped in Placement	1	0	2	0	0	3
Died in Holding Tubes	7	4	71	9	2	93
Drowned in Ovi-tubes	5	4	8	1	0	18
Number Surviving to Oviposit	167	159	62	19	2	--
Number Oviposited	0	16	33	15	2	66
					TOTAL FLIES	180

[a] Artificial lighting was used since trial was conducted at night.

Table 15 - Fate of 180 bloodfed Simulium metallicum subjected to 5 consecutive oviposition trails in the laboratory.

	Hour at Trial (Postfeeding)					
	42[a]	48	72	96	120	TOTAL
Escaped in Placement	0	1	0	0	0	1
Died in Holding Tubes	4	8	6	19	19	56
Drowned in Ovi-tubes	10	3	5	4	3	25
Number Surviving to Oviposit	166	154	121	40	2	--
Number Oviposited	0	22	58	16	2	98
				TOTAL FLIES		180

[a] Artificial lighting was used since trial was conducted at night.

Table 16 - Comparative percents mortality for S. exiguum with S. metallicum at 24-hour intervals postfeeding/preoviposition. Flies retained eggs through period of survivorship.[1]

Hour of Count Postfeeding	Simulium exiguum[2] (1) Percent Mortality	Simulium exiguum[2] Cum. % Mortality	Simulium metallicum[3] (2) Percent Mortality	Simulium metallicum[3] Cum. % Mortality	S. metallicum[4] (3) Adjusted % Mortality
36	5.0	5.0	1.6	1.6	3.1
60	0.0	5.0	0.0	1.6	2.5
84	18.3	23.3	13.3	15.3	15.8
108	40.0	63.3	31.6	46.8	34.1
132	30.0	93.3	30.0	76.8	32.5
156	6.7	-100.0	21.6	98.4	12.0
180	---	---	1.6	-100.0	---

Hour of Count Postfeeding	Chi-Square Contribution Columns 1 & 2 Compared	Chi-Square Contribution Columns 1 & 3 Compared
36	7.23	1.16
60	0.00	2.50
84	1.87	0.39
108	2.23	1.02
132	0.00	0.19
156	10.27	2.34
180	1.60	----
Total Contribution	23.20*	7.60**

[1] Data from Table 14 and 15 Controls.

[2] 60 flies in test collected 12 Sept. 1979: Parity 40%

[3] 60 flies in test collected 3 Oct. 1979: Parity 25%

[4] Mortality adjusted by 2½% per day (15% overall) to account for difference in parity as it related to mortality.

* 6 degrees of freedom; $P < 0.01$ that they are the same.

** 5 degrees of freedom; $P > 0.10 < 0.25$ that they are the same.

Table 17 - Comparison of mortality rates of gravid and postoviposition Simulium exiguum and Simulium metallicum[1].

Day of Mortality	S. exiguum Percent Dead		S. metallicum Percent Dead		Columns (2) and (4) compared Chi-Square Contribution
	Gravid Control Retained eggs[2] (1)	Oviposited[2] (2)	Gravid Control Retained eggs[3] (3)	Oviposited[3] (4)	
1	16.5	35.3	14.6	17.1	9.38
2	15.4	21.9	18.1	22.9	0.05
3	22.3	21.9	20.5	27.1	1.23
4	21.8	13.4	27.5	17.9	1.51
5	8.5	5.9	11.7	10.7	3.91
6	8.0	1.6	4.8	3.6	4.56
7	5.3	---	2.3	0.7	
8	1.6	---	0.5	---	
9	0.6	---	---	---	
TOTAL	100.00	100.00	100.00	100.00	20.64[a]

[1] Rates are based upon the number of surviving flies that were placed in oviposition tubes 3 days after a bloodmeal and either oviposited or retained eggs. The first rate is derived for the 4th day after the bloodmeal or 1 day after the oviposition trial.

[2] Cumulative results of 8 oviposition trials (376 controls and 374 oviposited flies) with an overall parous rate of 48.6 percent.

[3] Cumulative results of 11 oviposition trials (343 controls and 280 oviposited flies) with an overall parous rate of 56.4 percent.

[a] Probability is greater than .995 that rates in columns (2) and (4) are significantly different.

Table 18 - Expected mortality of a population of Simulium exiguum passing through 2 gonotrophic cycles over 7 days.[a]

Interval in Days	Actual Percent Mortality[1]	Percent Subtraction of Weighted Adjustment[2]		Percent Combined Adjusted Mortality	Percent Death Rate/ Interval[3]		Percent Secondary Mortality/ Interval[4]		Percent Tertiary Mortality/ Interval[5]		Percent Expected Mortality/ Interval[6]	Theoretical Population Decline of 10,000 Female Simulium exiguum
1	5	0	=	5	5.0	+	0.0	+	0.0	=	5.0	9,500
2	0	0	=	0	0.0	+	0.0	+	0.0	=	0.0	9 500
3	18	0	=	18	18.9	+	0.0	+	0.0	=	18.9	7,704[a]
4	35	-7	=	28	36.4	+	5.0	+	0.0	=	36.9	4,902
5	22	-5	=	17	34.7	+	0.0	+	0.0	=	34.7	3,201
6	22	-5	=	17	53.1	+	18.9	+	0.0	=	72.0	896[b]
7	13	-4	=	9	60.0	+	34.7	+	5.0	=	99.0	3
8	6	-2	=	4	50.0	+	53.1	+	0.0	=	Extinct	Extinct
9	2	0	=	2	50.0	+	60.0	+	18.9	=	Extinct	Extinct
10	0	0	=	0	100.0	+	50.0	+	34.7	=	Extinct	Extinct
TOTAL	123	-23	=	100								

[a] Footnotes on following page

[1] Column includes two sets of observations; one set is for Days 1-3 and includes mortality during the period of oogenesis; the other set is for Days 4-10 and includes mortality during the period of postoviposition.

[2] Since 23% of the flies would have already died on Days 1-3, this percent was subtracted from the percents of mortality on Days 4-10 proportionate to the observed mortalities for each day.

[3] Mortality rates at each interval of individuals surviving each one-day period.

[4] Additional mortality increment is added since flies actually passing through a second gonotrophic cycle and surviving thereafter would be subject to mortality connected with additional risks associated with oogenesis, oviposition and postoviposition. Mortalities associated with the primary gonotrophic cycle and thereafter were applied in estimation of these risks.

[5] Mortality associated with the primary gonotrophic cycle and thereafter were again applied in estimation of the risks of death due to passage through a third gonotrophic cycle.

[6] Expected mortality rate at each interval of individuals surviving through each one day period. Mortality includes risks associated with passing through 3 gonotrophic cycles.

[7] Example of the decline of a population of 10,000 female flies, all passing through 2 gonotrophic cycles and beginning a third when the population would become extinct.

[a] Number surviving oogenesis of the first gonotrophic cycle up to oviposition.

[b] Number surviving oogenesis of the second gonotrophic cycle up to oviposition.

Table 19 - Expected mortality of a population of Simulium metallicum passing through 2 gonotrophic cycles over 7 days.[a]

Interval In Days	Actual Percent Mortality[1]	Percent Subtraction of Weighted Adjustment[2]		Percent Combined Adjusted Mortality	Percent Death Rate/ Interval[3]		Percent Secondary Mortality/ Interval[4]		Percent Tertiary Mortality/ Interval[5]		Percent Expected Mortality/ Interval[6]	Theoretical Population Decline of 10,000 Female Simulium metallicum
1	2	0	=	2	2.0	+	0.0	+	0.0	=	2.0	9,800
2	2	0	=	2	2.0	+	0.0	+	0.0	=	2.0	9,604
3	15	0	=	15	15.6	+	0.0	+	0.0	=	15.6	8,106[a]
4	17	-3	=	14	17.3	+	2.0	+	0.0	=	19.3	6,542
5	23	-4	=	19	28.4	+	2.0	+	0.0	=	30.4	4,554
6	27	-5	=	22	45.8	+	15.6	+	0.0	=	61.4	1,758[b]
7	18	-3	=	15	57.7	+	28.4	+	2.0	=	88.1	210
8	10	-2	=	8	72.7	+	45.8	+	2.0	=	Extinct	Extinct
9	4	-1	=	3	100.0	+	57.7	+	15.6	=	Extinct	Extinct
10	1	-1	=	0	100.0	+	72.7	+	28.4	=	Extinct	Extinct
TOTAL	119	-19	=	100								

[a] Footnotes on following page

[1] Column includes two sets of observations; one set is for Days 1-3 and includes mortality during the period of oogenesis; the other set is for Days 4-10 and includes mortality during the period of postoviposition.

[2] Since 23% of the flies would have already died on Days 1-3, this percent was subtracted from the percents of mortality on Days 4-10 proportionate to the observed mortalities for each day.

[3] Mortality rates at each interval of individuals surviving each one-day period.

[4] Additional mortality increment is added since flies actually passing through a second gonotrophic cycle and surviving thereafter would be subject to mortality connected with additional risks associated with oogenesis, oviposition and postoviposition. Mortalities associated with the primary gonotrophic cycle and thereafter were applied in estimation of these risks.

[5] Mortality associated with the primary gonotrophic cycle and thereafter were again applied in estimation of the risks of death due to passage through a third gonotrophic cycle.

[6] Expected mortality rate at each interval of individuals surviving through each one day period. Mortality includes risks associated with passing through 3 gonotrophic cycles.

[7] Example of the decline of a population of 10,000 female flies, all passing through 2 gonotrophic cycles and beginning a third when the population would become extinct.

[a] Number surviving oogenesis of the first gonotrophic cycle up to oviposition.

[b] Number surviving oogenesis of the second gonotrophic cycle up to oviposition.

Table 20 - Comparison of cumulative percents mortality of fluorescein-marked and unmarked groups of laboratory maintained, bloodfed Simulium exiguum demonstrating the effects of marking.

Hour postfeeding/marking	Simulium exiguum[a] Unmarked Control[1]	Fluorescein Compound Orange[2]	Blue[1]
36	5.0	17.2	46.6
60	5.0	24.1	49.9
———Midday Minimum Oviposition Deadline[3]———			
84	23.3	53.4	64.9
———Midday Maximum Ovoposition Deadline[4]———			
108	63.3	75.8	79.9
132	93.3	-100.0	94.9
156	-100.0	---	-100.0

[a] Collected 3 October 1979: Parity 40%

[1] 60 Flies tested

[2] 58 Flies tested

[3] The minimum deadline corresponds with the end of the period of the time needed for egg development and falls at a time after bloodfeeding upon which most gravid S. exiguum were found to be oviposition ready. See Results II.F. and Figure 25.

[4] The maximum deadline of S. exiguum falls at a time after bloodfeeding upon which the vast majority of laboratory-maintained, gravid flies oviposited. See Results II.F. and Figure 25.

Table 21 - Comparison of cumulative percents mortality of fluorescein-marked and unmarked groups of laboratory maintained, bloodfed Simulium metallicum demonstrating the effects of marking.

	Simulium metallicum					
	Fluorescein Compounds					
	Test I[a]			Test II[b]		
Hour post-feeding/marking	Control[1]	Orange[1]	Blue[1]	Blue Repeat[2]	Orange/Blue[3]	Control[1]
36	1.6	1.6	35.0	13.5	6.5	1.6
60	1.6	8.2	40.0	21.9	8.6	1.6
Midday Minimum Oviposition Deadline[4]						
84	15.2	24.8	56.6	43.9	41.2	19.2
Midday Maximum Oviposition Deadline[4]						
108	46.8	54.8	76.6	81.2	71.6	68.2
132	76.8	93.1	96.6	98.1	91.1	91.5
156	98.4	-100.0	-100.0	98.1	97.6	-100.0
180	-100.0	---	---	-100.0	-100.0	---

[a] Collected 12 September 1979: Parity 25%

[b] Collected 26 September 1979: Parity 25%

[1] 60 Flies tested

[2] 59 Flies tested

[3] 46 Flies tested

[4] See footnotes 3 and 4 on Table 20.

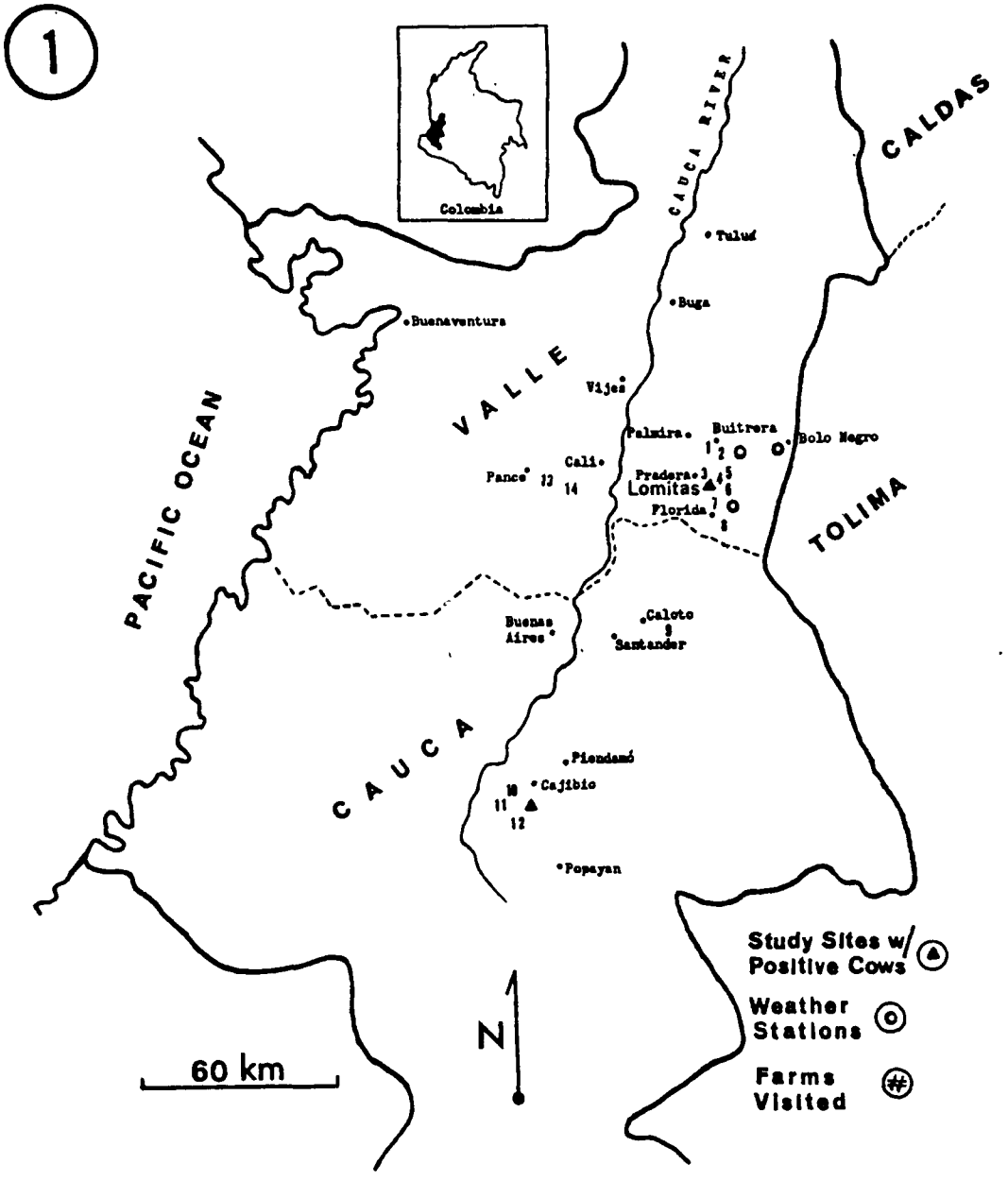
Table 22 - Number of Simulium exiguum marked, released and recaptured in a nine day trial, June 30 - July 8, 1970.

Marking Day	Marking Color	No. Marked & Released	Number Marked Flies Recaptured	Days Elapsed (Post-marking) until Return of Recaptured Flies	Percent Recaptured
1	Orange	856	1	6	0.117
2	Blue	911	8	2,3,3,3,3,4,4,5	0.878
	TOTAL	1,767	9		0.509

Table 23 - Number of Simulium metallicum marked, released and recaptured in a nine day trial, July 19 - July 28, 1979.

Marking Day	Marking Color	No. Marked & Released	Number Marked Flies Recaptured	Days Elapsed (Post-feeding) until Return of Recaptured Flies	Percent Recaptured
1	Orange	274	4	3,3,3,4	1.459
2	Blue	399	8	2,3,3,3,3,3,4	2.001
	TOTAL	673	12		1.783

Figure 1 - Map showing black fly study sites, farms where cows were skin snipped in search of Onchocerca gutturosa infections and weather stations near Lomitas, Valle, Colombia.


1
Colombia
CAUCA RIVER
CALDAS
Tuluá
Buga
Buenaventura
PACIFIC OCEAN
VALLE
Vijes
Palmira
Buitrera
Bolo Negro
Cali
Pance
Pradera
Lomitas
Florida
TOLIMA
Caloto
Buenos Aires
Santander
CAUCA
Piendamó
Cajibio
Popayan
Study Sites w/ Positive Cows
Weather Stations
Farms Visited
N
60 km

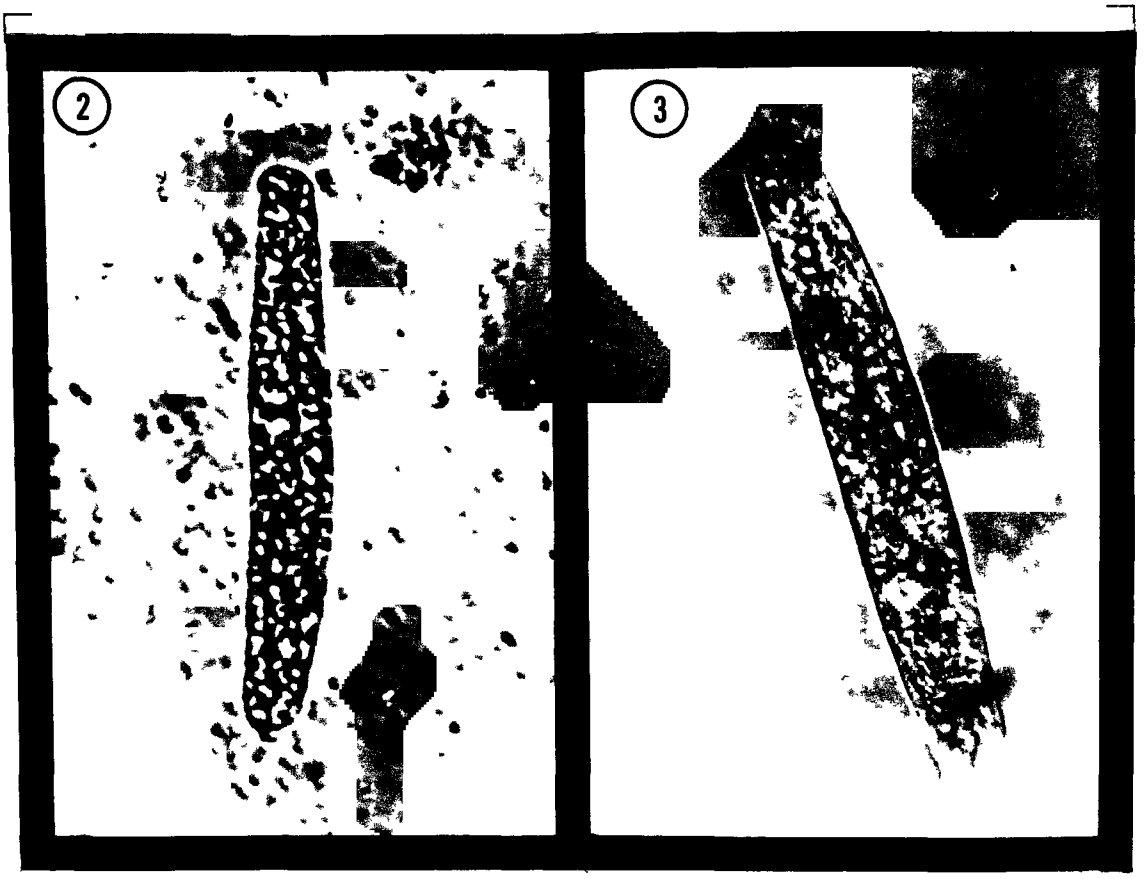
Figures 2 to 5 - O. gutturosa development in black flies.

Figure 2 - Degenerating L_1 (Sausage Stage) of Onchocerca gutturosa found in the thorax of Simulium exiguum after 108 hours (Day 5) of development. (X590)

Figure 3 - Degenerating L_1 (Sausage Stage) of Onchocerca gutturosa found in the thorax of Simulium metallicum after 120 hours (Day 5½) of development. (X470)

Figure 4 - Viable, but abnormally developing late L_1 Stage of Onchocerca gutturosa found in the thorax of Simulium exiguum 108 hours (Day 5) after infection: Note several hypodermal nuclei (HP) as well as what appears to be a formative anal plug (AP) are visible. Cuticle is abnormally crenate. (X850)

Figure 5 - Viable, but abnormally developing early L_2 Stage of Onchocerca gutturosa found in the thorax of Simulium exiguum 156 hours (Day 7) after infection: Note melanization (Me) in the area of the esophagus and the arrows demonstrating the profound constriction in the cuticle in the caudal region. (X375)


2
3

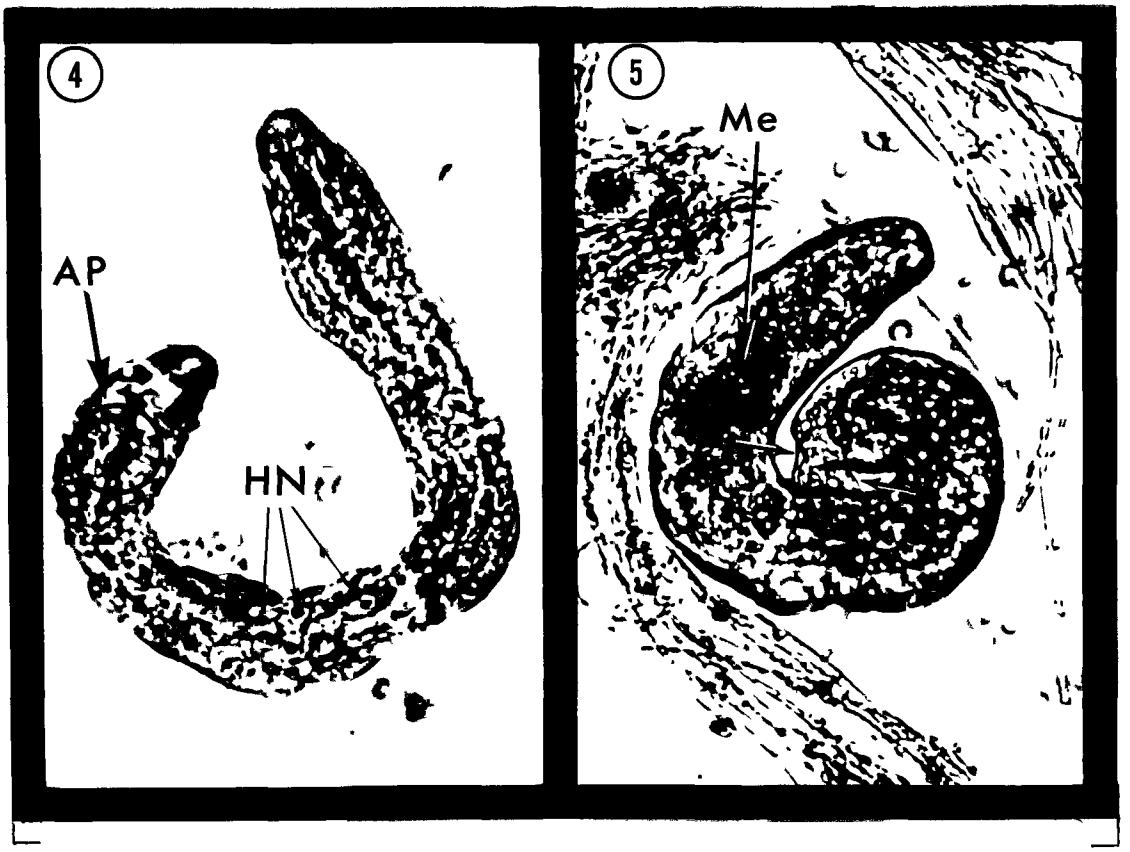

4
AP
HN
5
Me
C

Figure 6 - Normally developing early L_3 stage (543 μ) of Onchocerca gutturosa found in the thorax of Simulium exiguum 60 hours (2½ days) after infection. (X480). Arrows show intestinal-esophageal junction and nerve ring (NR), glandular esophagus (GE) and intestine (In) are visible.

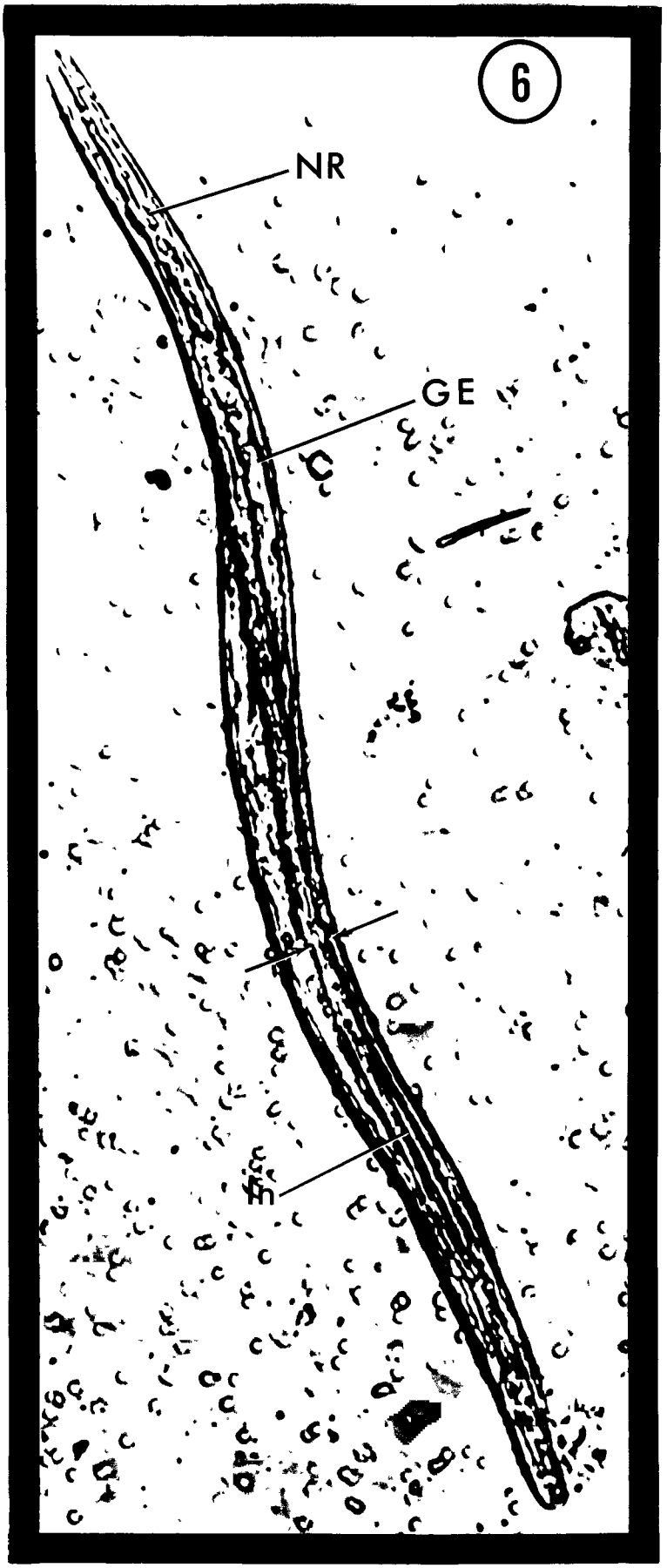

6
NR
GE

Figure 7 - Profile of the gonotrophic state of the Simulium exiguum population in Lomitas, Valle, Colombia as represented by 500 flies dissected between October, 1979 and March, 1980.

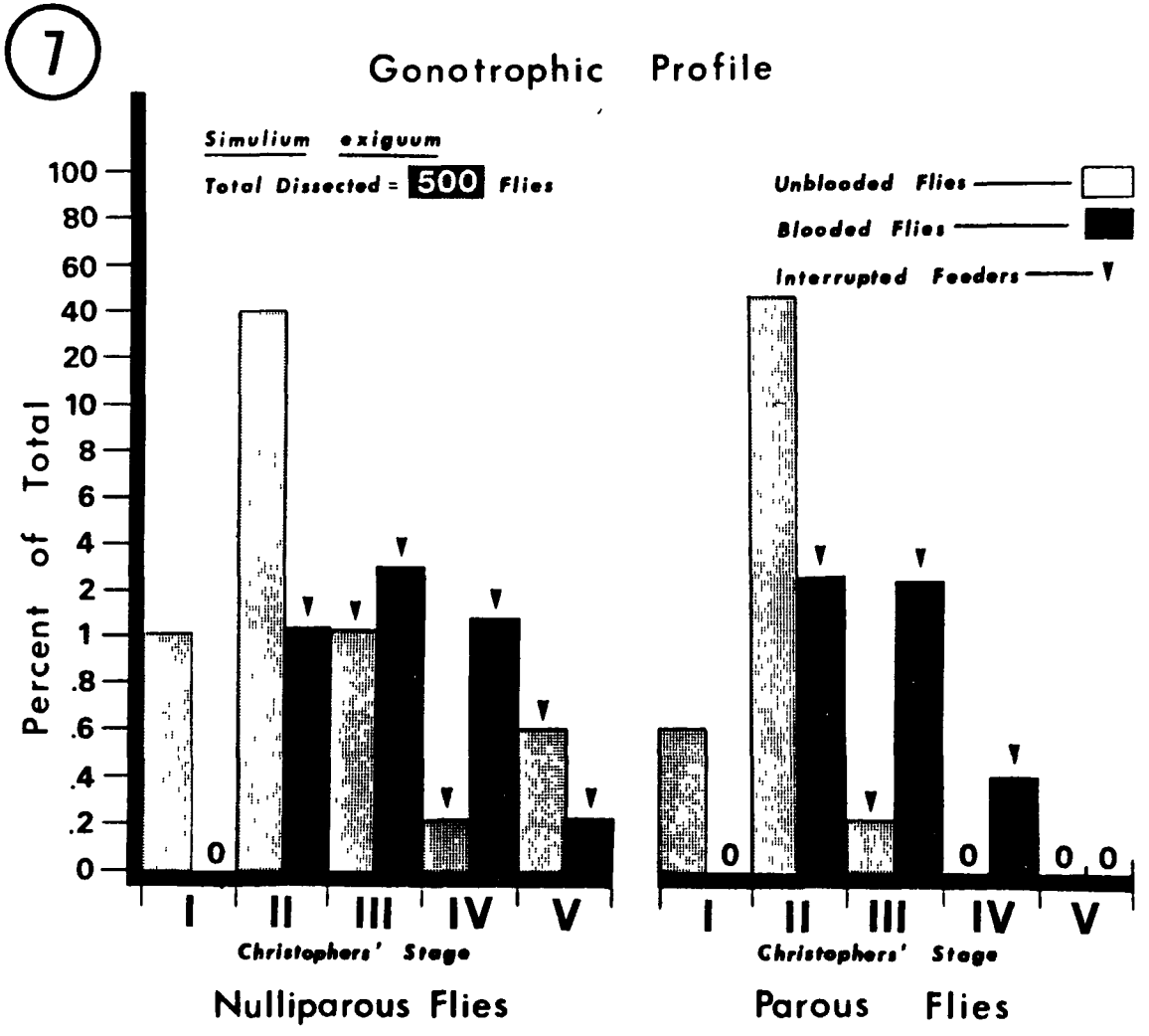

7
Gonotrophic Profile
Simulium exiguum
Total Dissected = 500 Flies
Unblooded Flies
Blooded Flies
Interrupted Feeders
Percent of Total
100
80
60
40
20
10
8
6
4
2
1
.8
.6
.4
.2
0
0
0
0
0
0
I
II
III
IV
V
Christophers' Stage
Nulliparous Flies
Parous Flies

Figure 8 - Profile of the gonotrophic state of the Simulium metallicum population in Lomitas, Valle, Colombia as represented by 353 flies dissected between October, 1979 and March, 1980.

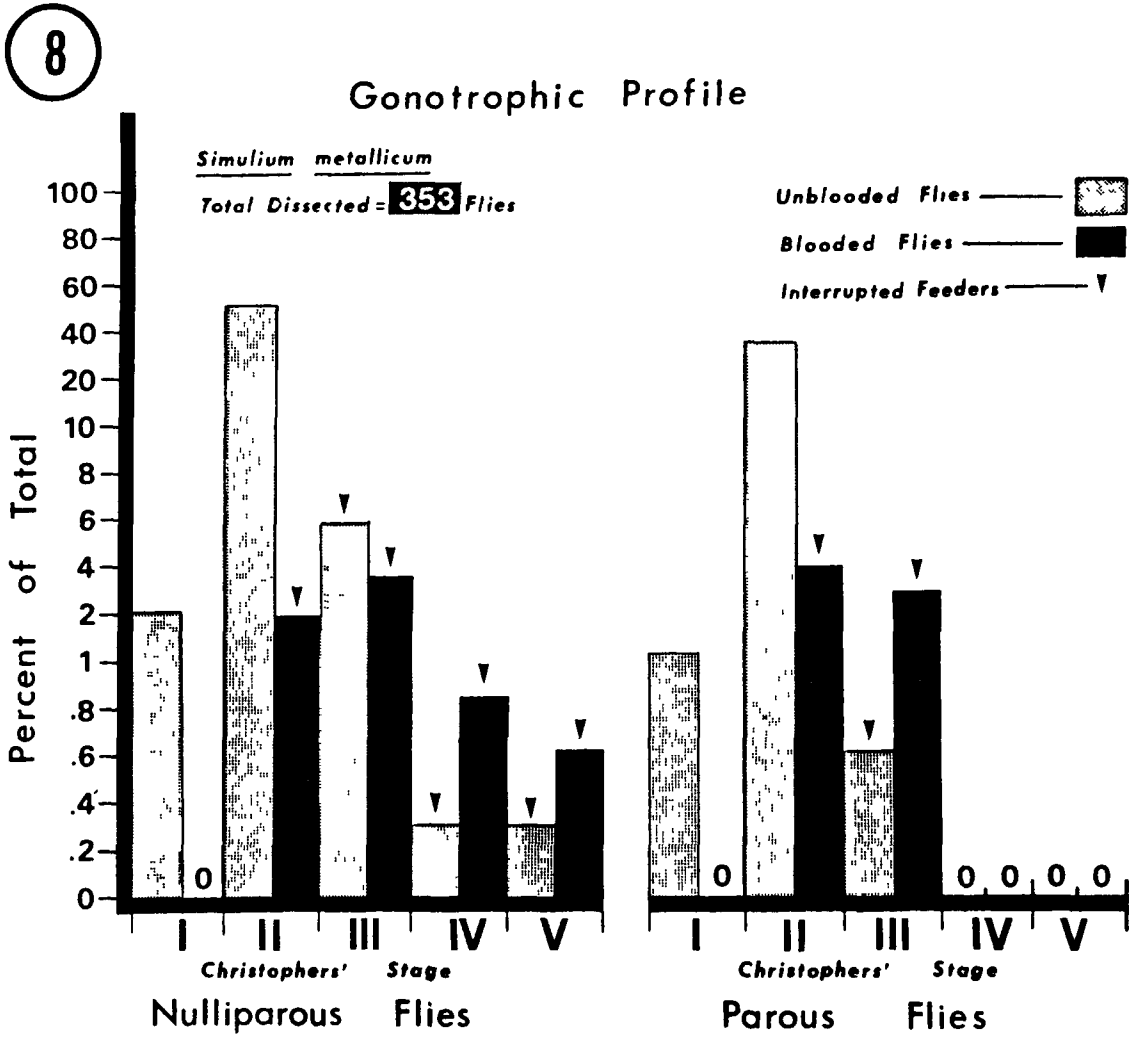

8
Gonotrophic Profile
Simulium metallicum
Total Dissected = 353 Flies
Unblooded Flies
Blooded Flies
Interrupted Feeders
Percent of Total
100
80
60
40
20
10
8
6
4
2
1
.8
.6
.4
.2
0
0
0
0 0 0 0
I II III IV V
Christophers' Stage
Nulliparous Flies
I II III IV V
Christophers' Stage
Parous Flies

Figure 9 - Screening test of unknown bloodmeal No. 30 from Simulium metallicum showing positive reaction to anticow (AC).

Center well (30) : Unknown No. 30

1 - Antihuman : negative reaction

2 - Anticow : positive reaction

3 - Antibird : negative reaction

Figure 10 - Control test using the gel-diffusion, precipitin test to identify 6-hour-old bloodmeals of fully engorged Simulium exiguum and Simulium metallicum.

Center well (AC) - Anticow (1:16,000)

1-3 : Known cow bloodmeals of Simulium exiguum

4-6 : Known cow bloodmeals of Simulium metallicum

3, 5 and 6 are positive

Figure 11 - Sensitivity titration of anticow (AC) against the maximum serum-meal (MSM) of Simulium metallicum and dilutions of the MSM.

Center well (AC) : Anticow

1-6 : Serial dulutions of cow serum

1 - 1/5th of MSM positive at 1:400

2 - 1/10th of MSM positive at 1:800[a]

3 - 1/20th of MSM positive at 1:1,600

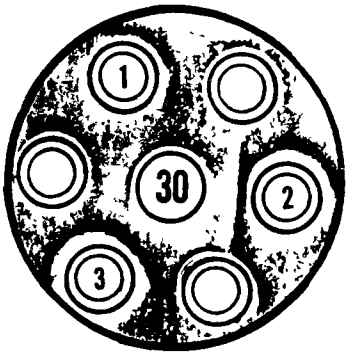
4 - 1/40th of MSM positive at 1:3,200

5 - 1/80th of MSM positive at 1:6,400

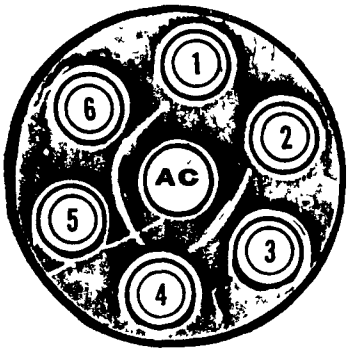
6 - 1/160th of MSM negative at 1:12,800

[a] Dilutions of 2-6 represent 1/5th, 1/10th, 1/20th, 1/40th and 1/80th, respectively, of a maximum serum-meal (MSM) of Simulium exiguum. Therefore, the test is only sensitive enough to identify 1/40th of the MSM of S. exiguum.

9



10



11

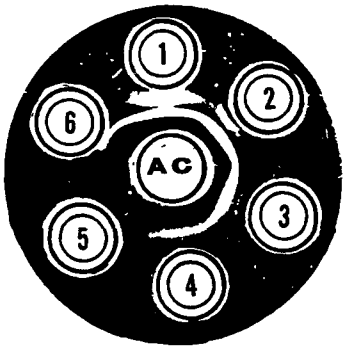


Figure 12 - Linear comparison of landing rates versus feeding rates for Simulium exiguum and Simulium metallicum indicating a higher feeding efficiency for S. exiguum.

12
Feeding Rates/Hour
Landing Rates/Hour
Simulium exiguum
Y = −3.02 + .512X
Simulium metallicum
Y = 53.1 + .277X

Figures 13 to 24 - Oogenesis of Simulium metallicum.

Figure 13 - Christophers' N Stage. (X150)

Figure 14 - Christophers' IIA Stage: Note yolk vacuole (YV) without yolk granules. (X150)

Figure 15 - Christophers' IIB Stage: Note yolk vacuole is filled and occupies 1/3 of oocyte. Nurse cells (NC) are clearly visible. (X150)

Figure 16 - Christophers' IIIA Stage: Yolk occupies 1/2 of oocyte. (X150)

Figure 17 - Christophers' IIIB Stage: Yolk occupies 2/3 of oocyte; and see follicular granules (Gr) denoting parity. (X150)

Figure 18 - Christophers' IVA Stage: Yolk occupies 3/4 of oocyte. (X150)

Figure 19 - Christophers' IVB Stage: Yolk occupies 7/8 of oocyte; nurse cells (NC) occupy the proximal portion of the oocyte. (X150)

Figure 20 - Christophers' VA Stage: Fully developed oocyte with opposing sides flat and convex. (X150)

Figure 21 - Christophers' VB Stage: Fully developed egg ready for oviposition as indicated by stretched tunica (T) and sloughing chorion (C). Secondary oocyte (2^{o}) is in Christophers' I Stage: Note nurse cells (NC) and absence of yolk sac. (X150)

Figure 22 - Egg one hour post-oviposition; Note chorion (C) has filled with water; micropyle (M) is evident. (X150)

Figure 23 - Secondary oocyte (2^{o}) with scar (S), one hour post-oviposition. (X150)

Figure 24 - Rarely observed degenerated primary follicle (DF(1^{o})) with developing secondary follicle (2^{o}) in Christophers' IIB Stage with nurse cells (NC). (X150)

Key to Letter Abbreviations in Figures 13 to 24.

1^{o}	Primary Follicle	Gr	Follicular Granules
2^{o}	Secondary Follicle	M	Micropylar area
3^{o}	Tertiary Follicle	NC	Nurse Cells
C	Chorion	T	Tunica
DF	Degenerate Follicle	YV	Yolk Vacuole
G	Germarium	Y	Previtellogenic Yolk

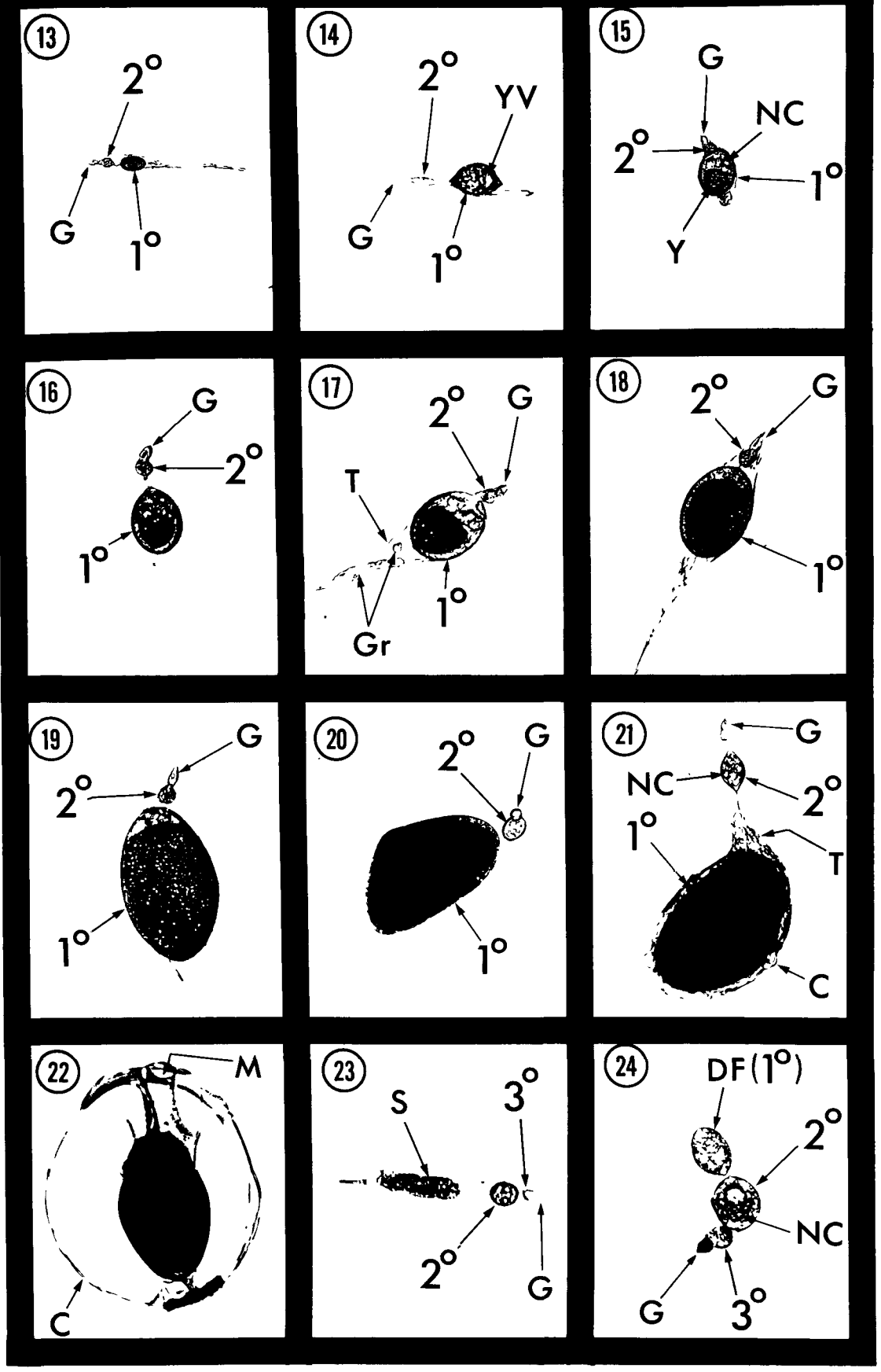

13
2°
G
1°
14
2°
YV
G
1°
15
G
NC
2°
1°
Y
16
G
2°
1°
17
2°
G
T
1°
Gr
18
2°
G
1°
19
G
2°
1°
20
2°
G
1°
21
G
NC
2°
1°
T
C
22
M
C
23
S
3°
2°
G
24
DF(1°)
2°
NC
G
3°

Figure 25 - Frequency of oviposition of field collected and laboratory maintained Simulium exiguum and Simulium metallicum at five consecutive trials postfeeding.

25

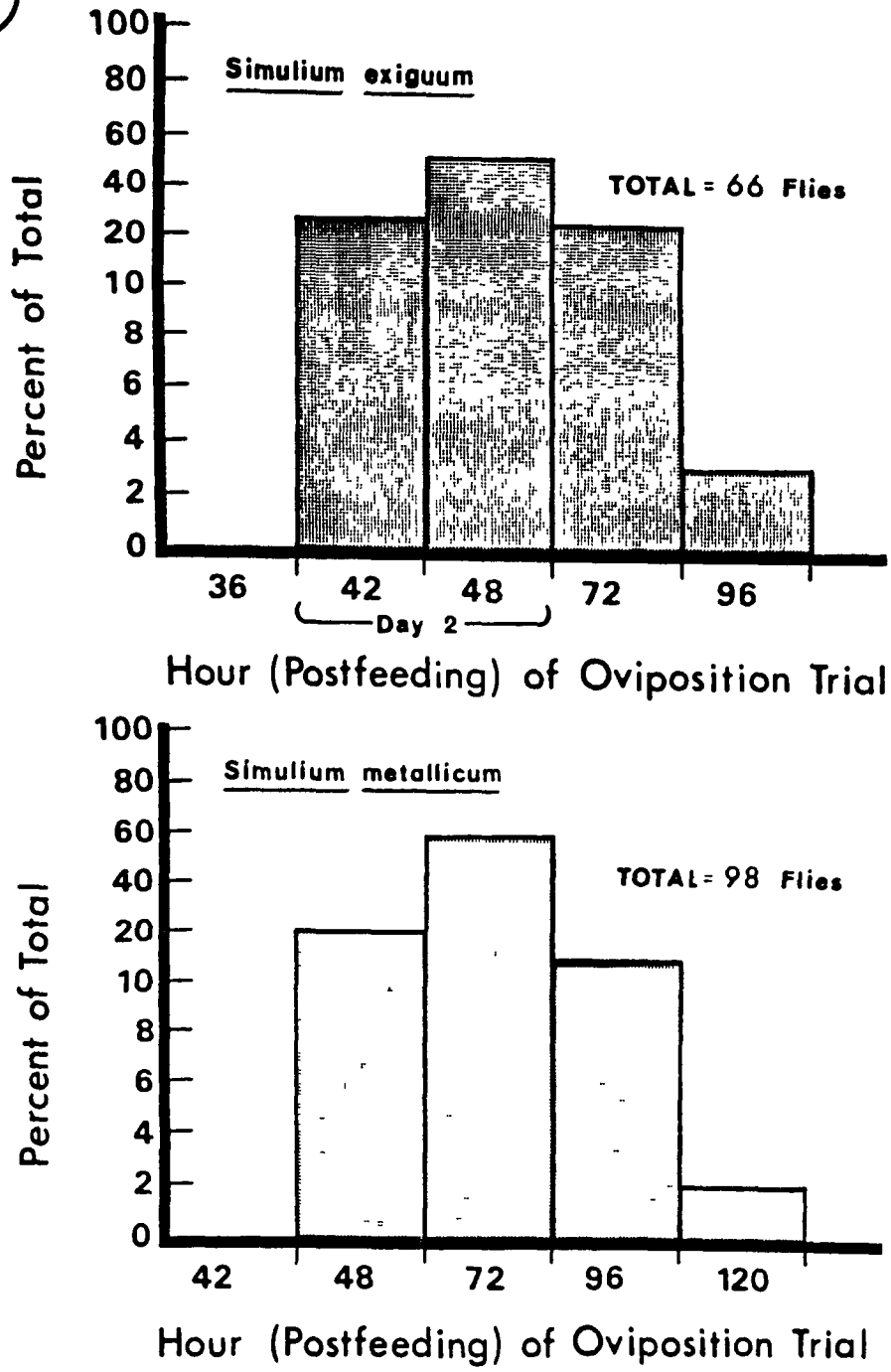
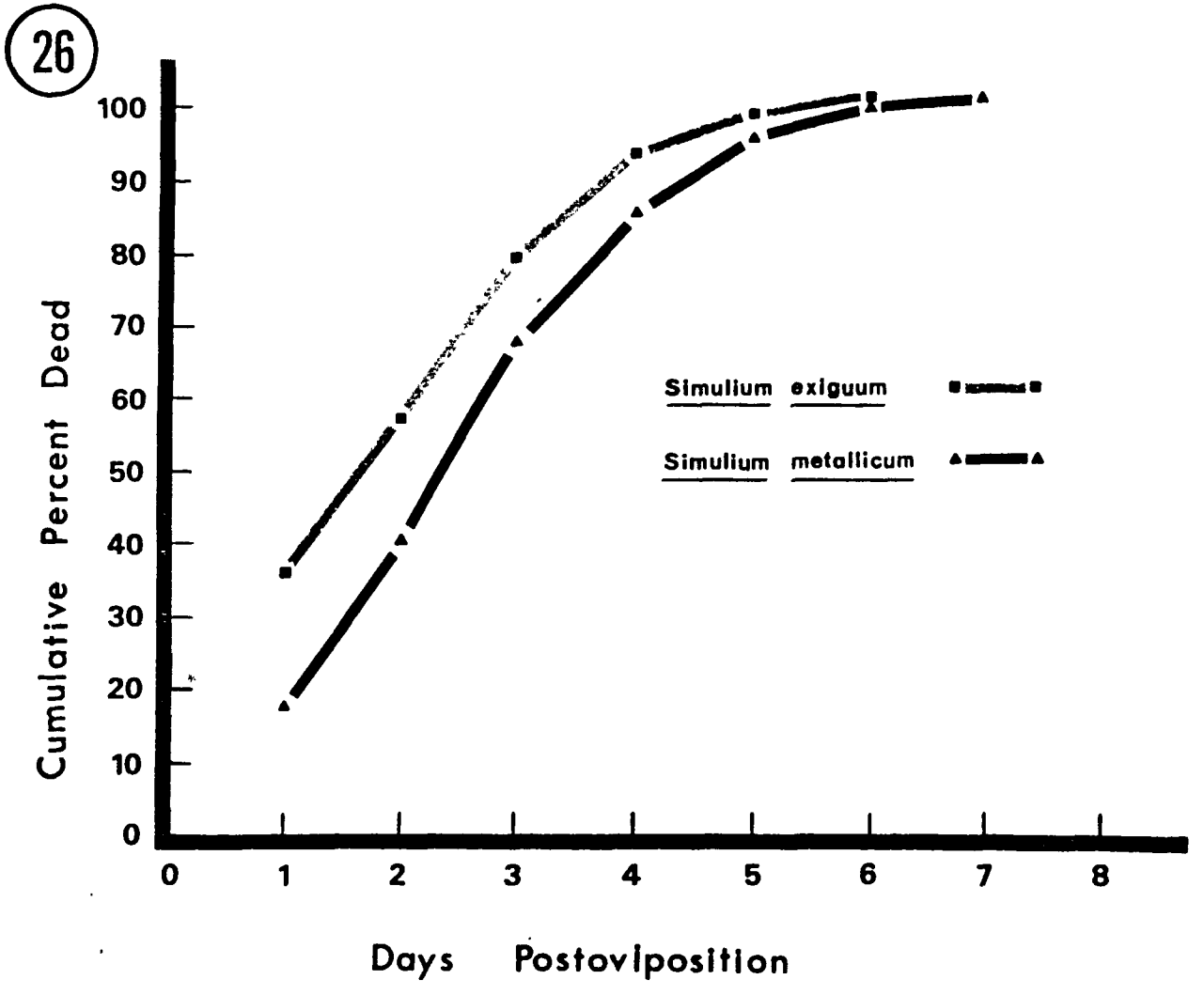

Simulium exiguum
TOTAL = 66 Flies
Percent of Total
100
80
60
40
20
10
8
6
4
2
0
36
42
48
72
96
Day 2
Hour (Postfeeding) of Oviposition Trial
Simulium metallicum
TOTAL = 98 Flies
Percent of Total
100
80
60
40
20
10
8
6
4
2
0
42
48
72
96
120
Hour (Postfeeding) of Oviposition Trial

Figure 26 - Comparison of mortality rates of laboratory-maintained groups of Simulium exiguum and Simulium metallicum after oviposition.


26
Cumulative Percent Dead
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Days Postoviposition
Simulium exiguum
Simulium metallicum

Figures 27 to 30 - Ovarian scar reduction.

Figure 27 - Ovariole of Simulium metallicum one hour post-oviposition showing scar (S) of parous fly.

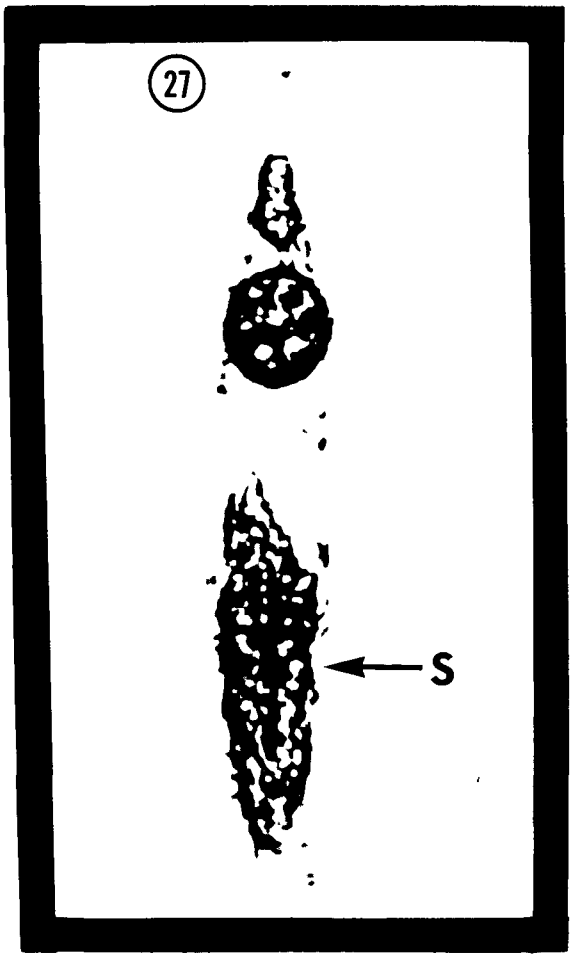
Figure 28 - Ovariole of Simulium metallicum 18 hours post-oviposition showing coalescence of granules (Gr) which form follicular relic (FR) of parous fly.

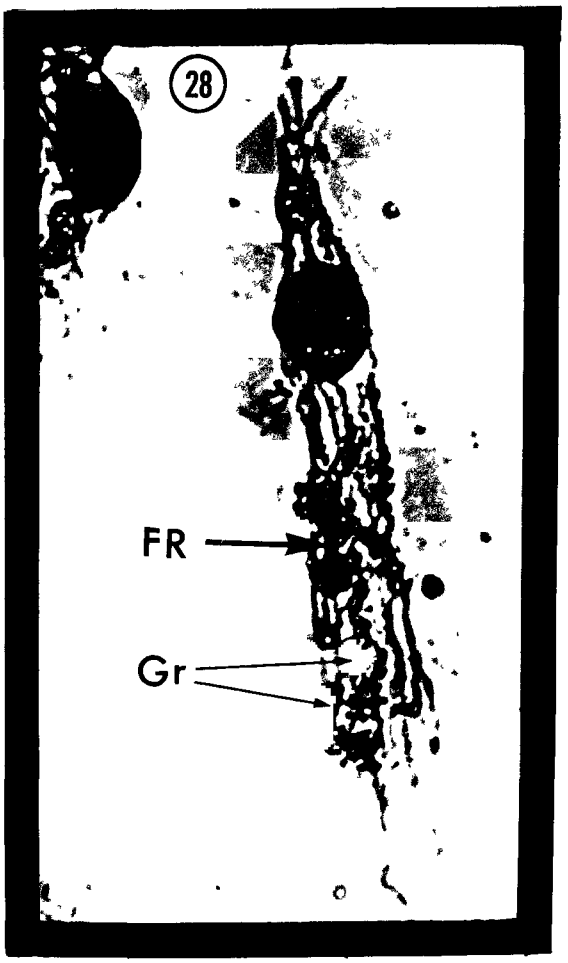
Figure 29 - Ovariole of Simulium metallicum 36 hours post-oviposition showing granules (Gr) of parous fly.

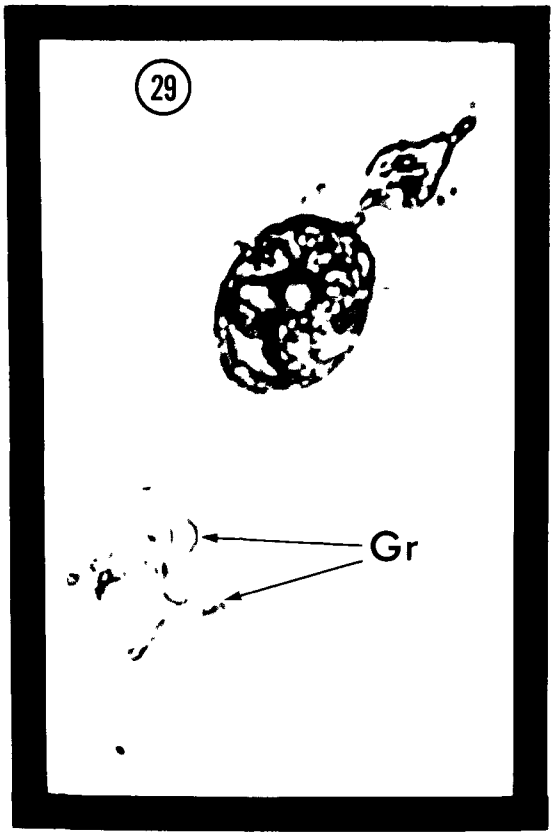
Figure 30 - Ovariole of Simulium metallicum 48 hours post-oviposition showing remnants of granules (Gr) of parous fly.

Key to Letter Abbreviations in Figures 27 to 30.

Gr - Granules also called yellow bodies
FR - Follicular Relic
S - Scar containing relic debris


27
S


28
FR
Gr


29
Gr

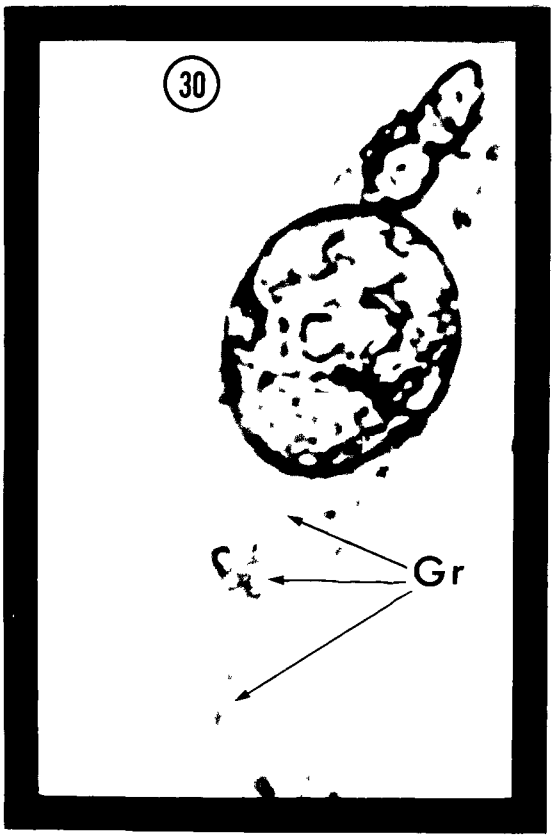
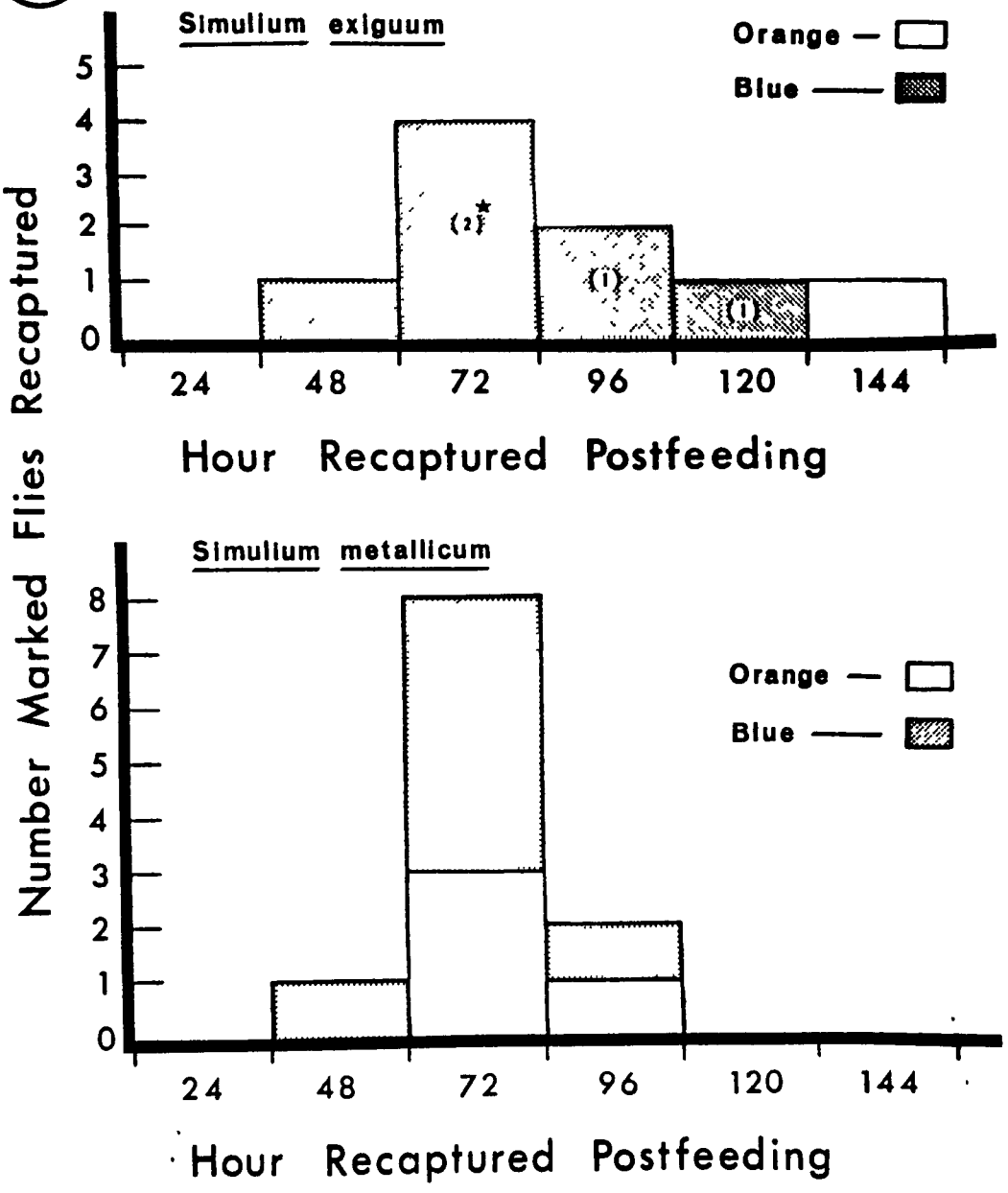

30
Gr

Figure 31 - Numbers of Simulium exiguum and Simulium metallicum recaptured after feeding on preceding days and being marked with fluorescein dusts.

31



*(No.) Apparently Good Marks.

Figure 32 - Daily activity of Simulium exiguum compared with fluctuations of temperature and relative humidity in Lomitas, Valle, Colombia.

32

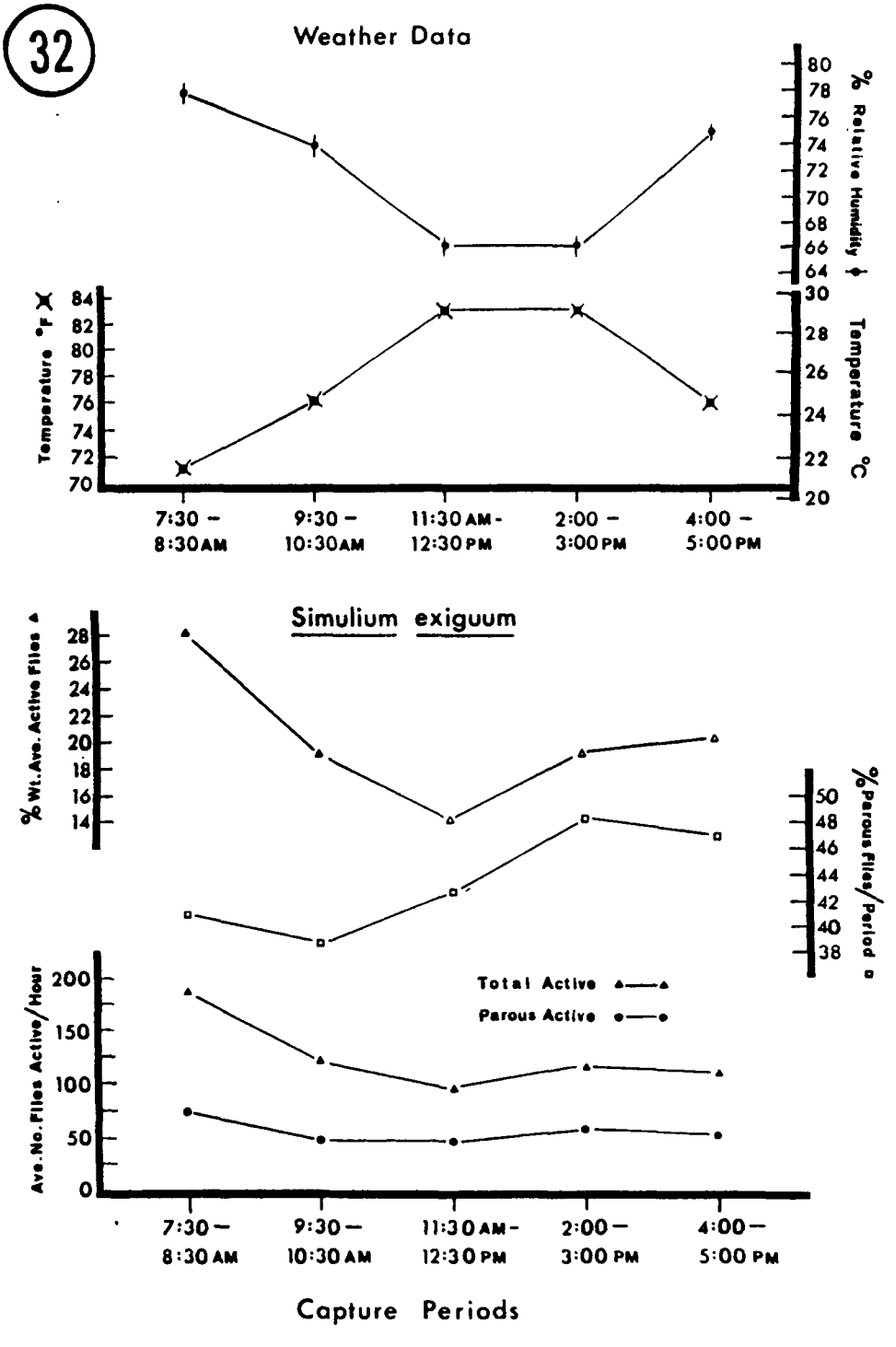

Weather Data
% Relative Humidity
Temperature °F
Temperature °C
80
78
76
74
72
70
68
66
64
30
28
26
24
22
20
84
82
80
78
76
74
72
70
7:30 – 8:30 AM
9:30 – 10:30 AM
11:30 AM - 12:30 PM
2:00 – 3:00 PM
4:00 – 5:00 PM
Simulium exiguum
% Wt. Ave. Active Flies
% Parous Flies/Period
Ave. No. Flies Active/Hour
Total Active
Parous Active
28
26
24
22
20
18
16
14
50
48
46
44
42
40
38
200
150
100
50
0
7:30 – 8:30 AM
9:30 – 10:30 AM
11:30 AM - 12:30 PM
2:00 – 3:00 PM
4:00 – 5:00 PM
Capture Periods

Figure 33 - Estimated curves of activity for Simulium exiguum in relation to changes in humidity and temperature.

33

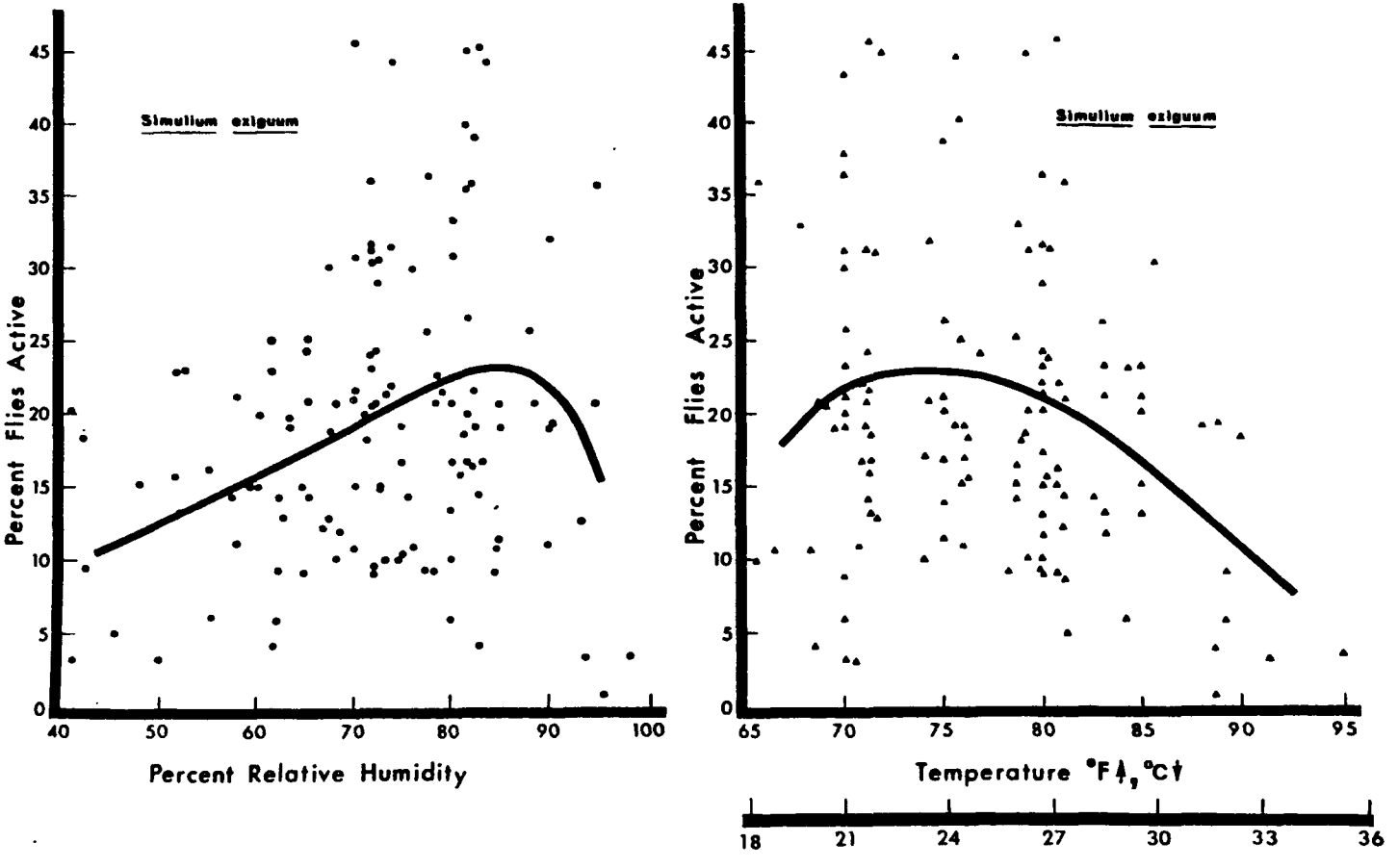

Simulium exiguum
Percent Flies Active
0
5
10
15
20
25
30
35
40
45
40
50
60
70
80
90
100
Percent Relative Humidity
Simulium exiguum
Percent Flies Active
0
5
10
15
20
25
30
35
40
45
65
70
75
80
85
90
95
Temperature °F ½, °C †
18
21
24
27
30
33
36

Figure 34 - Daily activity of Simulium metallicum compared with fluctuations of temperature and relative humidity in Lomitas, Valle, Colombia.

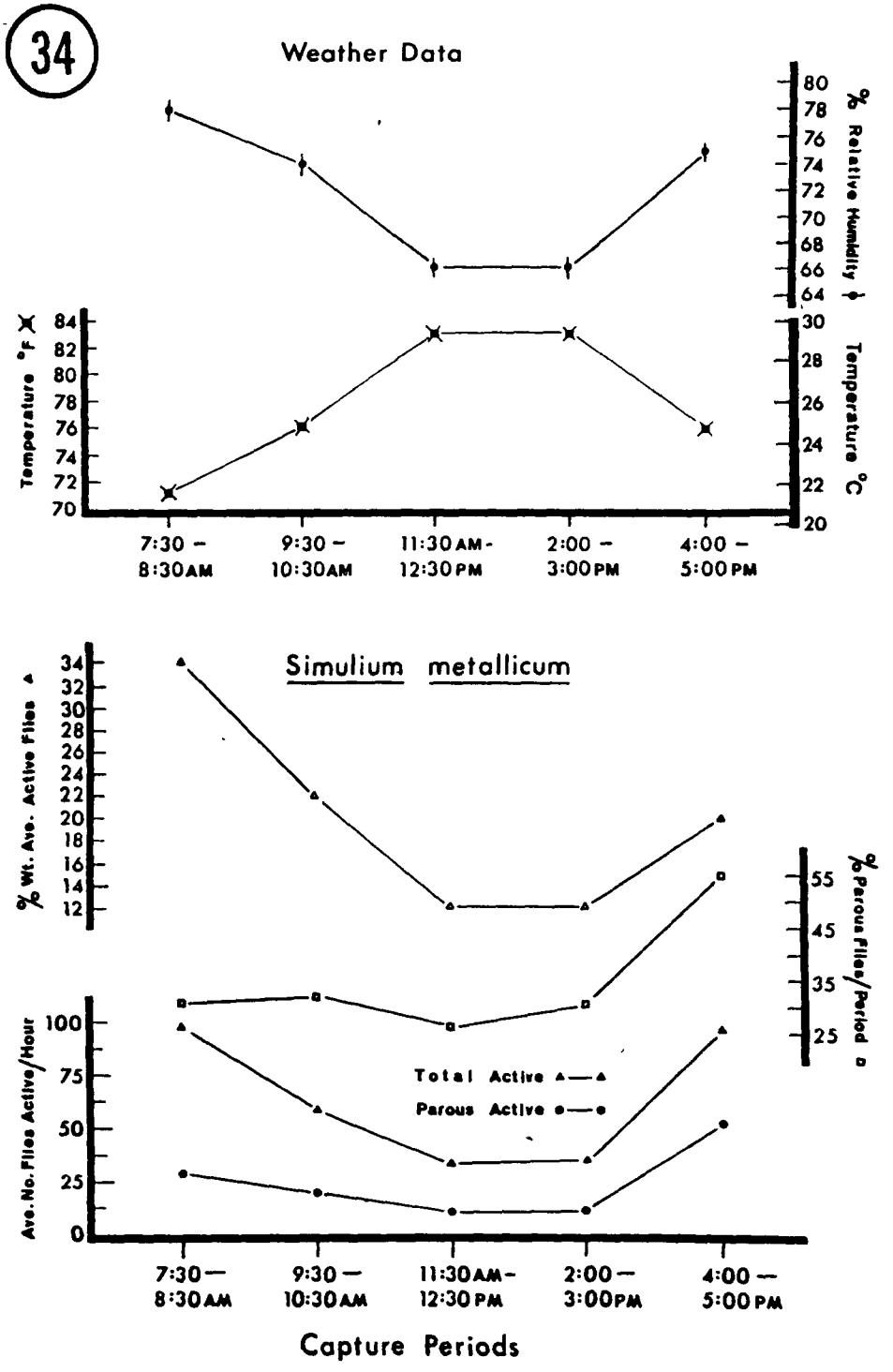

34
Weather Data
% Relative Humidity
Temperature °F
Temperature °C
7:30 – 8:30AM
9:30 – 10:30AM
11:30AM – 12:30PM
2:00 – 3:00PM
4:00 – 5:00PM
Simulium metallicum
% Wt. Ave. Active Flies
% Parous Flies/Period
Ave. No. Flies Active/Hour
Total Active
Parous Active
Capture Periods

Figure 35 - Estimated curves of activity for Simulium metallicum in relation to changes in humidity and temperature.

35

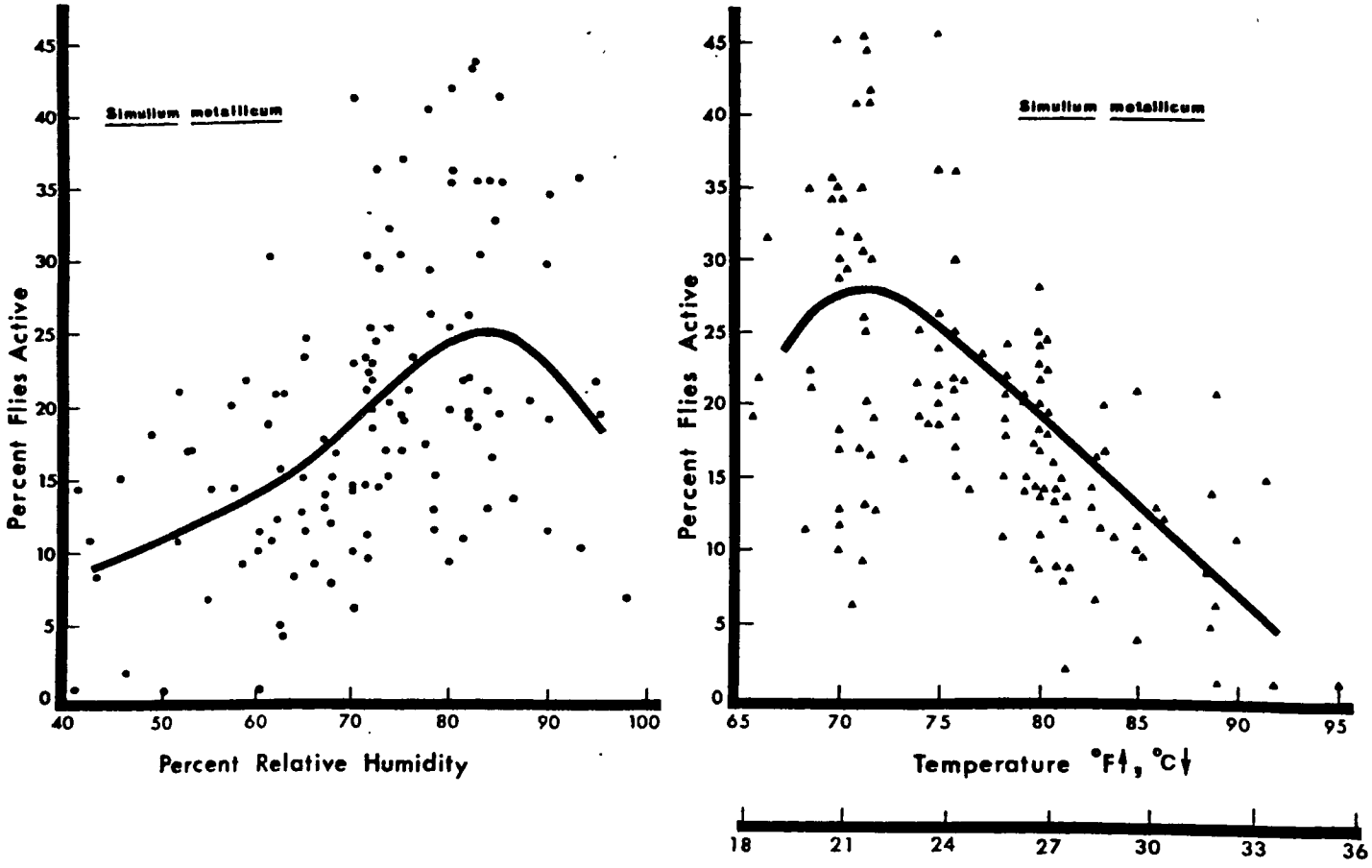
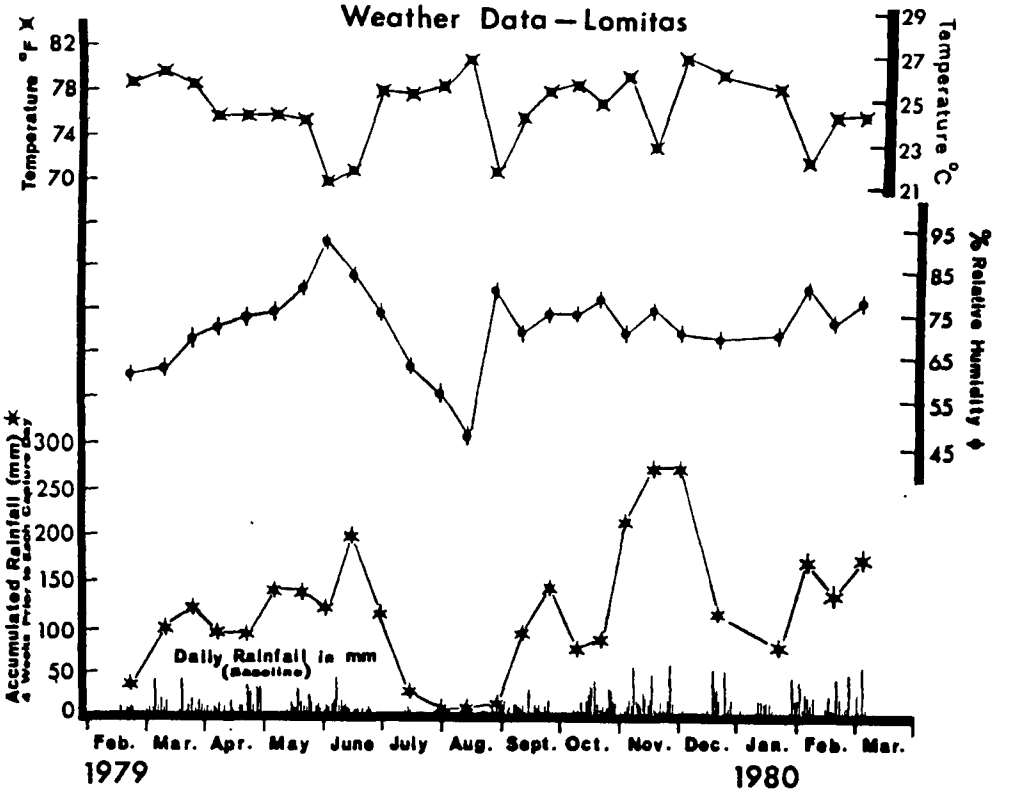

Simulium metallicum
Percent Flies Active
0
5
10
15
20
25
30
35
40
45
40
50
60
70
80
90
100
Percent Relative Humidity
Simulium metallicum
Percent Flies Active
0
5
10
15
20
25
30
35
40
45
65
70
75
80
85
90
95
Temperature °F↑, °C↓
18
21
24
27
30
33
36

Figure 36 - Seasonal activity of Simulium exiguum in Lomitas, Valle, Colombia compared with local fluctuations of temperature, humidity and rainfall.

36


Weather Data — Lomitas
Temperature °F
82
78
74
70
Temperature °C
29
27
25
23
21
% Relative Humidity
95
85
75
65
55
45
Accumulated Rainfall (mm)
300
250
200
150
100
50
0
Daily Rainfall in mm
(Baseline)
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1979
1980

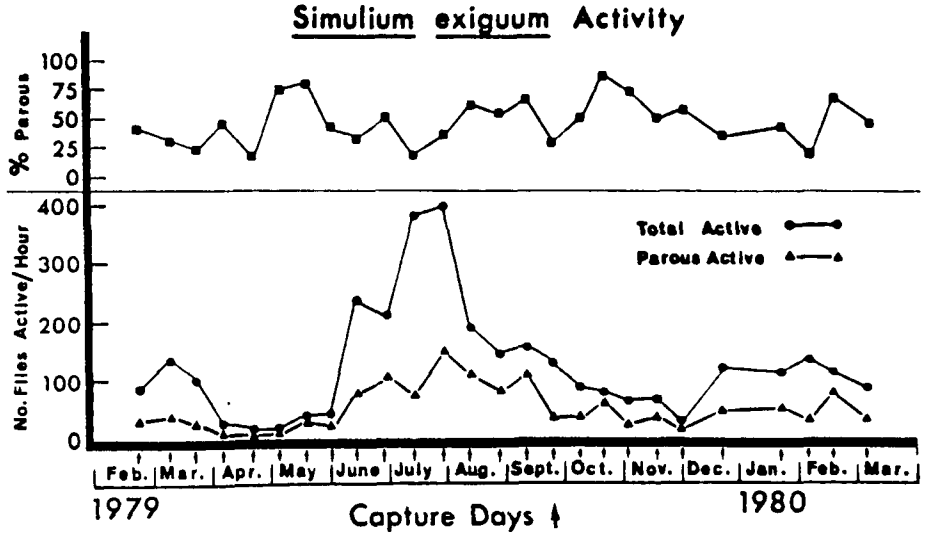
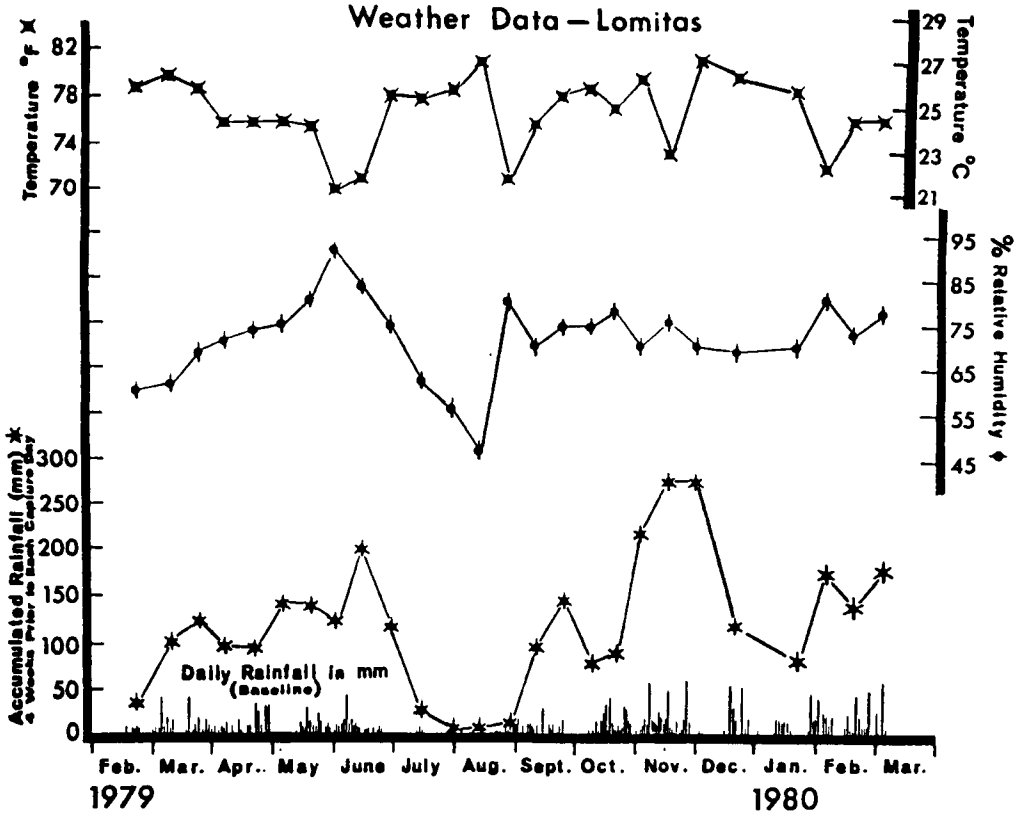

Simulium exiguum Activity
% Parous
100
75
50
25
0
No. Flies Active/Hour
400
300
200
100
0
Total Active
Parous Active
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1979
Capture Days
1980

Figure 37 - Seasonal activity of Simulium metallicum in Lomitas, Valle, Colombia compared with local fluctuations of temperature, humidity and rainfall.

37


Weather Data — Lomitas
Temperature °F
82
78
74
70
Temperature °C
29
27
25
23
21
% Relative Humidity
95
85
75
65
55
45
Accumulated Rainfall (mm)
4 Weeks Prior to Each Capture Day
300
250
200
150
100
50
0
Daily Rainfall in mm
(Baseline)
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1979
1980

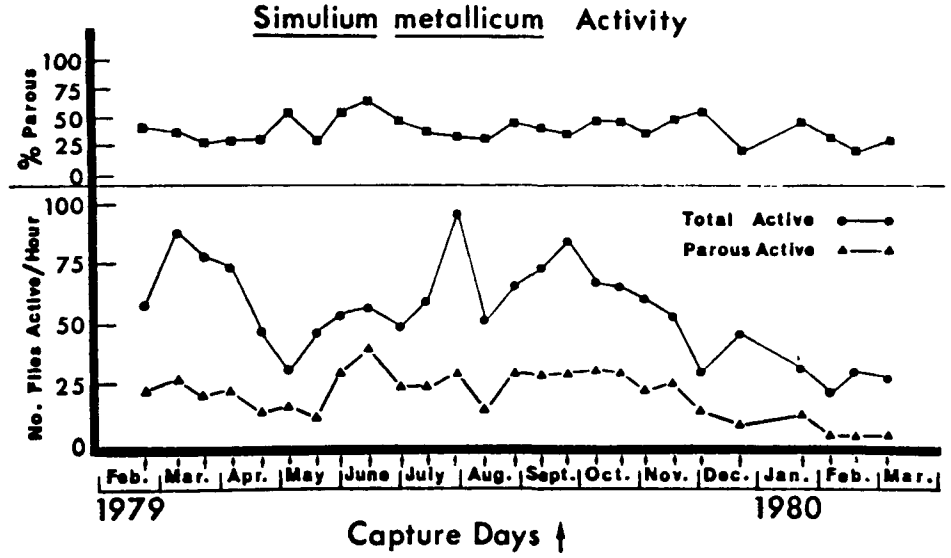
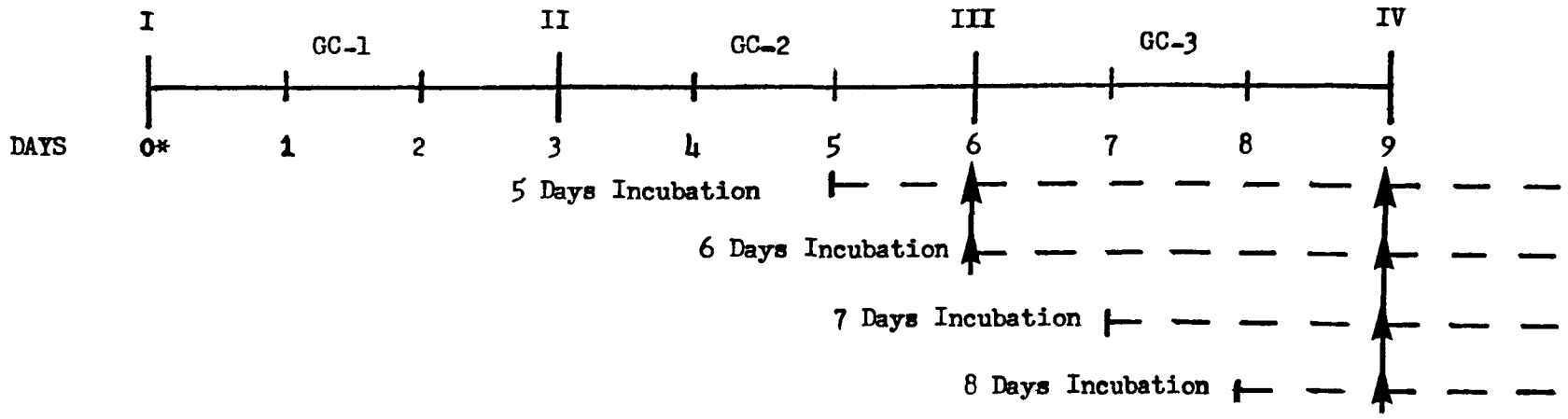

Simulium metallicum Activity
% Parous
100
75
50
25
0
No. Flies Active/Hour
100
75
50
25
0
Total Active
Parous Active
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1979
1980
Capture Days

Figure 38 - Feeding sequence and days upon which transmission may occur given that the incubation period may vary in Simulium exiguum and Simulium metallicum.

38



I-IV: Bloodmeals stimulating each gonotrophic cycle.

GC 1-3: Gonotrophic Cycles.

* Time zero of infection at first bloodmeal of nulliparous fly.

▲: Possible feeding upon which transmission may occur depending upon the length of incubation.

═: Duration of fly infectivity

REFERENCES

Abdelnur, O.M. 1968. The biology of some black flies (Diptera: Simuliidae) of Alberta. Quaest. Entomol. 4: 113-174.

Ash, L.R. and J.R. Schacher. 1971. Early life cycle and larval morphogenesis of Wuchereria bancrofti in the jird, Meriones unguicalatus. J. Parasitol. 57: 1043-1951.

Bain, O. 1979. Transmission de l'onchocerque bovine, Onchocerca gutturosa, par Culicoides. Ann. Parasitol. (Paris). 54: 483-488.

Bain, O., A.M. Denke, Y. Amegee and A.G. Chabaud. 1977. Les onchocerques des bovins au Togo: Les microfilaries et leurs distributions. Ann. Univ. Benin, Togo. III: 117-123.

Bain, O., G. Petit and B. Poulain. 1978. Validite des deux especes Onchocerca lienalis et O. gutturosa chez les bovins. Ann. Parasitol. (Paris). 53: 421-430.

Bloom, W. and D.W. Fawcet. 1968. A Textbook of Histology. 9th edition. W.B. Saunders Co., Philadelphia.

Carlson, G. 1967. Environmental factors influencing blackfly populations. Bull. Wld. Hlth. Org. 37: 139-150.

Chauhan, P.P.S. and B.P. Pande. 1978. Morphological variations within the species of Onchocerca armillata and O. gutturosa in buffaloes and cattle in India, with special reference to the male tail and cuticular ornamentation. J. Helminthol. 52: 343-353.

Clements, A.N. 1963. The Physiology of Mosquitoes. MacMillan Co., New York.

Collins, R.C. 1979. Onchocerciasis transmission potentials of four species of Guatemalan Simuliidae. Am. J. Trop. Med. Hyg. 28: 72-75.

Collins, R.C., C.C. Campbell, D.P. Wilton and L. Newton. 1977. Quantitative aspects of the infection of Simulium ochraceum by Onchocerca volvulus. Tropenmed. Parasitol. 28: 235-243.

Crosskey, R.W. 1962. Observations on the uptake of human blood by Simulium damnosum: The engorgement time and size of the bloodmeal. Ann. Trop. Med. Parasitol. 56: 141-148.

Crosskey, R.W. 1962. Simuliidae, German: Kriebelmucken in Insects and Other Arthropods of Medical Importance edited by K.G.V. Smith. British Museum of Natural History, London.

Cupp, E.W. and R.C. Collins. 1979. The gonotrophic cycle in Simulium ochraceum. Am. J. Trop. Med. Hyg. 28: 422-426.

Dalmat, H. 1952. Longevity and further flight range studies on black flies (Simuliidae: Diptera) with the use of dye markers. Ann. Entomol. Soc. Amer. 45: 23-39.

Dalmat, H.T. 1955. The black flies (Diptera: Simuliidae) of Guatemala and their role as vectors of onchocerciasis. Smithson. Misc. Collections. 125 (1): 1-425.

Davies, L. 1957. Study of the age of females of Simulium ornatum attracted to cattle. Bull. Entomol. Res. 48: 535-552.

Deleon, J.R. 1957. Simulium vectors of Onchocerciasis in Guatemala. Bull. Wld. Hlth. Org. 16: 523-529.

Denke, A.M. and O. Bain. 1978. Donnees sur le cycle d'Onchocerca ochengi chez Simulium damnosum s.l. au Togo. Ann. Parasitol. (Paris). 53: 757-760.

Detinova, T.S. 1962. Age-Grouping Methods in Diptera of Medical Importance. Wld. Hlth. Org., Geneva.

Duke, B.O.L. 1962. Studies on factors influencing the transmission of Onchocerciasis. II. The intake of O. volvulus microfilariae by Simulium damnosum and the survival of the parasite in the fly under laboratory conditions. Ann. Trop. Med. Parasitol. 56: 255-263.

Duke, B.O.L. 1968a. Studies on factors influencing the transmission of Onchocerca. IV. The biting cycles, infective biting density and transmission potential of "forest" Simulium damnosum. Ann. Trop. Med. Parasitol. 62: 95-106.

Duke, B.O.L. 1968b. Studies on factors influencing the transmission of Onchocerciasis. V. The stages of Onchocerca volvulus in wild "forest" Simulium damnosum, the fate of the parasites in the fly and the age distribution of the biting population. Ann. Trop. Med. Parasitol. 62: 107-115.

Duke, B.O.L. 1968c. Studies on the factors influencing the transmission of Onchocerca. VI. The infective biting potential of Simulium damnosum in different bioclimatic zones and its influence on the transmission potential. Ann. Trop. Med. Parasitol. 62: 164-170.

Duke, B.O.L. 1970. Onchocerca-Simulium Complexes. VI. Experimental studies on the transmission of Venezuelan and West African strains of Onchocerca volvulus by Simulium metallicum and Simulium exiguum in Venezuela. Ann. Trop. Med. Parasitol. 64: 421-431.

Duke, B.O.L. and D.J. Lewis. 1964. Studies on factors influencing the transmission of Onchocerciasis III. Observations on the effect of the peritrophic membrane in limiting the development of Onchocerca volvulus microfilariae in Simulium damnosum. Ann. Trop. Med. Parasitol. 58: 83-88.

Duke, B.O.L., J. Anderson, and H. Fuglsang. 1975. The Onchocerca volvulus transmission potentials and associated patterns of onchocerciasis at four Cameroon Sudan-Savanna villages. Z. Tropenmed. Parasitol. 26: 143-154.

Eberhard, M.L. 1976. The Morphology of Onchocerca Species (Nematoda: Filarioidea) Found in Cattle in the United States. Dissertation, Tulane University, pp. 1-62.

Eberhard, M.L. 1979. Studies on the Onchocerca (Nematoda: Filarioidea) found in cattle in the United States. I. Systematics of O. gutturosa and O. lienalis with a description of O. stilesi sp. N. J. Parasitol. 65: 379-388.

Eberhard, M.L. and T.C. Orihel. 1978. Equine and bovine onchocerciasis in Colombia, S.A. J. Parasitol. 64: 191-192.

Eichler, D.A. 1971. Studies on Onchocerca gutturosa (Neumann, 1910) and its development in Simulium ornatum (Meigen, 1818). II. Behavior of S. ornatum in relation to the transmission of O. gutturosa. J. Helminthol. 45: 259-270.

Eichler, D.A. 1973a. Studies on Onchocerca gutturosa (Neumann, 1910) and its development in Simulium ornatum (Meigen, 1818). III. Factors affecting the development of the parasite in its vector. J. Helminthol. 47: 73-88.

Eichler, D.A. 1973b. Studies on Onchocerca gutturosa (Neumann, 1910) and its development in Simulium ornatum (Meigen, 1818). IV. Systematics of O. gutturosa. J. Helminthol. 47: 89-96.

Eichler, D.A. and G.S. Nelson. 1971. Studies on Onchocerca gutturosa (Neumann, 1910) and its development in Simulium ornatum (Meigen, 1818). I. Observations on O. gutturosa in cattle in Southeast England. J. Helminthol. 45: 245-258.

Elbihari, S. and H.S. Hussein. 1978. Onchocerca gutturosa (Neumann, 1910) in Sudanese cattle. I. The microfilariae. Rev. Elev. Med. Vet. Pays Trop. 31: 179-182.

Figueroa, M.H., R.C. Collins, and W.J. Kozek. 1977. Post-prandial transportation and maintenance of Simulium ochraceum infected with Onchocerca volvulus. Am. J. Trop. Med. Hyg. 26: 75-79.

Fuglsang, H., J. Anderson, T.F. deC. Marshall, S., S. Ayange and O. Fisiy. 1976. Seasonal variation in the concentration of Onchocerca volvulus microfilariae in the skin. Z. Tropenmed. Parasitol. 27: 363-369.

Garms, R. 1973. Quantitative studies on transmission of Onchocerca volvulus by Simulium damnosum in Bong, Range, Liberia. Z. Tropenmed. Parasitol. 24: 358-372.

Garms, R. 1975. Observations of filarial infection and parous rates of anthropophilic blackflies in Guatemala, with reference to the transmission of Onchocerca volvulus. Z. Tropenmed. Parasitol. 26: 169-182.

Gibson, C.L. 1965. Experimental studies on the parasites and vectors of onchocerciasis in Guatemala. Bol. Sanit. Guatemala. 36: 246-287.

Gibson, C.L. and H.T. Dalmat. 1952. Three new potential intermediate hosts of human onchocerciasis in Guatemala. Am. J. Trop. Med. Hyg. 1: 848-851.

Guttman, D. 1972. The biting activity of black flies (Diptera: Simuliidae) in three types of habitats in Western Colombia. J. Med. Entomol. 9: 269-276.

Holdridge, L.R. 1967. Life Zone Ecology - Tropical Science Center, San Jose, Costa Rica.

Hussein, M.F., O. Abdel-Nur, M.S. Gassouma and G.S. Nelson. 1975. Onchocerca gutturosa infection in Sudanese cattle. Br. Vet. J. 131: 76-84.

Ivanov, I.V. 1964. Histological changes in the skin of cattle due to Onchocerca. Trudy vses. Inst. Gelmint. 11: 59.

James, M.T. and R.F. Harwood. 1969. Herm's Medial Entomology. 6th Edition. MacMillan Co., London.

Jamnback, H., J.D.M. Marr, B.B. Woddy, and L. Kartman. 1971. VI. Blackflies. WHO/VBC/ 71.238: 1-18.

Jones, C.M. and D.J. Richey. 1956. Biology of the black flies in Jasper County, South Carolina, and some relationships of Leucocytozoon disease in turkeys. J. Econ. Entomol. 49: 121-124.

Kartman, L. 1953. Factors influencing infection of the mosquito with Dirofilaria immitis (Leidy, 1856). Expl. Parasitol. 2: 27-78.

Kershaw, W.E., D.G. Jamison, D. Nugent and B.O.L. Duke. 1956. Preliminary observations on the depth distribution of O. volvulus in the skin and its relation to the reservoir of infection. Trans. Roy. Soc. Trop. Med. Hyg. 50: 6.

Kershaw, W.E., B.O.L. Duke and F.H. Budden. 1954. Distribution of the microfilariae of Onchocerca volvulus in the skin. Its relation to the skin changes and to the eye lesions and blindness. Brit. Med. J. 2: 724-729.

Khamis, Y., N. Helmy and L. Fahmy. 1973. Filariasis in buffaloes and cattle. Vet. Med. Rev. 4: 305-318.

Kolstrup, N. 1975. Onchocerca gutturosa in Danish cattle: prevalence, geographic distribution and host-vector relationships. Acta. Vet. Scand. 16: 1-13.

Laurence, B.R. 1966. Intake and migration of the microfilariae of Onchocerca volvulus in Simulium damnosum. J. Helminthol. 40: 337-342.

Lewis, D.J. 1957. Aspects of the structure, biology and study of Simulium damnosum. Trop. Med. Parasitol. 51: 340-358.

Loc, J.B., E.W. Cupp, E.I. Braide, and M.J. Bernardo. 1980. Aedes aegypti as a surrogate host for Onchocerca species. Am. J. Trop. Med. Hyg. 29: 382-388.

Lowrie, R.C. and M.L. Eberhard. 1980. A new technique for immobilizing filaria larvae using dimethyl sulfoxide (DMSO). J. Parasitol. 66: 169-170.

Mazzotti, L. 1951. Observations based on cutaneous biopsies in onchocerciasis. Am. J. Trop. Med. 31: 624-627.

Mellor, P.S. 1973. Studies on Onchocerca cervicalis Railliet and Henry, 1910. I. Onchocerca cervicalis in British horses. J. Helminthol. 47: 97-110.

Neumann, L.G. 1910. "Un nouveau nematode parasite du boeuf (Onchocerca gutturosa n. sp.). Rev. Vet. 67: 270-278.

Omar, M.S. and R. Garms. 1975. The fate and migration of microfilariae of a Guatemalan strain of Onchocerca volvulus in Simulium ochraceum and Simulium metallicum, and the role of the buccopharyngeal armature in the destruction of the microfilariae. Tropenmed. Parasitol. 26: 183-190.

Oothuman, P., M.C. Simpson, and B.R. Laurence. 1974. Abnormal development of a filarial worm, Brugia patei, in a mosquito host, Anopheles lambrachiae atroparvus. J. Helminthol. 48: 161-165.

Ottley, M.L. and D.E. Moorhouse. 1978. Bovine onchocerciasis: Aspects of carcass infection. Australian Vet. J. 54: 528-529.

Rabalais, F.C. 1974. Studies on Onchocerca cervicalis Railliet and Henry, 1910 microfilariae in the jird, Meriones unguiculatus. J. Helminthol. 48: 125-128.

Rabalais, F.C., D.C. Ashley and M.L. Eberhard, 1973. Bovine onchocerciasis in northwest Ohio. J. Am. Vet. Med. Assoc. 162: 839.

Ramirez-Perez, J.E. 1977. Estudio sobre la morfologia de Simulium metallicum, vector de la onchocercosis humana en Venezuela. Of. Sanit. Panam. Pub. Sci. No. 338, pp. 1-140.

Ramirez-Perez, J.E., E. Rassi, J. Convit and A. Ramirez. 1976. Importancia epidemiologica de los grupos de edad en las poblaciones de Simulium metallicum (Diptera: Simuliidae) en Venezuela. Bol. Of. Sanit. Panam. 80: 105-120.

Sanmartin, C., R. Mackenzie, H. Trapido, P. Barreto, C. Mullenax, E. Gutierrez and C. Lesmes. 1973. Encefalitis equina venzolana en Colombia, 1967. Bol. Of. Sanit. Panam. 64: 108-137.

Saravia, N.G. 1973. Feeding Patterns of Mosquito Species of Carmelo Determined by Gel Diffusion. Master of Science Thesis, Johns Hopkins Univ., Baltimore, Maryland. pp. 1-40.

Scholtens, R.G., S.R. Adams, and J.R. Broderson. 1977. Evidence of onchocerciasis in Georgia cattle: Prevalence at slaughter. Am. J. Vet. Res. 38: 1093-1097.

Schoop, G. and J. Lamina. 1959. Uber die vermizide wirkung von Neguvon aug Trichenella spiralis in experimental infizienten Mausen. Monatschrft f. Tierbeilkunde Bd. 11: 167-171. (Cited in Campbell, W.C. and A.C. Cuckler. 1964. J. Parasitol. 50: 481-488.

Service, M.W. 1977. Methods for sampling adult Simuliidae, with special reference to the Simulium damnosum complex. Trop. Pest Bull. 5: 1-48.

Shelley, A.J., R.R. Pinger, M.A.P. Moraes and J. Hayes. 1979. Concentration of microfilariae on Onchocerca volvulus by Simulium sanguineum during feeding: Use in mapping parasite distribution in the skin. J. Med. Entomol. 16: 48-51.

Steward, J.S. 1937. The occurrence of Onchocerca gutturosa (Neumann, 1910) in cattle in England, with an account of its life history and development in Simulium ornatum Mg. Parasitol. 29: 212-219.

Supperer, R. 1952. Uber das Vorkommen der filariae (sl.) Onchocerca gutturosa (Neuman, 1910) in Rinden in Osterreich and ihre Entwicklung in der Kriebelmuche Odagmia (Simulium ornata Mg.). Wien Tierarztl. Mschr. 39: 173-179.

Tidwell, M.A., M.A. Tidwell, 1979. Simulium exiguum, the vector of Onchocerca volvulus on the Rio Micay, Colombia. Am. J. Trop. Med. Hyg. 29: 377-381.

Tidwell, M.A., M.A. Tidwell, P.M. Hoyos, H. Corredor, P. Barreto. 1980. Studies on the factors of Onchocerca volvulus and Mansonella ozzardi in Colombia. Acta Med. Valle (Colombia) 12: (In press).

Thompson, B.H. 1976. The intervals between the bloodmeals of manbiting Simulium damnosum (Diptera: Simuliidae). Ann. Trop. Med. Hyg. 70: 329-341.

Trapido, H., A. D'Alessandro, and M.D. Little. 1971. Onchocerciasis in Colombia. Historical background and ecologic observations. Am. J. Trop. Med. Hyg. 20: 104-108.

Wanson, M. 1950. Contributions a la etude de le onchocercose africaine humaine (problemes de prophylaxie a Leopoldville). Ann. Soc. Belge Med. Trop. 30: 667-678.

Webber, W.A.F., G. Crip, A.I. Wright, and P. Williams. 1957. Records of Onchocerca found at postmortem examination of Welsh cattle. Trans. Roy. Soc. Trop. Med. Hyg. 51: 295-296.

Williams, C.B. 1962. Studies on black flies (Diptera: Simuliidae) taken in a light trap in Scotland. III. The relation of night activity and abundance to weather conditions. Trans. Roy. Entomol. London 114: 28-47.

Wilton, D.P. and R.C. Collins. 1977. A comparison of individual dissection and mass separation for recovery of Onchocerca larvae from vector black flies. Z. Tropenmed. Parasit. 28: 384-386.

Wilton, D.P. and R.C. Collins. 1978. An entomologic and parasitologic survey of onchocerciasis in Western S. Salvador. Am. J. Trop. Med. Hyg. 27: 499-502.

Vargas, L. 1945. Simulidos del Nuevo Mundo. Monogr. No. 1, Inst. Salud Enferm. Trop. Mex.

Venkataratnam, A. and W.E. Kershaw. 1961. Distribution of the microfilariae of Onchocerca in the skin of cattle. Ann. Soc. Belge Med. Trop. 4: 323-328.

Venkataratnam, A. and W.E. Kershaw. 1960. Distribution of the microfilariae of Onchocerca in the skin of cattle. Coll. International Filarioses, Ins. Trop. Med. (Prince Leopold), France, Ed. P.G. Janssens, pp. 87-91.

Voltava, C.L. and P.E. Thompson. 1973. Onchocerca lienalis of cattle in Georgia. J. Parasitol. 59: 938-939.

BIOGRAPHICAL SKETCH

Kirby O. Kloter was born on March 24, 1945 in Hartford, Connecticut. After receiving his high school education at the Gunnery in Washington, Connecticut he entered the University of Vermont in 1963 and received a B.A. degree in Zoology in 1967. He became a Peace Corps volunteer and served in Lesotho, Southern Africa as an inspector of schools and secondary school biology and mathematics teacher from 1967 to 1969. After termination, he entered the University of Connecticut Graduate School, Department of Biology and received a M.S. degree in Parasitology in 1972. In the same year he entered the Yale Medical School, Department of Epidemiology and Public Health and received a M.P.H. degree in Medical Entomology in 1974. He entered the School of Public Health and Tropical Medicine of Tulane University in 1975 and in the same year went to Colombia, South America to undertake parasitological studies connected with the program of the Tulane International Center for Medical Research (ICMR). In 1976 he took a leave of absence and served as a Research Associate at the Yale Laboratory of Epidemiology and Public Health and spent a short tenure connected with A.I.D. in Senegal, Africa as a Medical Zoologist. He returned to Tulane in 1977 to complete coursework and in 1978 went back to South America to carry out his dissertation project with ICMR in Cali, Colombia. At present he is a candidate for the Doctor of Science Degree in Parasitology from the Department of Tropical Medicine of Tulane University.