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IMPACT OF WASTEWATER IRRIGATION ON THE POPULATION OF
THE TREE HOLE BREEDING MOSQUITO, AEDES TRISERIATUS
(DIPTERA: CULICIDAE)

presented by

Morteza Zaim

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Entomology

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Major professor

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IMPACT OF WASTEWATER IRRIGATION ON THE POPULATION OF
THE TREE HOLE BREEDING MOSQUITO, *AEDES TRISERIATUS*
(DIPTERA: CULICIDAE)

By
Morteza Zaim

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ABSTRACT

IMPACT OF WASTEWATER IRRIGATION ON THE POPULATION OF
THE TREE HOLE BREEDING MOSQUITO, *Aedes triseriatus*
(DIPTERA: CULICIDAE)

By

Morteza Zaim

The impact of wastewater irrigation on the population of the tree hole breeding mosquito, *Aedes triseriatus*(Say), was studied. Unlike several other mosquito species which have been attracted to and established in this spray-irrigated woodlot, no appreciable number of *Ae. triseriatus* females were attracted from the neighboring woodlot.

The two year ovitrap collections at the Michigan State University Water Quality Management Project spray irrigation complex, as well as the human biting collections conducted in this area, revealed that there was significantly less mosquito activity in the sprayed zones compared to nonsprayed plots. Adult females moved out of the sprayed zones rather than changing their vertical distribution.

The reduction in air temperature and ovitrap water temperatures in sprayed plots as a result of spray irrigation operations were not great enough to be responsible, at least as a sole factor, for the observed behavioral differences in the activity of adult females. In addition, the sprayed water did not seem to have any appreciable repellency effect on the oviposition behavior of gravid females. It also supported the development of a relatively high percentage of larvae without much

Morteza Zaim

mortality. The habitat alteration, changes in food sources and their availability to female mosquito may be responsible, in part, for the observed behavioral differences.

To my parents

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INTRODUCTION

One of the major environmental questions is what to do with vast quantities of municipal wastewater. Conventional methods clean it up to some extent; but even after advanced treatments, the discharge of the remaining nutrients has caused lakes to eutrophy and waterways to be degraded. We are also becoming aware that another problem may be more important. People use many products that contain scarce and diminishing resources as elements or compounds that have a high energy demand for their manufacture. In an economy faced with both material and energy shortages, we can not condone manufacturing products with a high cost that are used once and then discarded; however this is exactly how we handle plant nutrients. We mine our increasingly limited supplies of phosphorus and use our scarce fossil energy to produce nitrogen fertilizers in order to grow food for the millions of people in our cities. Then these elements are dumped into the nearest stream. This gross mismanagement of our natural resources and the need for higher water quality have prompted an extensive search for alternative methods of removing the nutrients for reuse while making the renovated water acceptable for irrigation or other benefits.

The Michigan State University Water Quality Management Project is a pioneering effort in wastewater recycling. Through application of scientific understanding of aquatic and terrestrial ecosystems, the project strives to meet societal needs for wastewater disposal and

desires for clean water. The 2.3 million dollar facility includes a portion of East Lansing's sewage treatment plant, four and half miles of pipeline, four man made lakes, three marshes, and a spray irrigation area (Figure 1).

Although the wastewater effluent, which is piped from the East Lansing's secondary sewage treatment facility to the south end of MSU campus, meets public health standards, it is high in nitrogen and phosphorus compounds. These chemicals act as nutrients, fertilize natural water, and cause excessive growth of algae and aquatic plants. When these plants die and decay, they consume oxygen and thereby threaten many forms of aquatic life. In this project, the effluent material stays in the four interconnected ponds for about a month. Mature plants in these ponds take up large amounts of nitrogen and phosphorus, are then harvested and fed to animals. Dead algae and a variety of chemical compounds which settle to the bottom of the ponds are removed and used as fertilizer. This water, partially stripped of nutrients, is allowed to flow by gravity to the next pond in the chain and the same process is repeated. Thus water in the final pond is expected to be of high enough quality for sport fishing and other recreational uses. Also, water from the final pond or any combination of water from any of the four ponds may be applied to variety of field crops, and woodland environments. Nutrients remaining in the water will be absorbed by plants and soil, and water of drinking quality will eventually leach to the water table. In view of the massive water pollution problems of sewage origin that now exist in urban and suburban areas throughout the world, the need and practical values of the disposal techniques to be developed and evaluated in this project are self evident.

Figure 1. View of Water Quality Management Project area;
(A) Transmission line from East Lansing; (B)
Pump station; (C) Artificial channel to Herron
Creek; (D) Felton drain; (E) Lott Woodlot; (F)
Forage crop plots and; (G) Tree plantations.
(Courtesy of the Institute of Water Research,
Michigan State University).

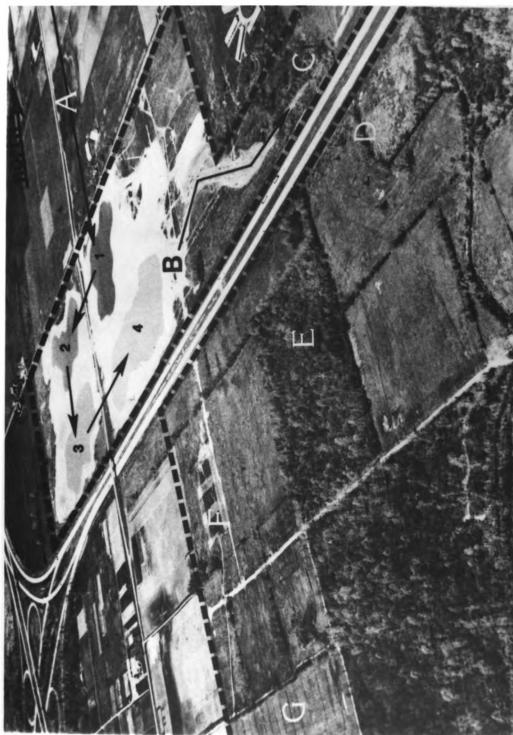


Figure 1

The spray irrigation in and around the wooded area, as the last step in removing undesirable chemicals from the water, however, may cause some potential health problems. Spraying in the wooded area will modify the water depth fluctuations in the vernal ponds, filling-up tree holes and artificial containers, as well as producing additional pockets of water, forming ideal breeding sites for certain mosquitoes. This may cause the excessive production of species that are of direct public health importance because of their ability to transmit disease organisms to man and his domestic animals. Involved, also, may be the excessive production of voracious species that cause serious annoyance and discomfort to man and other animals. This study was intended to evaluate the potential threat to public health created by such operations.

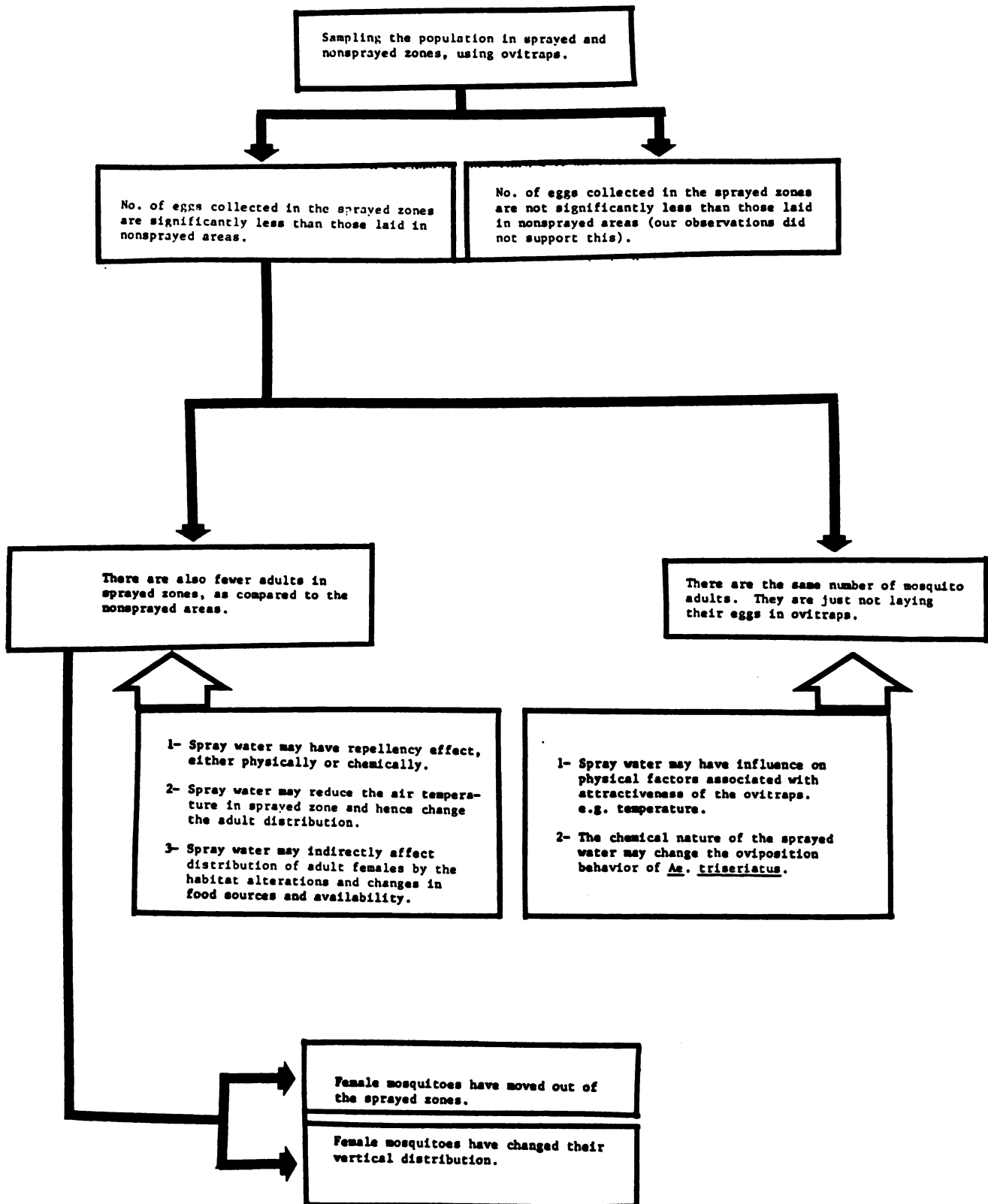
OBJECTIVES

The MSU Water Quality Management Project offers a possible solution to what is perhaps the most serious water pollution problem in the Great Lakes area. However, if such a sewage disposal system is to be accepted by the public for widespread use it not only must produce solutions to existing problems in the disposal of urban wastes, but it also must not create problems for the community in which it is located. Year-round spray irrigation operations in the wooded area may maintain water in tree holes and containers throughout the entire summer period and thus increase the breeding habitats of the indigenous mosquito, Aedes triseriatus (Say). If this should occur it could have important implications since this mosquito is a vector of several pathogens, as well as a potential pest. The attraction of Ae. triseriatus females from other areas into this spray irrigated woodlot may further complicate the problems.

The main objective of this research was to study the effect of the proposed irrigation project on the population of Ae. triseriatus in the project area. However, to better interpret the data generated in such a study a number of experiments were needed. The flow of thought in the development of this research project is shown in Figure 2. The direction of any specific experiment evolved as the results of the preceding experiments were obtained rather than all being designed at the outset.

Figure 2. Sequential development of experiments to determine the impact of sewage spray irrigation on the population of the tree hole breeding mosquito, Ae. triseriatus.

EXPERIMENTAL DESIGN



(Figure 2)

LITERATURE REVIEW

Mosquito problems associated with irrigation are of long standing and major concern in many areas of the United States. They date from the early part of the 18th century when residents of the South Atlantic seaboard recognized an association between the flood irrigation of rice fields and hyperendemic malaria (Hess et al., 1970). Although this was prior to knowledge of the role of Anopheles mosquitoes in transmission of malaria, citizens of Savannah, Georgia, early in the 19th century purchased easements for certain wetlands to prohibit rice culture for the stated purpose of malaria control (Henderson, 1952). Today, it is reported that mosquito production arising from irrigation-related sources varies from 75 to 90% of the total mosquito problem in many areas of the western states (Hess et al., 1970).

Sewage oxidation ponds are widely used and becoming increasingly popular for the disposal of liquid waste generated in urban communities. The mosquito breeding potential of sewage lagoons has been investigated by many workers. Such field studies have shown that the breeding of mosquitoes in waste disposal lagoons and/or oxidation ponds creates a potential danger to the inhabitants within a particular area (Eads and Menzies, 1956; Beadle and Harmston, 1958; Rapp, 1960; Beadle and Rowe, 1960; Rapp, 1961; Mykleburst, 1962; Rapp and Emil, 1965; Smith and Enns, 1967; Steelman et al., 1967; Smith, 1969a, b; Carestia et al., 1971; and Newson, 1974). An increase in the number of oxidation ponds and waste

lagoons along with recent reports of encephalitis has demonstrated the need for more detailed studies of the impact of such systems on public health and the need for adequate mosquito control measures.

Zorka (1975) obtained baseline data concerning the indigenous mosquito fauna at Lott Woodland, a beech (Fagus sp.)-maple (Acer sp.) climax forest area, used as one of the spray irrigation sites in the MSU Water Quality Management Project. He found that three species of major pest mosquitoes were present in large numbers before the irrigation became operational. These were: Aedes stimulans (Walker), Ae. vexans (Meigen), and Ae. triseriatus (Say). In that study he did not distinguish between Ae. hendersoni Cockerell, a species very similar to Ae. triseriatus, so data on the latter species are open to question because of the likelihood that some of the specimens collected were probably Ae. hendersoni. He also reported that Ae. stimulans occurred in large numbers in vernal water accumulations in a semipermanent wooded pond. Ae. vexans, however, was not found breeding in the project area but apparently migrated from an outside breeding site subjected to intermittent flooding during the summer months. Ae. stimulans is a univoltine species, the eggs of which hatch in March with adults emerging in mid-May. Although some published accounts state that this is a relatively short-lived species, in the project area as well as other parts of southern Michigan it persists in large numbers in wooded localities until late August or early September and avidly feeds upon anyone who intrudes into its habitat during day or early evening. Both Ae. stimulans and Ae. vexans are severe biting pests and during the preirrigation study at the project site they caused extreme discomfort to anyone who frequented the wooded area during the summer. In the evening, both species readily

leave their shade habitats and may fly relatively long distances in search of blood meal, so they undoubtedly would constitute a significant problem should any of the ponds in the project area be developed, as contemplated, for boating or other types of recreation.

The third species, Ae. triseriatus, is primarily a treehole breeder whose range includes the deciduous forests of the eastern United States and southern Canada (Zavortink, 1972). It is a known vector of the La Crosse strain of California encephalitis virus (Sudia et al., 1971), and is considered to be a potential vector of dog heartworm (Phillips, 1939 and Intermill, 1973), bird malaria (Huff, 1932) and eastern equine encephalomyelitis (Davis, 1940; Chamberlain and Sudia, 1961; and Whitfield et al., 1971). Previous studies on small mammals in the sewage spray irrigation area have indicated the presence of California encephalitis virus activity in at least three species of vertebrates: chipmunks, red and fox squirrels (Wildie, 1975).

Ae. triseriatus is a mosquito which has, until relatively recently, been neglected as a species of concern by medical entomologists. However, isolation of La Crosse virus from pools of field collected adult mosquitoes (Masterson et al., 1971; Sudia et al., 1971; and Thompson et al., 1972) and laboratory demonstration of its vector capacity by Watts et al. (1972, 1973a) has changed this situation. More recently there has been a large number of publications dealing with this vector-virus relationship.

Assessment has been made of the sylvan cycle of La Crosse virus transmission and amplification. Wright and DeFoliart (1970) demonstrated that both squirrels and chipmunks served as acceptable blood meal sources for Ae. triseriatus. Moulton and Thompson (1971) found high California

virus group antibody rates in chipmunks and squirrels in areas with Ae. triseriatus populations. Finally, the isolation of La Crosse virus from chipmunks and delineation of the amplification system of La Crosse virus were reported by Gauld et al. (1974, 1975) and Ksiazek and Yuill (1977).

Laboratory infection and subsequent transmission of La Crosse virus by Ae. triseriatus to suitable animal hosts was accomplished by Pantuwatana et al. (1972) and Watts et al. (1973a).

In 1974, Pantuwatana et al. reported isolation of La Crosse virus from field-collected Ae. triseriatus larvae. In the laboratory Watts et al. (1973b) demonstrated transovarial transmission of La Crosse virus by Ae. triseriatus females to their offspring. These two findings demonstrated the mechanism for the overwintering of La Crosse virus in the temperate zone, first reported by Watts et al. (1974) and confirmed by Berry et al. (1974) in Ohio, Balfour et al. (1975) in Minnesota, and Beaty and Thompson (1975, 1976) and Lisitza et al. (1977) in Wisconsin.

Additional research on the association between La Crosse virus and Ae. triseriatus has been reported. Most important among these are the reports of transovarial transmission through at least eight generations without appreciable loss of virulence (Miller et al., 1977), the use of fluorescent antibody techniques to determine the site and extent of viral infection within the mosquito body (Beaty and Thompson, 1975, 1976), and geographic variations in vector susceptibility and ability to transmit (Grimstad et al., 1977). There have even been reported the unusual infection routes of Ae. triseriatus by venereal transmission from males to females (Thompson and Beaty, 1977) and by ingestion of infected larval tissues by uninfected larvae (Miller et al., 1978), although the latter

may not be an important method of horizontal amplification of the virus (Miller et al., 1978).

Ae. triseriatus overwinters in the egg stage in Michigan although larvae may overwinter in more southern areas (Love and Whelchel, 1955). Gravid females exhibit a high degree of selectivity in oviposition site selection, but the relative importance of each of the various factors involved is not fully understood. Wilton (1968) examined factors influencing oviposition of Ae. triseriatus and found that the presence of decayed organic matter or a dark oviposition substrate consistently increased oviposition. Prior work by Williams (1962) has established that this species prefers a dark medium (water of high optical density) for oviposition.

Within recent years, it has become increasingly evident that chemical attractancy and stimulation are important components of the web of factors influencing the choice of oviposition sites by gravid females of Ae. triseriatus. Bentley et al. (1976) reported that larvae of Ae. triseriatus produce a substance that attracts and significantly enhances oviposition by females of this species. The importance of such larval-produced attractants were further confirmed by McDaniel et al. (1976).

Numerous studies have been published on the biology and ecology of mosquitoes that breed in tree holes. However, only a few investigators have considered the water chemistry of this unusual breeding site and its relation to gravid female attraction, egg hatching properties and larval development. Petersen and Chapman (1969) analyzed samples of water from cavities which were producing mosquitoes, including Ae. triseriatus, for pH, conductivity, carbonate, bicarbonate, potassium, sodium, calcium, magnesium, chloride, and sulfate. They concluded that

a definite relationship existed between the chemical makeup and the mosquito species present, however, they were not able to say whether those chemical differences were themselves responsible for the differences in the species breeding in such habitats. Perhaps, as they have mentioned, they were an indirect result of more important physical factors as has been true with Orthopodomyia californica and Ae. sierrensis in California (Chapman, 1964). However, both Petersen and Chapman (1969) and Petersen and Willis (1971) reported that Ae. triseriatus was generally restricted to habitats with low salinity.

Studies of the vertical distribution of mosquitoes have shown that the behavior and preference of individual species dictate specific vertical activity levels under natural conditions. Several workers (e.g. Corbet, 1961; Rachou et al., 1949) have demonstrated that ovipositional vertical distribution exists for some mosquito species. These species are primarily tropical tree hole or container breeders (Mattingly, 1969), and selection of oviposition sites may be determined more by the type of container than by a specific distance above ground.

The vertical oviposition of Ae. triseriatus has been studied by Loor and DeFoliart (1970), Sinsko and Grimstad (1977) and Scholl and DeFoliart (1977). Ae. triseriatus exhibited a strong preference for oviposition at ground level. It is not yet known if such preference is due to the chemical nature of the water, temperature, or the physical nature of the tree hole.

Studying the dynamics of mosquito populations and their biology is an essential part of epidemiologic investigations on arboviruses. Until 1970, the only significant report of the ecology of Ae. triseriatus in the field was a summary of casual observations by Jenkins and Carpenter

(1946). The dearth of information available from field studies was primarily due to the difficulty encountered in monitoring adults. They are not attracted to light traps, and thus were not included in light-trap survey results. Traps of any type, including animal-bait traps as employed by Wright and DeFoliart (1970) yielded low numbers of Ae. triseriatus. The dry-ice baited trap which DeFoliart and Morris (1967) used in their survey of hematophagous Diptera was equally inefficient for this species.

Ovitrap are considered a reliable tool in studying the population growth of container breeding mosquitoes (Evans and Bevier, 1969). They have been used extensively in the United States Aedes aegypti eradication program (Fay and Perry, 1965; Jakob and Bevier, 1969 a,b). These ovitraps consisted of a pint jar with the outer surface coated with a glossy black paint. Gravid females were attracted to the container and oviposited on a fiber-board paddle about 3/4 inch wide and five inches long, which was clipped inside the jar in a vertical position.

The reliability of ovitraps as a means of surveying Ae. triseriatus populations has been confirmed by Furlow and Young (1970). Beer cans with black muslin sleeves, a modification of Fay and Eliason's (1966) method, were also successfully used for detecting Ae. triseriatus populations by Loor and DeFoliart (1969).

In a preliminary study on feeding behavior, Benach et al. (1971) reported that Ae. triseriatus was a general feeder, taking blood from almost any animal (from amphibian to mammal) which was offered as a source of blood meal. This same pattern was observed previously by Wright and DeFoliart (1970). The feeding behavior was shown to be bimodal, with the highest intensity occurring before dusk (Loor and DeFoliart, 1970; Sinsko, 1976).

The ease with which a mosquito vector makes contact with a number of hosts, to provide opportunities for feeding, is significant in determining its effectiveness. Superior mobility aids in the rapid dissemination of disease over a wide area so that it does not remain limited and focal in nature. It has been generally believed that Ae. triseriatus has a limited flight range and usually does not leave the woodland area in which it occurs (Barr, 1958). However, no critical study has been done on the dispersal ability of this medically important species except those of Sinsko (1976) and Scholl (1978). Sinsko (1976) found no interchange between two woodlots in Indiana separated by 300 meters of open terrain. On the other hand, Scholl (1978) who did his studies in a woodlot, surrounded by cropland and 425 meters away from the nearest wooded area, noticed a considerable amount of movement into and out of that woodlot. Further studies need to be done to determine how the size of the woodlot influence movement of Ae. triseriatus populations within.

Ae. hendersoni is a sibling species, closely related to Ae. triseriatus. It was resurrected to species level by Breland (1960). Until that time, because of the close morphological and ecological similarity between Ae. hendersoni and Ae. triseriatus, Ae. hendersoni was regarded as a variety of Ae. triseriatus (Hedeen, 1963). The importance of Ae. hendersoni to studies of Ae. triseriatus can be summarized briefly as follows. According to Zavortink (1972), the geographical distribution of both species overlap through most of the United States east of 100° longitude (Figure 3). This distribution, plus close similarities in adult morphology and breeding habitats introduces the possibility of misidentification while working with field populations. In addition, Truman and Craig (1968) showed that the two species could be hybridized

Figure 3. Distribution of Ae. triseriatus (A) and Ae. hendersoni (B) in the United States. (shaded areas represent states from which these have been collected).

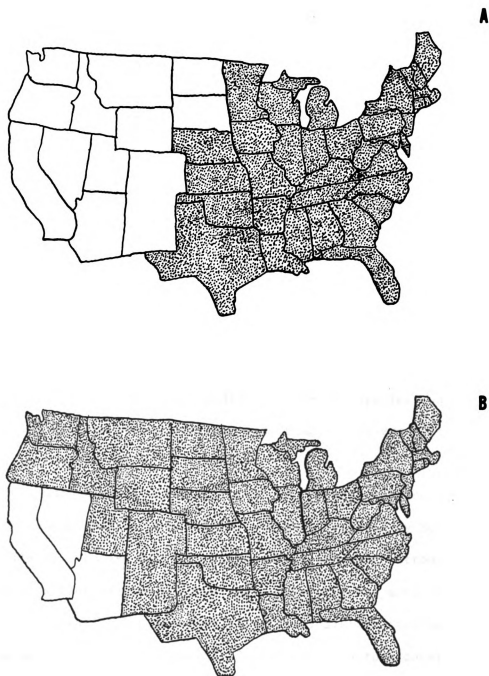


Figure 3

under laboratory conditions. The possibility that hybridization may occur in the field further complicates efforts at identification (Grimstad et al., 1974).

Final implication of Ae. triseriatus as the vector of La Crosse virus by Watts et al. (1972, 1973 a,b) was made through laboratory transmission of the virus. Watts et al. (1975) gave evidence that Ae. hendersoni is incapable of transmitting this virus. Thus, the treatment of the two species as a single taxonomic entity would give misleading results.

Grimstad et al. (1974) made an intensive characterization of diagnostic features of the larvae and adults of both species plus the hybrids in Wisconsin. A total of at least seven, and probably as many as eleven, morphological traits were later reported by Lunt (1977) for distinguishing the larvae of the two species in Nebraska. Harmston (1969) had previously shown differences in the tarsal claw between Ae. hendersoni and Ae. triseriatus. Finally, identification of the two species, using polyacrylamide gel electrophoresis for detecting the isozyme patterns, has been reported by Saul et al. (1977).

Vertical and temporal distribution of Ae. triseriatus and Ae. hendersoni, as measured by oviposition, has been reported by Scholl and DeFoliart (1977). Unlike Ae. triseriatus which exhibited strong preference for oviposition at ground level, Ae. hendersoni was more arboreal. Such habitat separation by differential vertical distribution has also been shown by Sinsko and Grimstad (1977), however, factors causing such strong preference remain to be investigated.

In an attempt to see what factors possibly limit the western distribution of Ae. triseriatus, and what stages of the mosquito are affected

by these factors, Lunt and Peters (1976) studied the influence of a number of ecological conditions on the distribution of these two sibling species, from western Iowa across Nebraska into eastern Wyoming and eastern Colorado. Nebraska is one of the states in which the tree hole mosquito Ae. triseriatus reaches its western limit; it is found in the eastern half of the state. Ae. hendersoni, however, is found throughout the state. They concluded that the average annual precipitation appeared to be important in the case of Ae. triseriatus. Generally, they found this species to be predominant where the average annual precipitation exceeded 50 cm per year. However, Ae. hendersoni was predominant where the precipitation was near 50 cm per year and it completely replaced Ae. triseriatus where the precipitation dropped below that amount.

MATERIALS AND METHODS

Laboratory colony maintenance

The mosquitoes used for the laboratory experiments were obtained from the eggs laid by wild females in ovitraps placed in two woodlots on the Michigan State University campus, East Lansing, Michigan. During the summer the mosquitoes were hatched from field-collected eggs. During the rest of the year, experiments utilized mosquitoes from a laboratory colony established during the late summer. A maximum of four to five generations were used from this colony before it was discarded.

Colonies were maintained at 24° C and 70% relative humidity. There was a 16-hour light period which included a half hour crepuscular period at the beginning and at the end of the 16-hour photophase. An eight-hour dark period followed the evening crepuscular period. Adult females were fed on guinea pigs. A ten percent sucrose solution was available to adults at all times and was replaced at least once a week.

Eggs were collected in black-painted beakers lined with strips of brown paper toweling and filled about 1/3 full of water. Oviposition beakers were placed in the colony cages on the sixth day after a blood feeding. When removed from a cage the beaker was sealed with plastic wrap. The sealed beaker provided a water-saturated atmosphere in which the eggs were kept for at least seven days to permit the completion of embryonation. The eggs were then stored on moist substrate in the insectary until needed.

Eggs were hatched at room temperature by submerging strips of towel-
ing with their attached eggs in beakers containing a Bacto-autolyzed yeast
nutrient broth (Difco Laboratories, Detroit) solution of .33 g/1000 ml
of tap water. The first instar larvae were transferred to white-enamel
pans, each pan containing 2000 ml of tap water and approximately 200
larvae. Larvae were fed on a diet consisting of beef blood, bacto-liver,
and autolyzed yeast (1:1:1), by adding 75 mg per pan every other day.

Population sampling and the effect of spray irrigation on the oviposition
behavior of *Ae. triseriatus*

Lott Woodland is a beech-maple climax forest of about 45 acres lo-
cated at the south end of the Michigan State University campus (T. 3N.,
R. 1W., sec. 6). It is connected to a big woodlot extending along
Sycamore Creek (Fig. 4). Population samplings of *Ae. triseriatus* were
conducted, using ovitraps consisting of quart jars, with the outer sur-
face coated with black paint. A tongue depressor covered with brown
paper toweling clipped inside each jar in a vertical position served as
an "oviposition board". Electric tape and a wooden stick were used to
keep each jar firmly on the ground (Figures 5 and 6).

Five separate oviposition study plots were established and in each
plot 24 ovitraps were placed in a grid pattern at about 20 m intervals
(Fig. 7). Plot B received a total of five cm of municipal wastewater
and plot E received a total of ten cm, each week, in two applications.
At each sampling trial 200 ml of water, previously kept for a week in
buckets containing leaves, were added to each jar and oviposition boards
were left in each jar for about 48 hours. All samplings were made when
no sprayers were in operation.

Figure 4. Map showing the location of Lott Woodland area.

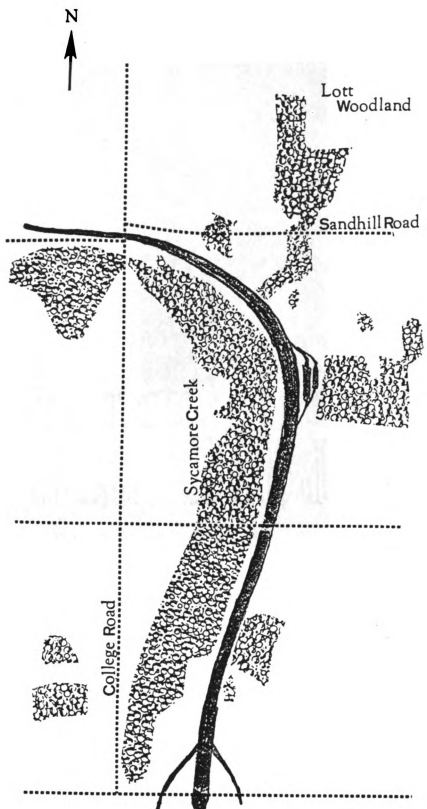


Figure 4

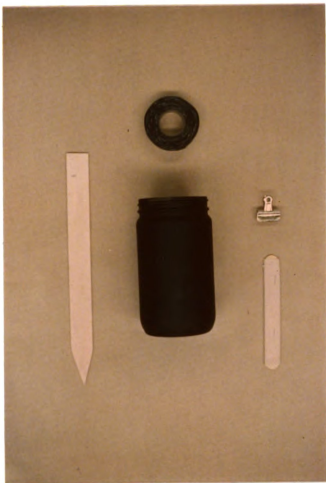


Figure 5. Equipment used for making an ovitrap



Figure 6. An ovitrap used for sampling eggs in 1976 and 1977.

Figure 7. Arrangement of ovitraps in the study plots.
Zone A, F, and G are the nonsprayed plots.
Zone B received total of 5 cm of municipal
wastewater, each week, in 2 applications.
Zone E received total of 10 cm of sprayed water,
each week, in 2 applications.

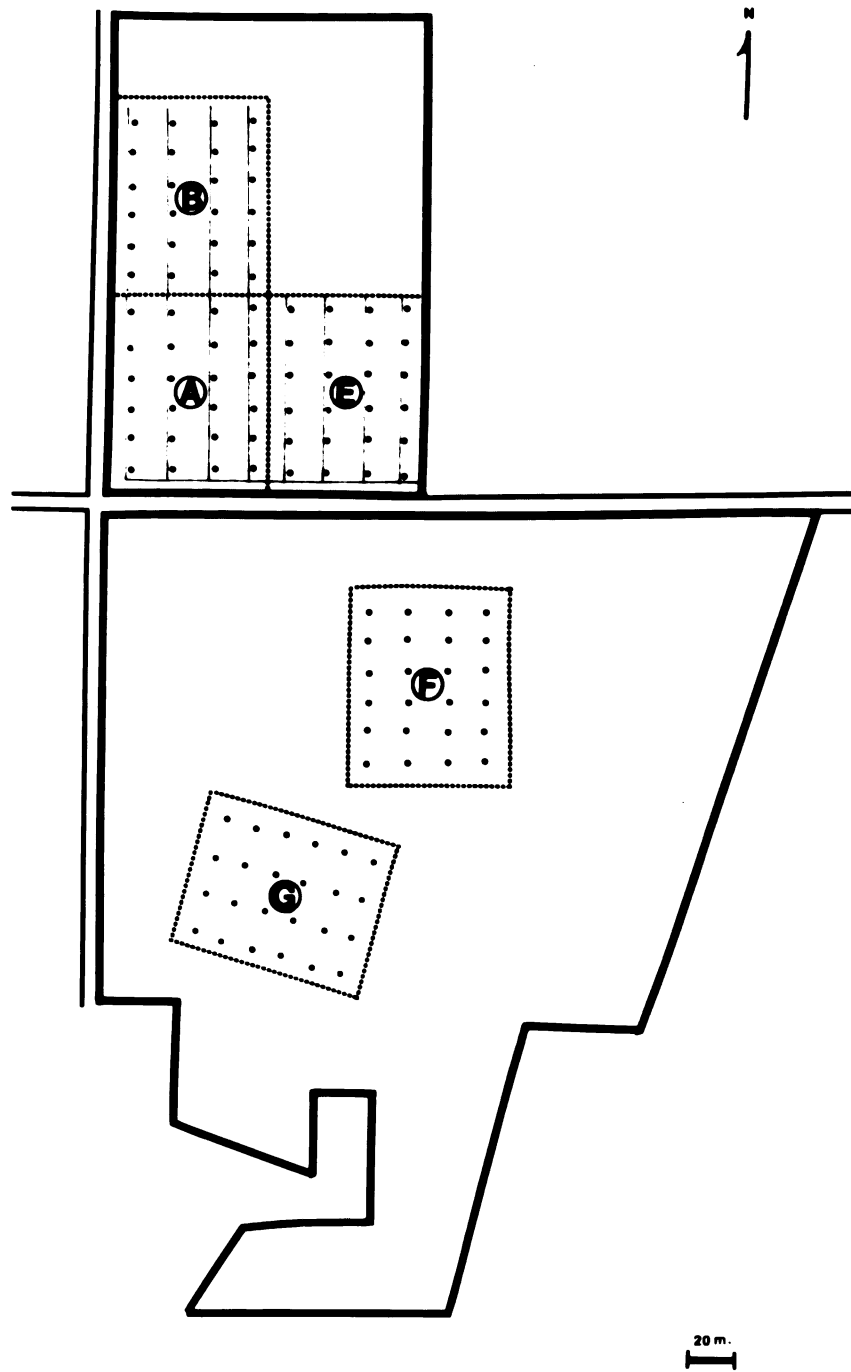


Figure 7

The weekly sampling and identification of eggs were conducted from June 25 to September 1, in 1976, and from May 1 to October 7-9, in 1977. Eggs laid on the oviposition boards were brought to the laboratory. They were kept in water-saturated atmosphere for at least seven days, to permit the completion of embryonation, after which they were hatched. Larvae were identified to species in the 4th instar. Also, as a double check, eggs were later removed from oviposition boards and were cleared and identified, using the technique described in Appendix II.

During the summers of 1976 and 1977, the total number of eggs collected during each week, in each plot, were tabulated. Analysis of variance (randomized complete-block design) was performed to determine the possible treatment effect of spray irrigation on the oviposition behavior of this mosquito species.

Adult activity-human biting collections

Human biting collections were made during the months of June, July and August of 1978, at different times of the day, to investigate the possible effects of the spray irrigation on the adult female population of Ae. triseriatus. Collections were made in zones A (a nonsprayed plot) and E (the plot receiving the highest amount of spray water per week). In each zone, collections were made at three stations, for ten minutes each. Figure 8 shows the position of the six collecting stations in the project site. Collections were made by having a colleague and myself aspirate mosquitoes from each others bodies. At the end of the collections in each zone, we would move to an open area and then into the other zone, from the other side, so that the possibility of taking mosquitoes with us to the new location would be minimized. The zone to be sampled first was chosen randomly each time.

Figure 8. Human biting collection stations in the non-sprayed zone A (A_1 , A_2 , A_3) and most heavily sprayed zone E (E_1 , E_2 , E_3), in 1978.

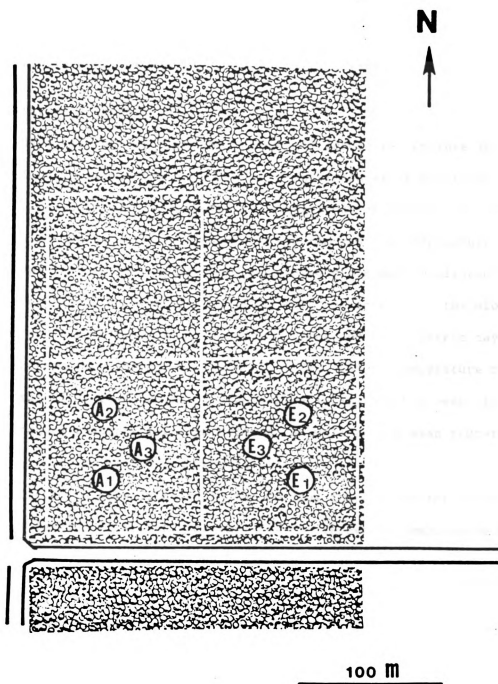


Figure 8

The collected mosquitoes from each zone were put in separate containers and were brought to the laboratory for identification. Since each biting collection lasted for one hour, it was assumed that the difference in time would not have a significant effect on the collection, and results could be analyzed, using paired t-test.

Temperature effects on adult activities

Overhead spray irrigation is known to reduce temperature and might therefore influence the selection of oviposition sites by gravid females of Ae. triseriatus. To determine the magnitude of temperature reduction and obtain data for later laboratory experiments the temperature of the ovitraps in both the sprayed and nonsprayed plots were monitored. Water temperatures of ten randomly selected ovitraps in each of the blocks A, B, and E were measured at different times and on different days, using telethermometers (Arthur H. Thomas Co., Pa., USA). Temperature recordings were made from 24 to 86 hours after spray irrigation had been stopped. Temperature readings for each ovitrap, as well as the mean temperatures for ovitraps in each block were tabulated.

Little is known concerning the optimum temperature for ovipositing gravid females of Ae. triseriatus, and the threshold temperature below which they will not attempt to lay their eggs. In order to test the relation between temperature and oviposition activity, the following experiment was conducted. A hole 7.6 cm in diameter was made in the bottom of a 60 x 60 x 60 cm mosquito cage. A cylinder, 20 cm long, painted black, was passed through the hole halfway, so that it could be partially submerged in an aquarium (Fig. 9). The temperature of the water in this aquarium was controlled, using a heating element. Lower

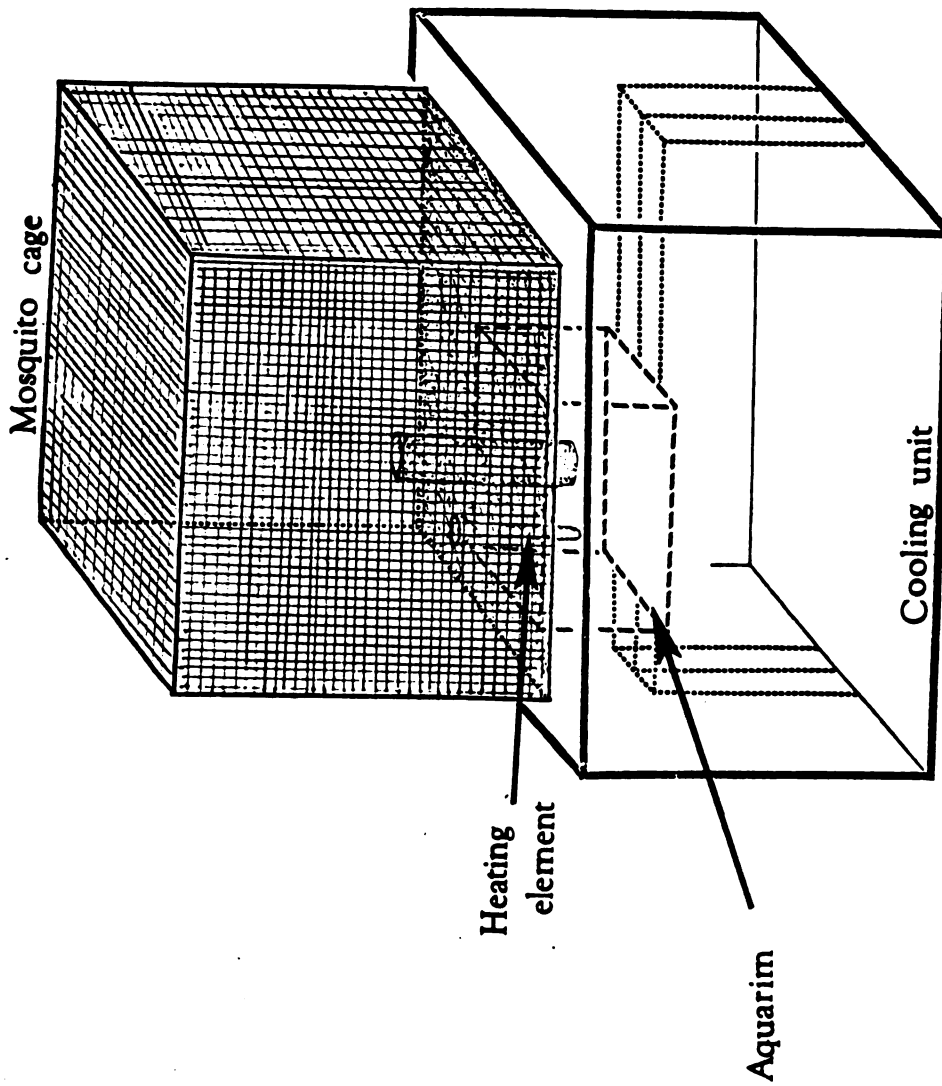


Figure 9. Equipment used for testing the oviposition activity in relation to water temperature.

temperature settings were achieved by placing the aquarium in a cooling unit. Fifteen different water temperature settings, ranging between 15.6° and 27.2° C were used in this experiment. The room temperature and humidity were set at 22.2° C and 50% relative humidity, respectively. Ten 10 to 15 day-old females were fed on a guinea pig and were released into this cage seven days later. Eggs were collected on a brown paper toweling lining the inside of the cylinder. The experiment was run only for 24 hours after which the paper toweling was removed and the number of eggs laid were counted. The total number of eggs laid at each trial was recorded. A linear regression analysis was performed on the data.

In addition, to investigate the possibility that spray irrigation lowered the air temperature, either driving mosquitoes out of the sprayed plots, or changing their vertical distribution, the air temperature was monitored both in the sprayed and nonsprayed plots. Hygrothermographs were installed in the two sprayed zones (Plots B and E) and a nonsprayed zone (Plot A) at a height of 1.5 m. The recorded maximum and minimum temperatures of each plot, for each day, were tabulated and correlated with the results of the ovitrap population studies previously described.

Oviposition behavior as affected by spray irrigation

1- Attractiveness of sewage water of varying "ages" to gravid females.

Year-round spray irrigation in the wooded area may maintain water in tree holes and containers throughout the summer period and thus increase the potential breeding habitats of Ae. triseriatus. The following laboratory experiment was designed to determine the relative attractiveness of sprayed water of varying "ages" as oviposition sites for gravid females of Ae. triseriatus.

Female mosquitoes were fed on a guinea pig and were held in a 60 x 60 x 60 cm mosquito cage. Homogenous light was obtained in the cage by covering it with a piece of white cloth and providing an overhead light source (75 watt incandescent light bulb). At each trial, three black jars, each provided with an oviposition board were placed, in a random manner, inside the cage, 40 cm apart from one another. Two hundred ml of one-day old sewage water, 5-day old sewage water, and deionized water (as control) were added to jars 1, 2 and 3, respectively. Sewage water for this experiment was obtained by collecting it directly from spray nozzles at the Water Quality Management Project site. Five-day old sewage water was obtained by holding the collected water in the laboratory prior to each test. Each test trial was run for approximately 30 hours. The percentage of eggs laid in each type of water, at each trial, was tabulated. Results were analyzed, using analysis of variance (randomized complete-block design).

2- Oviposition behavior in relation to calcium nitrate and sodium chloride in water. The results of the routine chemical analysis of the sprayed water revealed high contents of sodium chloride and calcium nitrate. Previous reports by Petersen and Chapman (1969) and Petersen and Willis (1971) concerning the fact that Ae. triseriatus is generally restricted to habitats with lower salinity were the primary reasons for an investigation to determine how such chemicals may affect the oviposition behavior of Ae. triseriatus females. A factorial experiment was designed to study such relationships, as well as the interaction of the two chemicals. Nine 150 ml beakers, painted black and lined with brown paper toweling, were placed randomly in a grid pattern, about eight

inches apart, on the bottom of a 60 x 60 x 60 cm mosquito cage. Three concentrations of sodium chloride (2.00, 1.00, and 0 g/l) and three concentrations of calcium nitrate (5.00, 2.50, and 0 g/l) were used. A wider range of concentrations of salts, than has been reported in the wastewater was chosen for better understanding of the mosquito response to these two salts. One hundred ml of each of the nine different salt combinations were added to 250 ml beakers. The cage was surrounded by a white sheet of cloth, and an overhead light (fluorescent) was used to provide homogeneous light. Twenty five 10-15 day-old females were released in the cage with freedom to select a combination of salts for oviposition. For each trial, the experiment was run approximately 24 hours, after which the beakers were removed and eggs were counted. Results were analyzed, using analysis of variance (randomized complete-block design).

Larval development and sprayed water

In order to determine if the chemical nature of the sewage water would support larval development, the following experiment was conducted. Two thousand ml of the treated sewage water, directly collected from spray nozzles in the field, were placed in larval rearing pans and 200 newly hatched Ae. triseriatus larvae were added to each pan. The same number of larvae were reared in pans containing the same amount of tap water as the control. The food regimen was the same for both treatments, and is given in the section relating to colony maintenance. Larvae were maintained in the insectary, and larval mortality was obtained by comparing the number of pupae developed in each pan with the number of introduced larvae. Survival rates were tabulated and analyzed, using Student's t-test.

The results of the routine chemical analysis of the sprayed water by Dr. Burton of the Institute of Water Research, including the total amounts of nitrate, ammonium, nitrite, organic nitrogen, phosphorus and chloride, has shown that the sprayed sewage water has a high content of sodium chloride and calcium nitrate. In order to determine if larval mortality can be related to different concentrations of chloride and nitrogen in the water, a factorial experiment was designed to study the effect of each salt as well as their interactions on larval survival. Seven concentrations of sodium chloride (4.00, 2.00, 1.00, 0.50, 0.125, 0.063 and 0.031 g/l) and six different concentrations of calcium nitrate (0.250, 0.125, 0.063, 0.031, 0.016 and 0.008 g/l) were used for this experiment. A wider range of concentrations of salts, than has been reported in the wastewater, were chosen for better understanding of the larval response to these two chemicals. Two hundred ml of each of the 42 different salt combinations were dispensed in 250 ml beakers. Ten newly hatched larvae were placed in each beaker and were fed on standard diet consisting of beef blood, Bacto-liver and autolyzed yeast (Difco Laboratories, Detroit) (1:1:1). Mortality was tabulated for each treatment combination, and results were analyzed, using analysis of variance (randomized complete-block design).

Vertical distribution studies

1- Use of rotary traps. Rotary traps have been used by many mosquito ecologists for sampling mosquito populations (Williams and Milne, 1935; Stage and Chamberlin, 1945; Snow and Pickard, 1957; Love and Smith, 1957; Provost, 1952; and Nicholls, 1960). Such a device would not only enable continuous sampling in the absence of the operator which is

especially important where insects such as mosquitoes are attracted to humans, but quantitative data are also provided as the volume of air sampled can be calculated from the area of the opening of the net and the rate of travel.

In order to obtain an additional comparison of possible behavioral differences in the adult population of Ae. triseriatus (e.g. change in vertical distribution) due to the spray irrigation, two rotary traps were constructed. They were located in the center of the plots A (a non-sprayed plot) and E (the plot receiving the highest amount of wastewater per week), sampling adult mosquitoes at 0.3 and 2.4 m high. They were run at different times of the day for the total of 17 hours.

The rotary traps were constructed, incorporating some of the principles described by Williams and Milne (1935) and Nicholls (1960). Each trap consisted basically of two conical nets, one at 0.3 m and the other at 2.4 m high. The nets rotated in a horizontal plane around a central axis at 32 rounds per minute. The traps were driven by an electric motor.

The framework of each trap was made mainly from 1.9 cm steel tubing. The arms (A), each 1.2 m long were welded to a steel sprocket (S) which in itself was welded to the central axis (CA). Separate tubes (T), 1.32 m long, were used to support the two arms. They were also welded to the steel sprocket (Fig. 10).

The conical nets, 71 cm long and 43.2 cm in diameter at the mouth, were of 64 mesh nylon material reinforced at each end. The apex of each net was glued to a plastic funnel which had a 1.3 cm diameter tube leading into a removable 453.6 g polyethylene bottle. A hole was made in the screw cap of the bottle so that the funnel's tube could pass through. The funnel was then glued to the cap. A hole 3.8 cm in

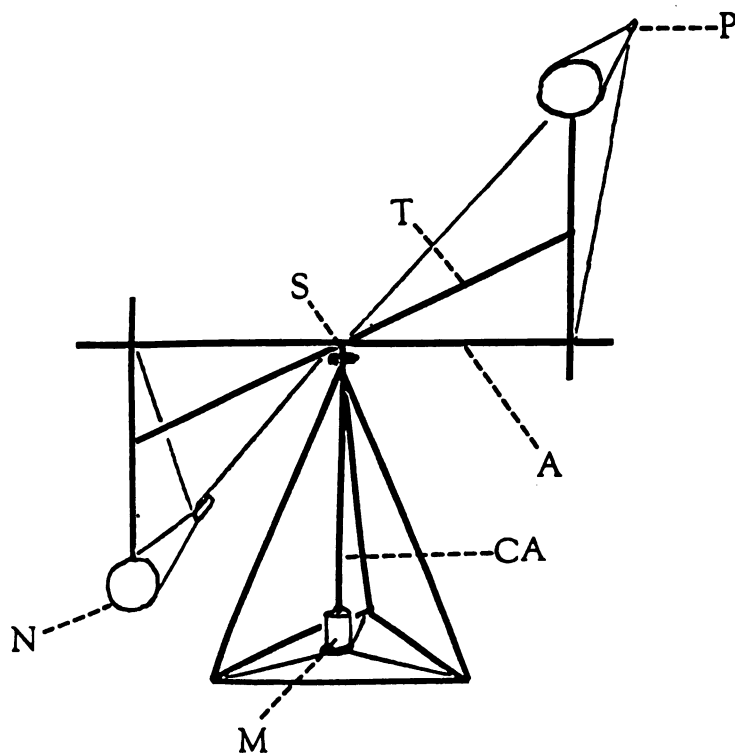


Figure 10. Diagram of rotary trap showing framework assembly. (A) arm; (CA) central axis; (M) motor; (N) collecting net; (P) polyethelene bottle; (S) the steel sprocket; (T) supporting tube.

diameter was cut in the bottom of each bottle and was covered with a piece of 32-mesh screen (Fig. 11).

The driving mechanism of each trap consisted of a 1/4 horsepower, 30 r.p.m., electric motor. A 3000 watt gasoline powered generator was used to run the motors in the field.

2- Use of ovitraps. Studies were made to determine whether or not the spray irrigation of wastewater may repel the mosquitoes and cause them to move out of the sprayed area or change their vertical distribution. To determine if the spray irrigation changed the vertical distribution of Ae. triseriatus in the project site, seven beech trees, 25.4 cm in diameter at belt line, which did not have natural water containing cavities, were chosen in the center of plots A, B, and E. In 1977 a set of beer can oviposition traps, each painted black inside and also outside, were arranged on each of the trees, one at ground level, and one at 5 m (Figures 12 and 13). Upper traps were raised into position by means of ropes and pulleys and then were brought into juxtaposition of the tree trunks. Cans at the basal level were situated beside the tree trunks, on a metal stick, less than 0.3 m above ground level. All ovitraps were facing the same direction to reduce the variation between experimental units. In the summer of 1978, however, 5 more trap sites were established in each zone, bringing the total number of traps, at each level to 12.

At each sampling trial, 150 ml of tap water, kept for a week in the buckets supplied with leaves, were added to each trap. An oviposition board was left in each trap for approximately 48 hours. The boards were then brought back to the laboratory where the eggs were identified and counted. The total number of Ae. triseriatus eggs collected at each

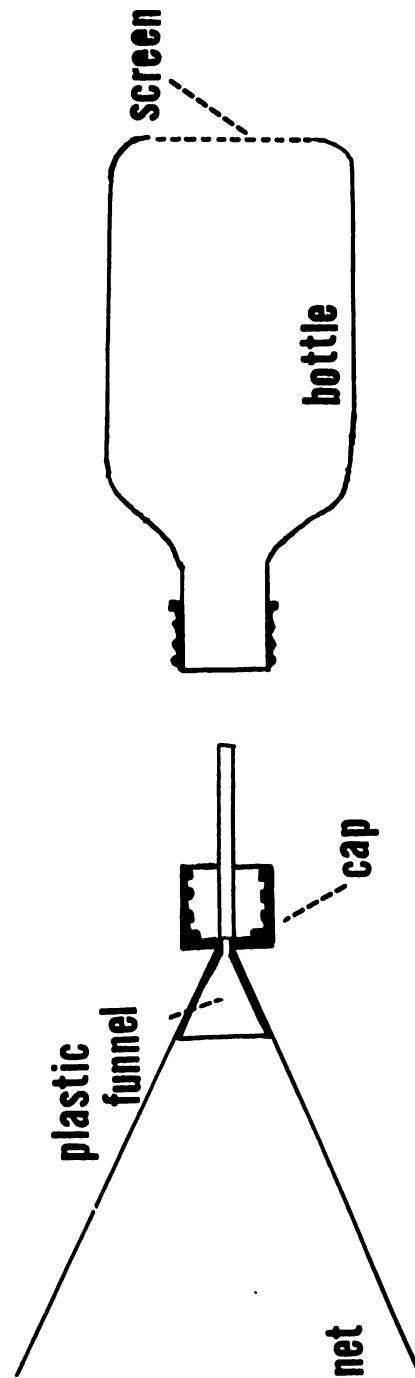


Figure 11. Sectional drawing of collecting bottle.



Figure 12. Pop-can type ovitrap placed at ground level.



Figure 13. Pop-can type ovitrap placed at
5 meter above the ground.

trial, at two different heights, were tabulated. The analysis of variance, Friedman's test, was performed.

Dispersal studies on *Ae. triseriatus*

Year-round spray irrigation in the wooded area of the Water Quality Management Project site may not only increase population of the indigenous mosquito, *Ae. triseriatus*, by increasing its breeding habitats, it may also attract females from a connecting woodlot extending along Sycamore Creek. The latter is a big woodlot separated by a cleared area of approximately 76 m from the project site, by Interstate Highway 127 (Fig. 14).

Many researchers working on *Ae. triseriatus* believe this species has a short flight range and usually does not leave the woodlot it inhabits. In his dissertation research at Notre Dame, Sinsko (1976) found no interchange between woodlots separated by 300 meters of open terrain. However, Scholl (1978) found a considerable amount of movement between two woodlots 425 meters apart in Wisconsin.

In order to see if any movement occurs from the Sycamore Creek woodlot to the project site, a total of 10,445 seven to nine day old fertilized females, were marked with a fluorescent powder, Rhodamine-B (Allied Chemical Co., New York) and were released on three different occasions at site R (Fig. 14) south of Interstate Highway 127 as follows:

<u>Date (1977)</u>	<u>Number released</u>
July 13	4800
August 4	3286
August 12	<u>2359</u>
Total =	10445

Figure 14. Location of release and the human biting collection sites for the mark-release-recapture studies of Ae. triseriatus.

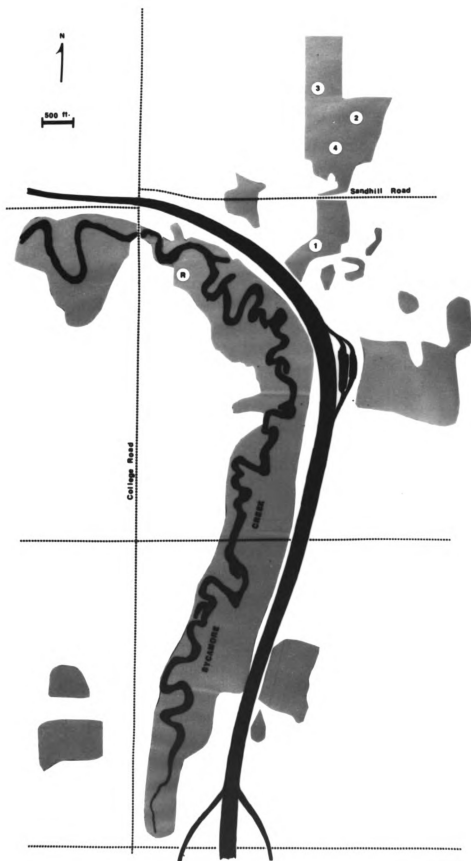


Figure 14

The females used for marking were obtained from a laboratory colony established from eggs collected in the field. For the marking procedure, small cardboard boxes (90 mm in diameter and 90 mm in height) were used. A small quantity (about 1 g) of the Rhodamine-B gum arabic dust mixture was placed on the bottom of each box and the mosquitoes introduced after being slightly anesthetized in a stream of CO₂. Rhodamine-B was mixed with gum arabic in the ratio of 1:3 and to this mixture 99% alcohol was added to form a fine paste. The paste was dried in an oven at 80° C and was then pulverized in a mortar before being placed in boxes. Mosquitoes were marked by gently shaking and rotating the box. The marking box was opened and placed in a larger cage (45 x 45 x 45 cm) and kept there until all the mosquitoes had recovered and flown into the cage. The larger cage was kept in the insectary with 70% humidity where the dyed gum arabic particles absorbed moisture and adhered to the mosquitoes. Mosquitoes were taken to field the next morning and were released at 6:30 A.M. by opening the cage and allowing females to fly out voluntarily. Biting collections were later made at four locations, as marked in Figure 14. This involved a fellow student and myself spending 30 minutes at site 1 and 20 minutes at sites 2, 3, and 4, aspirating mosquitoes from each others bodies. Collections were made between 4:00 and 6:00 P.M. Collecting was enhanced by placing a pan containing dry ice close to where we were standing. Biting collections were also made at the release site, on two different occasions, each time for 30 minutes, to determine if marked mosquitoes were still present after several major rainfalls had occurred in this area following the mosquito release.

Captured mosquitoes from each site were brought back to the laboratory in separate containers where they were sorted out to species. Ae.

triseriatus females were killed and placed more than 20 mm apart on a sheet of filter paper. A drop of alcohol was applied to each specimen. The pink-colored patch appearing around the mosquito immediately after the alcohol was applied was the indication of marked specimens (Fig. 15).

In the St. Louis encephalitis surveillance program, conducted by H. D. Newson and his co-workers (personal communication), around the Detroit area during the summer of 1976, larvae of Ae. triseriatus were occasionally found in automobile tires up to 68 m away from the closest woodlot. An investigation was undertaken at the project site to determine if gravid females of Ae. triseriatus actually leave the woodlot in search of oviposition sites. A set of beer can oviposition traps, each painted black inside and outside, were arranged on 30 bamboo sticks, at 1.2 m above the ground. Black electric tape was used to hold each can to a bamboo stick (Fig. 16). Fifteen mounted ovitraps were placed on the west side and 15 on the east side of the woodlot, 10 meters away from the wooded area and spaced 10 meters apart. They were kept approximately 1/3 filled with tap water, kept for a week in the buckets supplied with leaves, during the summer months. Oviposition boards were replaced every week, after which they were brought back to the laboratory and were checked for the presence of Ae. triseriatus eggs.



Figure 15. Detection of mosquitoes marked by Rhodamine-B-gum arabic mixture after application of alcohol.



Figure 16. An ovitrap used to study movement of females out of the woodlot.

RESULTS AND DISCUSSION

Results of the ovitrap collections in the sprayed as well as the nonsprayed plots, for summers of 1976 and 1977 are given in Appendices IV and V and are summarized in Tables 1 and 2, respectively. During the ten week sampling program in 1976, the main oviposition peak was observed in the third week of this study (June 9-11). The second peak was noticed in the 7th week (August 6-8), about a week and half after the major rainfall occurred in that area. Egg deposition increased between the 8th and 10th week (August 15-29) of that year, and at the end of August, in a 48 hour sampling period alone, 3000 eggs were collected in this woodlot (Fig. 17). Although there was a major rainfall in this area on July 31st and a part of the increase in the total number of eggs collected in the month of August may have been due to the emergence of young adults, most of the tree holes in this woodlot and other woodlots in East Lansing were completely dry by the end of the first week of August. In view of this there may be other explanations for the marked increase in Ae. triseriatus oviposition activity in that 48 hour sampling period. It may have been simply that gravid females were attracted to the only available oviposition sites present in the area, or, it could have been that Ae. triseriatus females were attracted from other dry areas into this spray irrigated woodlot. If it were the latter, it would have important implications in assessing vector potential of this mosquito species and this possibility was thought to warrant further investigation. As a result,

- Table 1 -
Aedes triseriatus oviposition activity in 1976
 (total no. of eggs per week)

Zones*	Dates											
	June				July				August			
	25-27	2-4	9-11	16-18	23-25	30-1	6-8	15-17	20-22	27-29		
A	255	335	529	211	214	299	234	263	691	1367		
B	103	62	309	189	310	157	0	218	278	114		
E	0	53	131	0	0	129	116	0	5	0		
F	293	200	1023	98	337	713	55	426	743	804		
G	155	0	567	187	113	187	49	837	497	682		

* Zones B and E are the sprayed and zones A, F, and G are the nonsprayed plots.

- Table 2 -
Aedes triseriatus oviposition activity in 1977
 (total no. of eggs per week)

Zones	Dates													
	June				July				August				Sept.	
	8-10	15-17	22-24	29-1	9-11	13-15	20-22	27-29	3-5	10-12	17-19	24-26	31-2	
A	118	1289	3078	305	322	402	261	279	457	0	49	78	19	
B	0	68	980	0	48	95	86	46	43	0	0	6	5	
E	0	519	1633	61	0	135	0	286	138	0	39	0	55	
F	0	178	970	369	298	228	450	288	109	78	186	103	6	
G	0	216	725	488	112	275	367	607	218	106	122	108	45	

* Zones B and E are the sprayed and zones A, F, and G are the nonsprayed plots.

Figure 17. 1976 Ae. triseriatus egg collections in the project site, correlated with daily maximum and minimum temperatures and rainfall data.

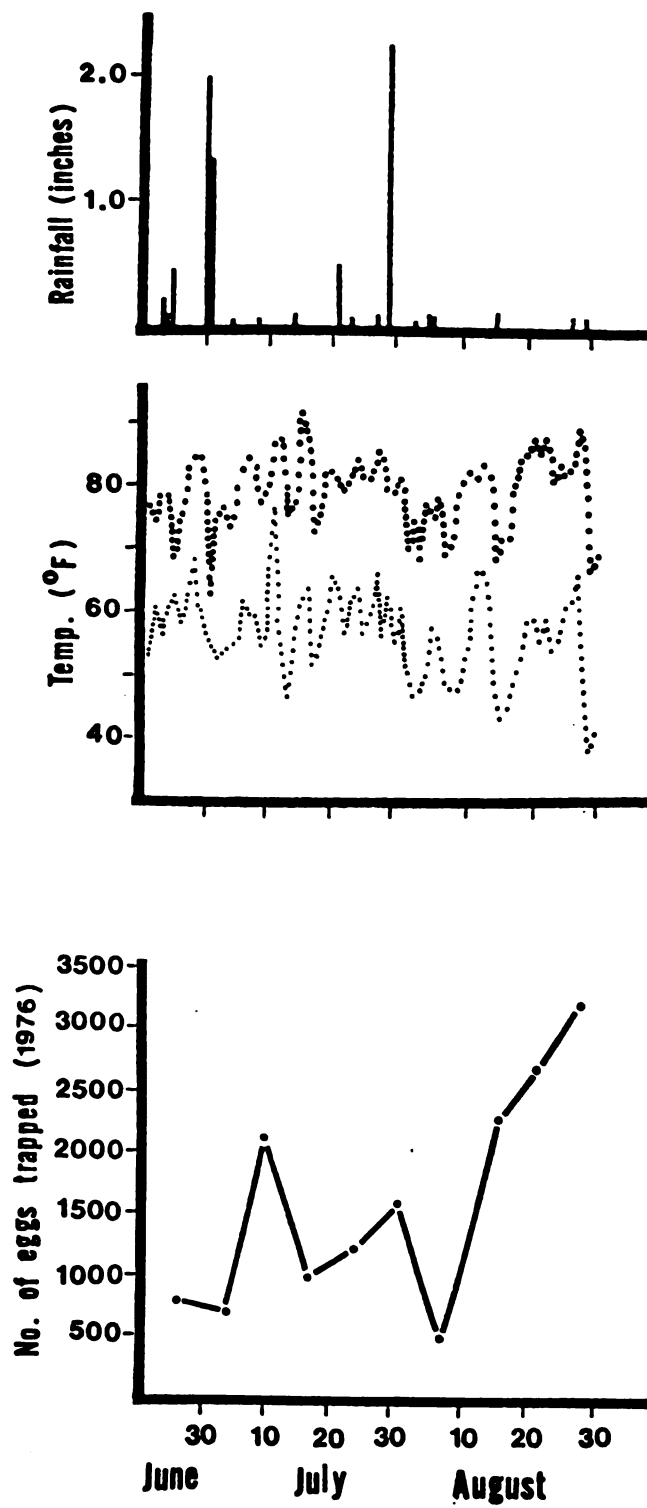


Figure 17

the possibility of movement of Ae. triseriatus females to the project site from the neighboring woodlot was studied more fully and the results are presented later in this chapter.

Although the 1977 weekly ovitrap collections were started in the first week of May, no eggs were deposited in the ovitraps until the first week of June, despite the presence of emerged adults. The main oviposition peak in 1977 was observed in the seventh week of this study (June 22-24). The second peak was noticed in the last week of July (July 27-29) (Fig. 18). Oviposition activity decreased noticeably in September, and no eggs were collected on the September 28-30 and October 7-9 sampling trials.

The long interval between pupation and oviposition in the population of Ae. triseriatus noticed in the field is largely explained by the early distortion of sex ratio. Sinsko (1976), in his Indiana study of Ae. triseriatus, sampled tree holes and derived sexual emergence patterns based on pupal collections. He found that there was a strongly male-dominated sex ratio early in the season which was reversed by the middle of the summer and favored females for the remainder of the year. This lack of oviposition despite the presence of emerged adults was also observed by Scholl (1978) in Wisconsin. The total weekly emergence of females did not begin to catch up with the number of emerged males until early July, and it was not until late July that female emergence remained ahead of males for the rest of the season (Scholl, 1978).

The analysis of variance which was performed on the $\sqrt{x + 1}$ transformed data of the ovitrap collections of 1976 and 1977, after they were combined, revealed that there was a highly significant difference in the total number of eggs collected in each zone (Table 3). The test of least

Figure 18. 1977 Ae. triseriatus egg collections
in the project site, correlated with
daily maximum and minimum temperatures
and rainfall data.

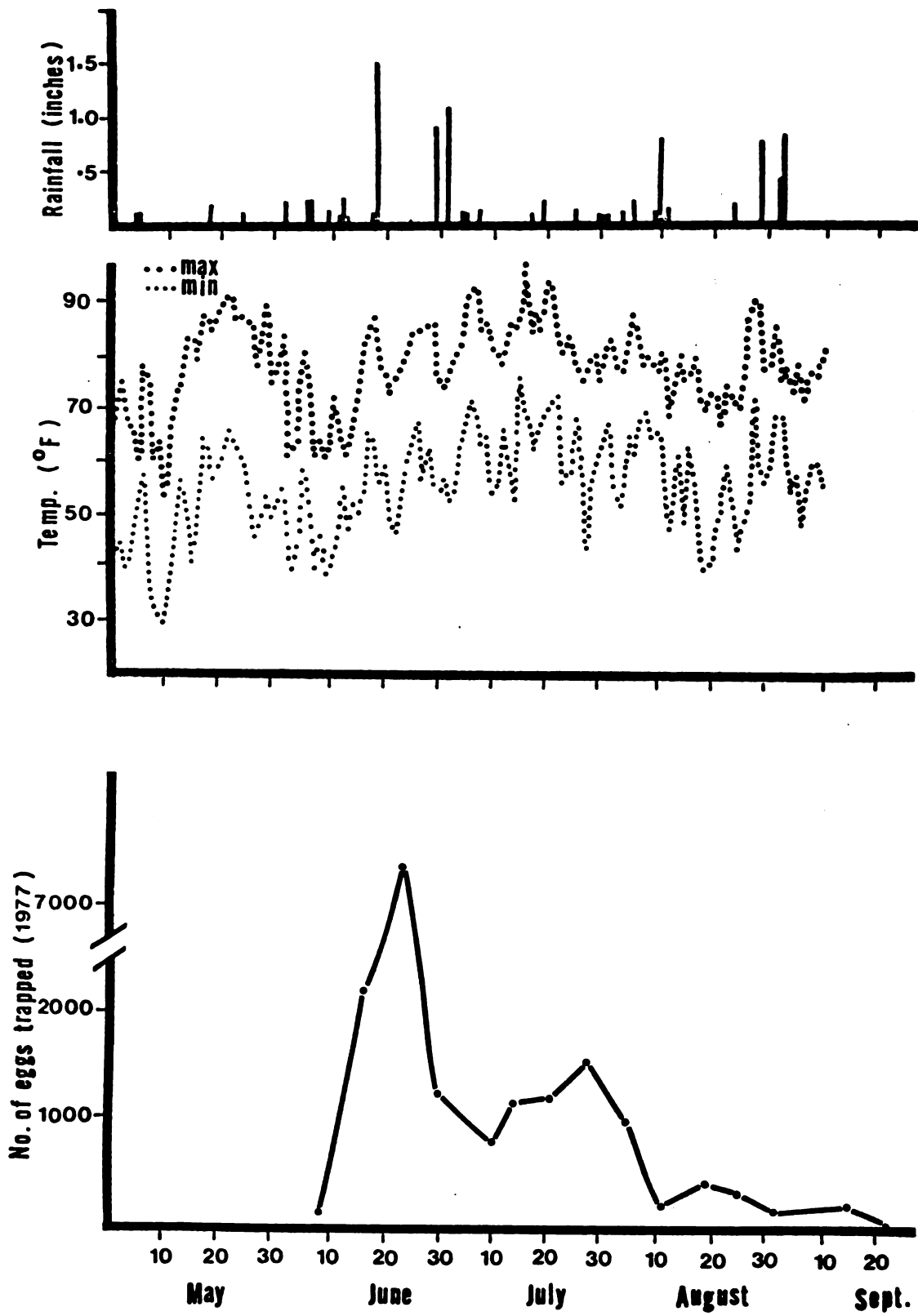


Figure 18

- Table 3 -
Analysis of variance on Ae. triseriatus oviposition
activity

Source of variation	df	SS	MS	F
Replicates	22	5864.07	266.55	
Treatments (zones)	4	2113.19	528.30	14.58 ***
Error	88	3188.55	36.23	
Total	114	11165.81		

*** significant at 0.1% level

significance difference (LSD) revealed that there was a significant difference between the total number of eggs collected in the sprayed vs. the nonsprayed zones, at the 95% significant level. However, no significant difference was detected between the two sprayed zones, B and E. This was due to the fact that unlike ovitrap collections in the summer of 1976, there were more eggs collected in the zone E than zone B, in 1977. As a result when the two year collection data were combined to obtain a better overall picture of the mosquito activity, no difference could be detected between these two zones. The difference between the two nonsprayed plots, F and G, were also not significant, at 95% significant level.

The above results brought up the next question, whether the spray irrigation actually affected the distribution of the adult females, or were they present in the sprayed zone but not laying their eggs in the ovitraps.

Table 4 shows the total number of Ae. triseriatus female mosquitoes as well as other species, taken in the biting collections, at the project site. There was a significant difference in the number of Ae. triseriatus females present in the two zones A and E (significance level of 99%). Indeed, only on two occasions were the number of the females taken in biting collections in the sprayed zone (plot E) greater than those captured in the nonsprayed plot (zone A). I believe these biting collections, which were made at different times of the day as well as before and after spray irrigation very well show the overall impact of the spray irrigation on the distribution of this mosquito species.

Ae. triseriatus does not seem to be the only mosquito species present at the project site whose distribution is apparently affected by the

- Table 4 -
Paired human biting collections in 1978

Date	<u>Ae. vexans</u>		<u>Ae. triseriatus</u>		<u>An. punctipennis</u>		<u>Ae. stimulans</u>		<u>Ae. cinereus</u>		<u>Ae. sticticus</u>	
	A	zone E	A	zone E	A	zone E	A	zone E	A	zone E	A	zone E
June 19	-	-	1	-	-	51	44	-	-	-	-	-
June 19	-	-	1	-	-	38	9	-	-	1	1	2
June 20	1	-	1	1	-	62	38	-	-	1	1	7
June 27	1	-	2	-	-	26	20	-	-	1	1	2
July 5	-	1	-	1	-	62	36	-	-	-	-	-
July 6	4	1	5	-	-	54	42	-	-	-	-	-
July 7	2	-	1	-	-	36	18	-	-	-	-	-
July 12	1	2	-	-	-	30	9	-	-	-	-	-
July 14	9	5	-	1	-	22	28	1	1	1	1	-
July 15	-	4	-	1	-	12	13	-	1	-	-	5
July 17	-	-	1	-	-	31	26	-	-	3	1	4
July 17	-	-	2	-	-	18	7	-	-	1	1	-
July 18	8	1	-	-	-	55	23	-	-	2	2	3
July 24	8	5	3	-	-	17	42	-	-	2	2	1
July 25	7	4	6	-	-	31	28	-	-	7	1	1
July 28	16	10	2	1	-	23	26	-	-	2	2	9
August 4	12	6	-	4	-	9	11	-	-	1	1	1
August 6	30	10	3	2	-	14	15	-	-	1	1	5
August 7	6	12	1	1	-	21	5	-	-	-	-	-
August 9	6	6	1	2	-	20	2	-	-	-	-	1
August 11	13	6	4	-	1	7	1	-	-	-	-	-
Totals	124	73	34	7	12	639	443	1	11	23	41	

spray irrigation operation. Ae. vexans females were also captured in significantly greater numbers (95% significant level) in the nonsprayed as compared to the sprayed plot. Although captured in small numbers, females of Anopheles punctipennis (Say) were also more prevalent in the nonsprayed zone.

On the other hand, females of Ae. stimulans were significantly more numerous in the sprayed zone. Females of Ae. cinereus Meigen, although caught in small numbers were also more numerous in the sprayed zone as compared to the nonsprayed plot. The only other mosquito species which was captured in significant numbers was Ae. sticticus (Meigen). However, there was no significant difference in the total number of females captured in the sprayed vs. the nonsprayed plot.

A possible explanation of this distinct difference in the distribution of the adult females of Ae. triseriatus, as indicated by human collections as well as the ovitrap collections is the fact that spray irrigation lowers the temperature and either drives mosquitoes out of the sprayed plot, or changes their vertical distribution. The recording of air temperature and water temperature in ovitraps, however, did not seem to support this hypothesis.

The maximum and minimum temperatures recorded by the three hygromographs, which were installed in the two sprayed plots (zones B and E) and a nonsprayed plot (zone A), as well as the reported maximum and minimum temperatures for E. Lansing, as reported by the National Oceanic and Atmospheric Administration, are tabulated in Appendix VI. The mean difference between zone A and the reported maximum and minimum temperatures for East Lansing were 2.06° (± 3.02) and 2.41° (± 2.12) C, respectively, with the woodlot being cooler during the day but warmer at

night. There was a significant difference in the maximum and minimum temperatures between the sprayed plot E and the nonsprayed plot A (Fig. 19), with the sprayed plot being cooler during the day, but warmer at night. However, I do not believe that such observed differences were great enough to be responsible, at least as a sole factor, for the observed behavioral differences in the activity of adult females.

The mean temperature of water in ovitraps, located on the ground, in plots A, B, and E, are shown in Table 5. The mean temperature of water in ovitraps in zone A was only slightly higher than those located in zones B and E, the sprayed plots. The temperature of ovitraps fluctuated with the air temperature and were a few degrees cooler than air temperature at the time of measurement.

The recording of water temperature in ovitraps brought up an interesting question. That is, how would the water temperature affect the oviposition behavior of Ae. triseriatus females. The results of the laboratory experiment conducted to study the ovipositional behavior of Ae. triseriatus females in relation to water temperature is given in Table 6. There was a linear relationship between temperature and the oviposition activity, within the range of temperatures tested. This was indicated by the analysis of variance and the coefficient of determination of $r^2 = .81$. No eggs were laid at temperatures below 16.67° C. The regression line as well as the 95% confidence belts for the true population regression line are shown in Figure 20.

In addition to temperature, the repellency effect of spray irrigation on the ovipositional behavior of Ae. triseriatus females, as a possible explanation to the observed behavioral differences in the field, was investigated. Table 7 shows the results of the relative attractiveness

Figure 19. Daily maximum and minimum temperatures at plots A and E, 1977. Plots A and E are the nonsprayed and the sprayed zones, respectively.

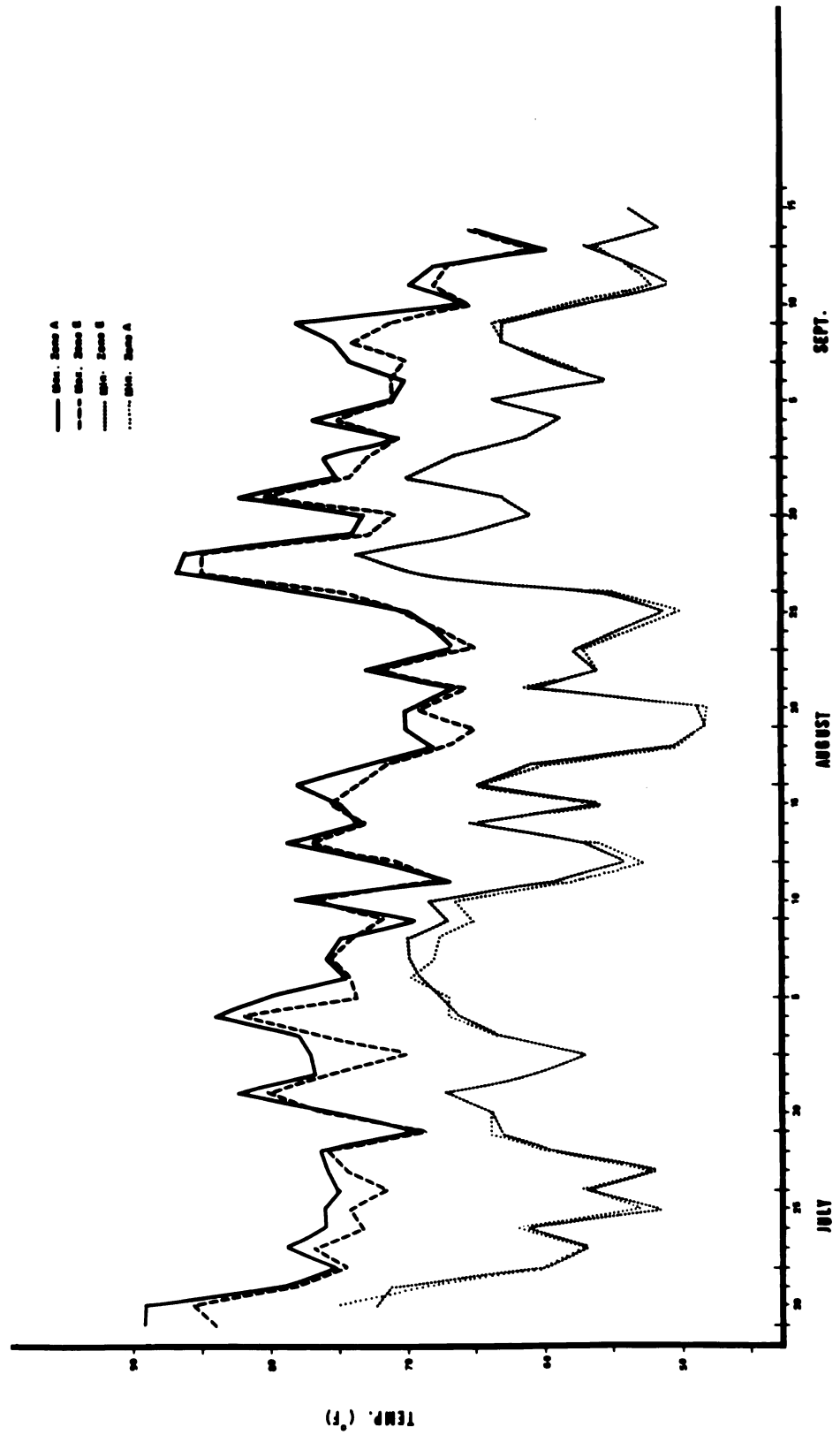


Figure 19

- Table 5 -
Mean temperature of water in ovitraps (°C), located
in study plots A, B, and E

Date (1977)	Time	Plots*		
		A	B	E
August 6	5:00 P.M.	22.03 ± .05	21.51 ± .03	21.68 ± .04
August 7	5:00 P.M.	27.72 ± .08	22.54 ± .04	22.55 ± .07
August 16	8:30 A.M.	18.16 ± .03	18.12 ± .02	18.05 ± .02
September 8	8:30 A.M.	16.58 ± .01	16.58 ± .01	16.59 ± .03

* Plots B and E are the sprayed and plot A is the nonsprayed zone.

- Table 6 -
Oviposition activity of Ae. triseriatus
in varying water temperatures

Temperature (°C)	Number of eggs laid*
15.56	0
16.67	0
20.42	44
20.44	70
21.67	148
22.22	68
22.44	346
22.50	56
22.78	145
23.33	187
23.61	223
24.17	192
24.44	286
26.67	377
27.22	822

* 10 female mosquitoes per test.

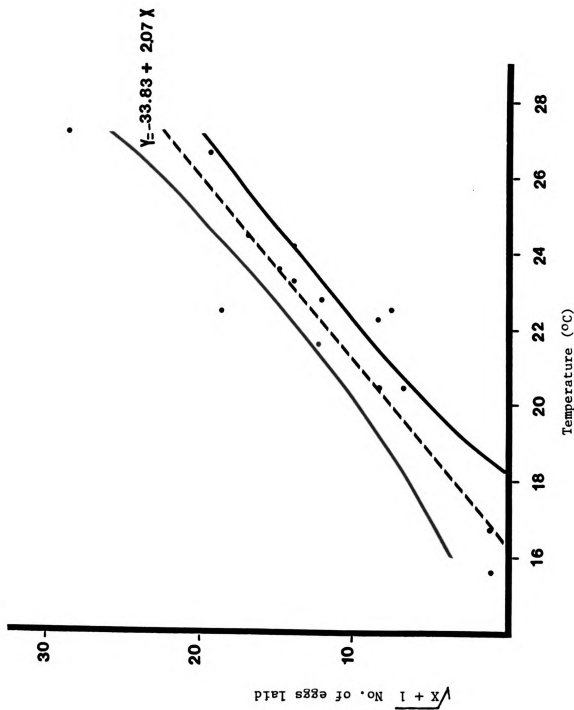


Figure 20. Oviposition behavior of *Ae. triseriatus* as affected by water temperature

- Table 7 -
 Relative attractiveness of different media
 as oviposition sites for gravid
 females of Ae. triseriatus

Repl.	Oviposition (in percentages)		
	Tap water	one-day old sewage water	five-day old sewage water
1	6.8	22.2	71.0
2	10.9	28.3	60.8
3	1.3	34.7	64.0
4	51.4	32.1	16.5
5	28.1	53.1	18.8
6	23.1	29.7	47.2
7	2.0	53.5	44.5
8	35.9	7.5	55.5
9	38.8	15.1	46.2
10	48.6	48.1	3.3
11	99.7	0.0	0.3
12	1.5	9.7	88.8
13	28.2	38.0	33.8
14	2.1	44.7	53.2
15	18.0	0.2	81.8
16	21.2	27.8	51.0
17	13.1	60.2	26.7
18	11.4	30.0	58.6
19	14.6	14.0	71.3
20	49.3	6.7	44.0
21	0.9	36.3	62.8
22	21.6	34.3	44.1
23	83.4	16.6	0.0
24	38.1	18.2	43.8
Mean	27.08	27.54	45.33
Coeff. of var.	94%	62%	54%

of wastewater of varying "ages" as oviposition sites for gravid females, presenting the percentage of eggs laid in tap water as well as in one-day old and 5-day old sewage spray water. This experiment was replicated 24 times. The analysis of variance, performed on the $\log (X + 1)$ transformed data, indicated that there was no difference in the attractiveness of the three media to the gravid females. However, the variation between treatments was quite high, as shown by coefficient of variation (Table 7). Such difference in the relative attractiveness of the sprayed water among different replicates was perhaps due to the great variation that occurred in the chemical properties of such water. The daily chemical analysis of the sprayed water, reported by Dr. Burton of the Institute of Water Research, at MSU, revealed that levels of two chemicals in the sprayed water, calcium nitrate and sodium chloride, varied markedly from time to time. An investigation was then undertaken to study the effect and interaction of each of these chemicals on the oviposition behavior of Ae. triseriatus females.

Table 8 shows the analysis of variance performed in the study of oviposition behavior as affected by varying concentrations of calcium nitrate and sodium chloride. The raw data is tabulated in Appendix VII. At the concentrations used in this study, sodium chloride was not significantly effective in repelling gravid females, but the repellent effect of calcium nitrate was highly significant (99.9% significant level). The interaction of the two salts was not significant.

The partitioning of the degrees of freedom, associated with the main effects of calcium nitrate, to one and one for linear and quadratic contrasts, revealed that the response of the gravid females to different concentrations of calcium nitrate is not exactly linear, but it is rather

- Table 8 -
Analysis of variance on Ae. triseriatus
oviposition behavior in varying water-
salt concentrations.

Source of variation	df	SS	MS	F
Replicates	9	2.36		
Ca(NO ₃) ₂	2	2.38	1.19	7 ***
linear	1	1.54	1.54	9.06 **
quadratic	1	0.84	0.84	4.94 *
NaCl	2	0.24	0.12	0.71
NaCl X Ca(NO ₃) ₂	4	1.09	0.27	1.59
Error	72	12.42	0.17	

*** significant at 0.1% level

** significant at 1% level

* significant at 5% level

a curve, as shown in Figure 21. Although there was no significant discrimination between distilled water and that containing 2.5 g/l of calcium nitrate, the difference between 2.5 and 5.0 g/l of this salt was highly significant, as indicated by the least significance difference (LSD) test.

Salinity of the sprayed water as measured by conductivity is about three to four times higher than that for most fresh water (0.8-1.02 mmhos/cm) (T. Burton, personal communication). Nevertheless, the last two experiments clearly show that the sprayed water has not much repellency effect, if any, on the gravid females of Ae. triseriatus. In this study, there was no significant discrimination between distilled water and that containing 2.5 g/l of calcium nitrate (electrical conductivity of approximately 2.0 mmhos/cm). Aging of the sprayed water, also, did not seem to decrease its relative attractiveness as oviposition sites for gravid females of Ae. triseriatus, as compared to distilled water or the one-day old sewage water.

Also of interest was whether or not the chemical nature of the sprayed water would support the larval development of Ae. triseriatus, should females lay their eggs in containers filled with such water. An investigation was undertaken to compare the larval mortality of Ae. triseriatus larvae reared in sprayed water as compared to those reared in tap water. The results of this experiment is shown in Table 9. There was a significant difference (95% level) between the larval mortality rate among those larvae reared in sprayed water and those reared in tap water, using Student's t-test. There was a great variation, however, in the larval mortality in those reared in the spray water. Coefficient of variation for the larval survival in sprayed water was 36.73% as compared to only 7.8% for those reared in tap water. Again this variation was

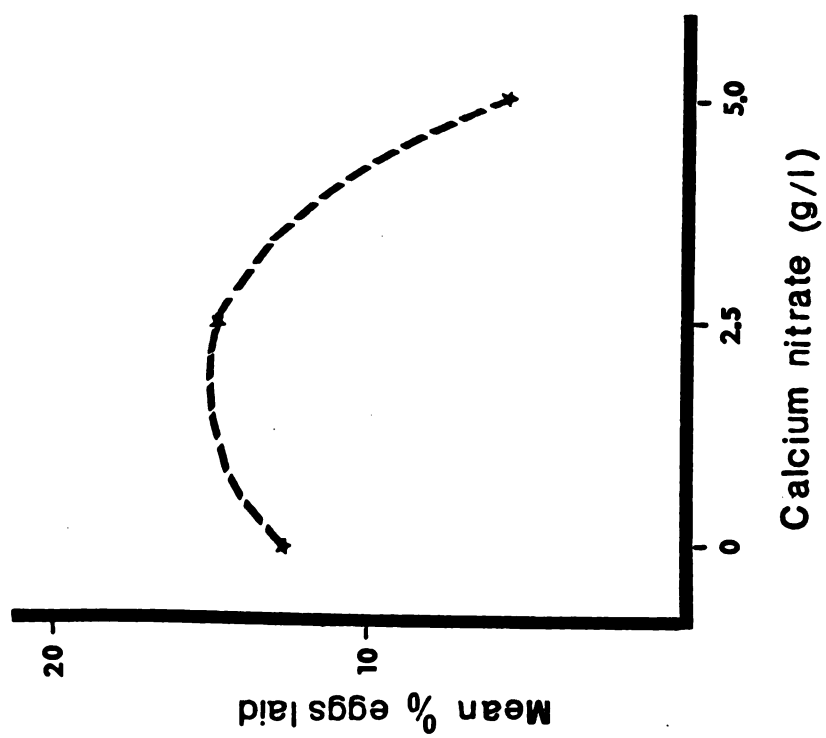


Figure 21. Oviposition rates of Ae. triseriatus in varying concentrations of calcium nitrate.

- Table 9 -
Larval survival in sewage effluent and
tap water

Replicates	Effluent water*	Tap water*
1	182	181
2	161	184
3	163	183
4	155	181
5	175	180
6	150	193
7	165	182
8	187	162
9	190	192
10	171	182
11	192	192
12	173	183
13	184	189
14	58	170
15	25	156
16	25	193
17	30	181
18	168	163
19	101	182
20	181	179
21	169	176
22	126	188
23	160	187
24	142	187
25	87	169
26	171	178
27	126	165
28	36	168
29	166	172
30	163	199
31	174	173
32	117	125
33	165	169
Totals	4638	5864
Means	140.55	177.7
Coefficient of variation	36.73%	7.81%

* 200 larvae per treatment replicates.

thought to be due to the change in the chemical properties of the sprayed water, and so, attempts were made to see how different concentrations of such salts may affect the larval development.

Table 10 shows the analysis of variance performed in the study of larval development in relation to various concentrations of sodium chloride and calcium nitrate. The raw data is tabulated in Appendix VIII. Calcium nitrate, at the concentrations used in this study, had no effect on the larval mortality. On the other hand, the effect of sodium chloride was significant at 5% level. The interaction of the salts was not significant. This means that salts acted independently of each other, and the simple effects of one salt were the same for all levels of the other, within chance variation as measured by experimental error. As a result, all the available data for each concentration of sodium chloride was lumped together and a regression analysis was performed, using the mean mortality rate corresponding to each concentration of sodium chloride. The regression line as well as the 95% confidence belts for the true population regression line are shown in Figure 22. This clearly shows that although higher concentrations of sodium chloride had a significant effect on the mortality of Ae. triseriatus larvae, they are able to tolerate a wide range of salinity in water.

Many papers have recently been published on the biology and ecology of Ae. triseriatus. However, only a few investigators have considered the water chemistry of tree holes and its relationship to gravid female attraction, egg hatching and larval development. Even among those published, the conclusions are not, in my opinion, substantiated by their observations. Petersen and Chapman (1969) reported that Ae. triseriatus was generally restricted to habitats with low salinity.

- Table 10 -
Analysis of variance on larval development of
A. triseriatus as affected by water salinity.

Source of variation	df	SS	MS	F
Replicates	3	27.62	9.21	
Ca(NO ₃) ₂	5	9.12	1.82	0.9
NaCl	6	25.45	4.24	2.1 *
Ca(NO ₃) ₂ X NaCl	30	39.05	1.30	0.64
Error	123	248.38	2.02	

* significant at 5% level

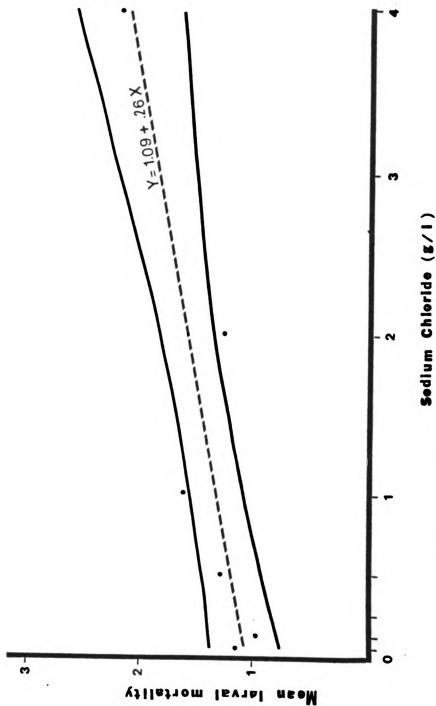


Figure 22. Mean mortality of *Ae. triseriatus* larvae (based on 10 larvae per replicate) exposed to different concentrations of sodium chloride.

However, these investigators did not establish whether the chemical differences or the physical factors causing the chemical differences were responsible for the distribution of this species. Petersen and Willis (1971) also studied the effect of salinity on site selection by ovipositing A. triseriatus, by providing ovitraps containing different dilutions with electrical conductivity ranging from 0.8 to 8 mmhos/cm. The ovitraps were checked every two weeks in summer and once a month in winter, when conductivity was measured and the number of Ae. triseriatus eggs laid in each trap were recorded. There is no question that conductivity of water in the containers could have changed during those periods between checks, causing a great variation in the data. They concluded that "Ae. triseriatus generally oviposited less frequently in traps as the conductivity of the water increased". However, as they have mentioned, the regression line they established was not significant.

Experiments dealing with the effect of the chemical composition of water on mosquito larvae have frequently presented a confusing picture, particularly when the water in which mosquito larvae are found or from which they are absent have been analyzed. This has led to the conclusion that certain solutes are harmful to the larvae, and when present, prevent the breeding of mosquitoes. The large number of variables existing between samples from different breeding sites, as well as wide variation of conditions which may occur over a short period of time in the same breeding site, may be responsible for this confusion. Perhaps only under controlled situations, with a limited number of variables can one actually study the effect of such variables and their interactions on the behavior of certain mosquito species.

It has been suggested that mosquitoes scatter their eggs indiscriminately on available aquatic sites, and that larvae of only certain species are able to survive in a given kind of habitat. However, intensive field and laboratory studies have shown that the restriction of a given species to a certain type of larval breeding place is largely a result of selective oviposition on the part of the adult female mosquito (Bates, 1940; Wallis, 1954; and Macan, 1961). The restricted nature of the larval habitat of Ae. triseriatus to waters of low salinity reported by Petersen and Willis (1971) results from the discrimination of the ovipositing female, for eggs are rarely found outside the usual larval habitat and the larvae are tolerant of a wider range of salinity than that they are found in, as shown in this study.

Within recent years, it has become increasingly evident that presence of decayed organic matter (Wilton, 1968), a dark medium for oviposition (water of high optical density) (Williams, 1962), and larval-produced attractants (Bentley et al., 1976; McDaniel et al., 1976) are important components of the web of factors influencing the choice of oviposition sites by gravid females of Ae. triseriatus. Based on my observations, I believe that salt content of the water may not be as important as has been previously speculated and that Ae. triseriatus will lay their eggs in such saline habitats if the other factors are favorable. The mean electrical conductivity of water from tree holes in Louisiana, as reported by Petersen and Chapman (1969), was about 2.0 mmhos/cm and Ae. triseriatus occurred in habitats with an average of 0.85 mmhos/cm. However, in this study, there was no significant discrimination against distilled water and that containing 2.5 g/l of calcium nitrate (electrical conductivity of approximately 2.0 mmhos/cm).

Inorganic salt solutions have been used in many experiments on selection of oviposition sites by gravid female mosquitoes, however, still much remains to be done on the mode of action as well as the understanding of the attractive properties of natural oviposition sites. Osmotic pressure has been suggested by many workers to be responsible for discrimination by the gravid female mosquito between different concentrations of various electrolytes. However, the fact that no significant interaction between different concentrations of calcium nitrate and sodium chloride was observed in this study, suggests that there is some influential factor other than osmotic pressure involved in such a discrimination.

The distinct difference in the distribution of the adult females of Ae. triseriatus between the sprayed and the nonsprayed plots, as indicated by the human biting as well as ovitrap collections, are not fully explained by changes in temperature due to the spray irrigation, or by the repellency of such water. However, still much remains to be done to determine the effects of the habitat alterations and changes that occur in food sources and availability, due to the spray irrigation operations.

Mosquitoes like many other organisms utilize protective shelters to avoid exposure either to unfavorable weather conditions or to natural enemies. During the four year history of the spray irrigation in the Lott Woodland, there has been quite a change in the ground vegetation as well as the canopy density. This difference may best be seen in the sprayed zone E, which receives the highest amount of sprayed water per week. The 10 cm of municipal wastewater being sprayed in two applications each week in this zone, as well as collection of runoff from the neighboring sprayed plot (zone B, receiving 5 cm of sprayed water each week) has now produced in much of this zone areas either with standing water

throughout most of the season, or with soil near saturation. The excessive irrigation and nutrients being added to the soil has caused the extensive growth of such plants as stinging nettle (Urtica dioica L.), broad leaved water leaf (Hydrophyllum canadense), Virginia creeper (Parthenocissus quinquefolia (L.) Planch.), violets (Viola spp.), spotted touch me not (Impatiens biflora, Watt), and Jack-in the pulpit (Arisaema triphyllum (L.) Schott), in this area (Figures 23 and 24).

In addition, there has been a great amount of tree mortality, causing a much thinner canopy and reduced amount of shade in this zone (Fig. 25). Barr (1958) reported that Ae. triseriatus is particularly common in dry woods. If this is true, then it is not surprising to find more Ae. triseriatus adults in the nonsprayed as compared to the sprayed plot. However, more studies on the behavior of Ae. triseriatus need to be done before one can definitely relate the changes that have occurred in this habitat to the distribution of this mosquito species.

In addition to the habitat alteration, changes in food sources and their availability to female mosquitoes, as affected by the spray irrigation operations, may also influence the distribution of Ae. triseriatus. At this time it is not yet known how the density and behavior of the avian and small mammal populations has been affected by the spray irrigation. A detailed study of the host preference of Ae. triseriatus females in the project site, as well as the impact of sewage spray irrigation on the population of each host animal species may explain, in part, the observed behavioral differences in the population of this mosquito species. Should the primary food source of the mosquito move out of the sprayed zone, it might not be surprising to find adult females having the highest activity in the nonsprayed plots, where suitable hosts are present.



Figure 23. Ground vegetation in plot A (the nonsprayed zone).



Figure 24. Ground vegetation in the most heavily sprayed zone E.



Figure 25. The canopy in the most heavily sprayed zone E.

Regardless of the cause, it seems that the adult females of Ae. triseriatus have either moved out of the sprayed plots or have changed their vertical distribution. In order to investigate this, rotary traps and ovitraps were used for sampling the population at two different heights.

Rotary traps proved to be of no value in the population sampling of this mosquito species. They were run for the total of 17 hours, in the late afternoon and early morning, when Ae. triseriatus was most active. Only one parous female Ae. triseriatus and two females of Ae. stimulans were captured. These were taken in the sprayed zone E, at a height of .3 m.

Low catches by rotary traps have also been reported by some other investigators. For example, in his study on the comparison of the sampling methods for adult mosquito populations in central Alberta, Graham (1969) trapped 44.6 adult female mosquito per 100 trap hours. Apparently these traps are only useful in trapping certain species of mosquitoes, especially when present in large numbers. Although there might be some attraction by female mosquitoes to ovitraps due to the movement of the nets, such traps collect mainly the active adults. There were not many adults of Ae. triseriatus around, as was shown in the biting collections (Table 4).

Ovitraps collections at the zero and 5 m were also used to see if any changes in the vertical distribution of Ae. triseriatus could be detected. Table 11 presents the total number of eggs collected at zero and 5 m high in each zone. The data from years 1977 and 1978 are included in the same table. The analysis of variance did not reveal any significant difference between the number of eggs laid at zero and 5 m in any of the zones.

- Table 11 -
Vertical distribution of Ae. triseriatus
(ovitrap collections)

Date	Zone A		Zone B		Zone E	
	0 m	5 m	0 m	5 m	0 m	5 m
June 26-29 1977	163	0	541	9	827	388
July 3-6	139	76	252	182	329	25
July 9-12	0	0	45	0	0	0
July 17-20	56	0	0	0	0	0
July 23-26	0	0	167	185	84	0
July 30-Aug. 2	0	0	86	0	77	35
Aug. 6-9	0	0	0	0	0	47
Aug. 13-16	58	0	0	0	0	85
June 21-23 1978	0	0	0	0	0	97
June 28-30	143	244	97	0	0	0
July 1-3	17	0	0	0	0	0
July 8-10	160	0	19	0	105	122
July 12-14	191	201	62	106	6	0
July 15-17	284	4	103	0	25	0
July 19-21	75	0	0	0	0	4
July 22-24	27	181	204	69	19	134
Totals	1313	706	1576	551	1472	937

Since the variation was quite high and homogeneity of variance, a basic requirement to the use of analysis of variance, was not met, Friedman's two way analysis of variance (a nonparametric statistical test) was performed. However, again no significant difference could be detected. Apparently, females of Ae. triseriatus utilized the ovitraps in both heights without any strong preference for either height, even in the zone which received the highest amount of spray irrigation. One should notice that this experiment has not been designed to compare the distribution of this mosquito species between the three zones, and such extrapolation from this data is not justified.

Eggs of Ae. hendersoni, although in small numbers, were also being laid in these traps. Unlike Ae. triseriatus, this mosquito species showed a very strong preference for ovitraps located at 5 m over those at ground level (Table 12).

The nonsignificant difference in the total number of eggs laid, by females of Ae. triseriatus, at ground level and a height of 5 m in the nonsprayed zone A, does not very well agree with what has been reported by Scholl and DeFoliart (1977) and Sinsko and Grimstad (1977). Scholl and DeFoliart (1977) studied the vertical distribution of Ae. triseriatus and Ae. hendersoni, using ovitraps. They reported that Ae. triseriatus showed a definite preference for ovipositing at ground level with 69% of its eggs laid there, while only 17, 8, and 6% were deposited, respectively, in traps at 3, 6, and 9 m above the ground. Ae. hendersoni, however, was strongly arboreal in its oviposition activity, depositing only 26% of its eggs at ground level and 74% in traps 3-9 m above the ground. Of course, these investigators recorded the number of eggs laid by each species, based on the larval counts and it is not known if the larval counts

- Table 12 -
Vertical distribution of Ae. hendersoni
(ovitrap collections)

Date	Zone A		Zone B		Zone E	
	0 m	5 m	0 m	5 m	0 m	5 m
June 26-29 1977	0	25	0	0	102	0
July 3-6	0	0	0	0	0	0
July 9-12	0	0	0	0	0	98
July 17-20	0	0	0	0	0	101
July 23-26	0	0	0	0	0	24
July 30-Aug. 2	0	0	0	0	0	70
Aug. 6-9	0	0	0	12	0	79
Aug. 13-16	0	49	0	0	0	53
June 21-23 1978	0	0	0	0	0	0
June 28-30	109	67	0	0	0	0
July 1-3	0	0	0	0	0	0
July 8-10	0	0	0	0	0	36
July 12-14	0	0	0	77	0	0
July 15-17	0	23	0	64	0	67
July 19-21	0	28	0	9	0	0
Totals	109	192	0	162	102	528

actually reflected the true egg density. They reported that the hatch of collected eggs dropped and remained as low as 42% after July 24th.

Sinsko and Grimstad (1977) also studied the vertical distribution of these two tree hole Aedes in Indiana. They reported that there was a linear relationship between the heights of the breeding place and the proportion of Ae. triseriatus and Ae. hendersoni eggs. Ae. triseriatus constituted 97.3% of all larvae and eggs from the basal traps, 57.8% from 3 m, and 38.3% from 6 m. This study, however, was done in one woodlot, using a total of only nine traps, placed at 3 different heights on three widely separated trees.

On the other hand, an intensive study conducted by J. Rogers (Michigan State University, personal communication) in 7 woodlots around the East Lansing area, using a total of 180 traps did not show a strong preference of Ae. triseriatus females for basal tree holes. Out of 12,079 eggs collected at zero and 6 m high, 8,251 were Ae. hendersoni and 3,828 of Ae. triseriatus. Although Ae. hendersoni showed a significant preference for traps at 6 m (7,040 eggs were laid at 6 m as opposed to only 1,211 at zero level), there was no significant difference between the number of eggs laid by Ae. triseriatus at either height. A total of 2,024 eggs were laid by Ae. triseriatus at zero level as opposed to 1,804 eggs laid at 6 m above the ground.

The vertical distribution of these two species is probably not yet as clear cut as one wants it to be. Probably the selection of oviposition sites is determined by the physical or chemical nature of the container rather than by elevation. Recent findings by Novak (University of Notre Dame, personal communication) in regard to trapping a great number of Ae. triseriatus adults in the canopy of a beech-maple climax forest contradicts

the previous findings on vertical distribution of Ae. triseriatus and shows the fact that more research needs to be done on the behavior of these two sibling mosquito species before any comparative conclusions can be made.

Results of the dispersal studies on Ae. triseriatus, investigating the possible movement of the females from Sycamore Creek woodlot to the project site, is represented in Table 13. This summarizes the results of the 18 two-hour human biting collections, conducted between July 17 (four days after the first release) and August 31 (19 days after the last release). A total of 404 Ae. triseriatus were collected, 13 of which were marked (3.2%). All of these marked mosquitoes were recaptured at sites 3 and 4, about 1159 and 1006 m away from the release site, respectively.

Biting collections were also conducted on two occasions at the release site to see what percentage of mosquitoes would still have their markings after several major rainfalls we received in that area. Out of 26 females captured on July 28, 13 were marked. Also, out of 28 females of Ae. triseriatus captured, on August 25, 13 days after the last release, 14 females were marked.

Though Ae. triseriatus has considerable powers of rapid and directive flight, it is not, like certain salt-water breeding mosquitoes and many anophelines, recognized as a species having a tendency to travel considerable distances. It seems that the activity of this mosquito species is largely restricted to the woodlots they inhabit. Sinsko (1976) found no interchange of this mosquito species between two woodlots in Indiana separated by 300 meters of open terrain. Grimstad et al.

- Table 13 -
Aedes triseriatus mark-release results
 (human biting collection)

Date	Total no. caught	No. marked*
July 17	4	
July 18	2	
July 19	10	
July 20	18	
July 21	2	
August 5	33	11
August 7	33	
August 8	29	
August 10	30	1
August 12	36	
August 15	44	
August 17	30	
August 19	18	
August 22	36	1
August 24	20	
August 26	20	
August 29	23	
August 31	16	
Totals	404	13

* marked mosquitoes were collected at sites 3 and 4, about 1159 and 1006 m away from the release site, respectively.

(1977) referring to an unpublished data believe that there is also a very little gene flow occurring between woodlots, 200-300 meters apart.

The small percentage of marked adults (3.2%) recaptured at the project site seems to indicate that there is not much movement from Sycamore Creek woodlot to our project site. The southern or the south-western prevailing winds in this area in the months of summer can bring small number of Ae. triseriatus females into this area. The fact that no eggs were collected in ovitraps arranged on bamboo sticks and placed about 10 meters away from the woodlot suggests that gravid females of this mosquito species probably do not frequency leave the woodlot in search for oviposition sites.

SUMMARY

- 1- The two-year ovitrap collections at the Michigan State University Water Quality Management Project spray irrigation complex revealed that there was significantly less oviposition activity in the wastewater sprayed zones as compared to the nonsprayed plots.
- 2- The human biting collections made in sprayed as well as in a non-sprayed plot revealed that there were also significantly fewer adult females in the zone receiving the highest amount of spray irrigation as compared to the nonsprayed plot.
- 3- The reduction in air temperature and ovitrap water temperature in the sprayed plots as a result of spray irrigation operations were not great enough to be responsible, at least as a sole factor, for the observed behavioral differences in the activity of adult females.
- 4- No significant difference was observed between the number of eggs laid in containers containing tap water, one-day old and 5-day old wastewater.
- 5- The relatively high salt content of the sprayed water has not much, if any, repellency effect on the gravid females. Indeed, salt content of the water may not be as important in the site selection by gravid females as has been previously speculated. It seems that Ae. triseriatus females will lay their eggs in such saline habitats if the other factors are favorable.

- 6- Although there was a significant mortality between those larvae reared in wastewater as compared to those reared in tap water, a relatively high percentage of individuals were able to complete their development, demonstrating that larvae are tolerant of a wide range of water salinity and the relatively high salt content of the sprayed water would not prevent their survival in sufficient numbers to maintain natural populations.
- 7- The habitat alteration, changes in food sources and their availability to female mosquito may explain, in part, the observed behavioral differences in the population of Ae. triseriatus. However, more studies need to be done before one can definitely relate all changes observed in this study to the distribution and behavior of Ae. triseriatus.
- 8- The excessive amount of spray irrigation has made the females of Ae. triseriatus move out of the sprayed zones rather than changing their vertical distribution.
- 9- The small percentage of marked adults (3.2%) recaptured at the project site indicates that there was not much movement from Sycamore Creek woodlot to the project site.

CONCLUSIONS

Entomological studies conducted at the Michigan State University Water Quality Management Project spray irrigation complex during the past three years have shown that marked changes have occurred in the mosquito fauna there as a result of the spray irrigation operations (see Appendix I). In 1973 and 1974, prior to the start of the spraying, only three mosquito species were collected in any appreciable numbers: Ae. stimulans, Ae. triseriatus and Ae. vexans. Adults of Anopheles punctipennis, Ae. canadensis (Theobald), Coquilleltidia perturbans (Walker) and Psorophora ferox (Humboldt) were also collected in landing counts, but in very small numbers (Zorka, 1975). Only the larvae of Ae. stimulans, Ae. triseriatus and An. punctipennis, however, were collected in this area.

The start of spray irrigation in 1975, however, seems to have caused a dramatic increase in both the numbers and the diversity of the mosquito species that began to use the wooded area for a breeding habitat. By the end of the summer of 1978, a total of 17 different mosquito species were collected in the wooded area, 11 of which are known to breed in this area. Among these are the known vectors of several arboviruses and other pathogens. The large numbers of these mosquitoes that could result from the numerous and varied breeding sites now available at Lott Woodland area would greatly enhance the

potential for outbreaks of these mosquito-borne diseases should the pathogens now be enzootic or later introduced into the area.

Until now, there has not been much, if any, increase in the number of Ae. triseriatus due to the wastewater irrigation. Although many tree holes are in the process of formation in the sprayed areas, no cavities containing larvae of this mosquito species have been found in such zones. The present tree holes are too small and shallow to be of any significance. However, this may change in the matter of few years, and coupled with possible expansion of the sprayed sites, more potential breeding sites may be created by the spray irrigation, and hence increase the population of this mosquito species.

Based on my observations, a container filled with wastewater may be used by gravid females of Ae. triseriatus given the other physical and chemical factors affecting the oviposition behavior of this mosquito species are acceptable. This water does not seem to have any appreciable repellency effect on the gravid females, and also supports the development of a relatively high percentage of larvae without much mortality.

The productivity of such tree holes and the magnitude of the increase in the population density, among other things, would be a function of the spray schedule and the amount of sprayed water. The spray irrigation operations may indirectly affect the distribution of Ae. triseriatus by altering the habitat and changing the source and availability of hosts. This may mean movement of adult females out of sprayed areas and hence less utilization of water-filled cavities

in the sprayed plots.

The changes that have occurred in the mosquito populations during the four year period the spray project has operated have been both impressive and dynamic. As of the end of 1978 season it does not appear that the mosquito species associated with the project site have stabilized or that the observations made in the past year can be considered to be typical of what may occur in future years. Observations must be made over a longer period to determine whether natural predators and other limiting factors in the area will effectively repress the mosquito populations without the employment of specific, directed mosquito control measures. It is certain, however, that the disposal of sewage oxidation pond effluent by spray irrigation creates a diversity of excellent mosquito breeding habitats that are suitable for use by a number of species. If the potential for major disease transmission and pest problems is to be minimized, the planners and designers should be fully aware of these potential problems and incorporate appropriate safeguards during the construction and operational phases of sewage spray irrigation disposal projects.

APPENDICES

- Appendix I -

Mosquito fauna at the MSU Water Quality Management
Project spray irrigation complex.

Appendix I

Mosquito fauna at the MSU Water Quality Management Project spray irrigation complex.

INTRODUCTION

A baseline study was conducted during the spring and summer months of 1973 and 1974 at the Michigan State University Water Quality Management Project spray irrigation complex (Zorka, 1975) to gather information concerning the human biting mosquitoes indigenous to the project complex prior to the initiation of spray operations. Methods of collection were landing counts, using human bait during the day, and CO₂ baited miniature light traps at night. Only three species of mosquitoes were collected in appreciable numbers: Ae. stimulans, Ae. vexans, and Ae. triseriatus. Adult females of An. punctipennis, Ae. canadensis, C. perturbans and Ps. ferox were also collected in landing counts but in very small numbers (Zorka, 1975). Only the larvae of Ae. stimulans, Ae. triseriatus and An. punctipennis, however, were collected in this area.

It was anticipated that spray irrigation in and around the wooded area of the project site, as the last step in removing undesirable chemicals from the water, might modify the water depth fluctuations in the vernal ponds, and produce additional pockets of water which would form ideal breeding sites for certain mosquito species; either voracious species which annoy and discomfort humans and domestic animals, or those of direct public health importance because of their ability to transmit pathogens. The purpose of the present investigation was to determine the possible long term effects of the wastewater spray irrigation

operations on the diversity of mosquito species present in the project site.

MATERIALS AND METHODS

Mosquito larval collections were made on weekly intervals during the spring and summer of 1978, at the project site. This included sampling of the two semipermanent ponds as well as the four snow pools in the wooded area. In addition, several extra ponds, produced by the spray irrigation, were also continuously monitored for the presence of mosquito larvae during the whole mosquito season.

Adult biting collections were made during the summers of 1977 and 1978. This involved a fellow student and myself, aspirating mosquitoes from each others bodies. In the summer of 1977, biting collections were conducted at three different sites (sites 2, 3, and 4 of Figure 14), spending 20 minutes per station. Collecting was enhanced by placing a pan containing dry ice close to where we were standing. In the summer of 1978, however, collections were made in zone A (a nonsprayed area) and E (the plot receiving the highest amount of wastewater per week). In each zone, collections were made at three stations, each for ten minutes (Figure 8). In order to have a better representation of the mosquito population in the area, collections were made at different times of the day.

RESULTS AND DISCUSSION

The results of the human biting collections, conducted during the summer of 1977 and 1978 are presented in Tables 14 and 15. The data on larval collections are presented in Table 16.

A total of 17 different mosquito species were collected in the wooded area of the Water Quality Management Project site, 11 of which

are known to breed in this area (Table 17). Two species of deer flies, Chrysops montana and C. pudica, and a species of black flies belonging to the Simulium vinistum complex were also taken in biting collections.

Aedes stimulans: This was the most common mosquito species encountered in our biting collections, showing a significant preference for aggregating in the sprayed zone as compared to the nonsprayed plots. Larvae were also collected in large numbers in snow pools and the two semipermanent ponds present in the wooded area. Larvae were also present in smaller numbers in the snow pools formed, in early spring, in the heavily sprayed zone.

This mosquito is among the most abundant and annoying of the woodlot mosquitoes in many of the northern states. They bite readily in the day time. There is only one generation a year but the adults live most of the summer. The winter is passed in the egg stage, hatching with the melting of the ice and snow in early spring.

Aedes vexans: This mosquito species was also found in great numbers, both in larval and human biting collections. Adults were more numerous in the nonsprayed zones as compared to the sprayed plots. Larvae were collected in very large numbers in late spring, in shaded rain pools in the wooded area.

This inland floodwater mosquito, is probably the most widely distributed species of Aedes in the United States and the most abundant and troublesome mosquito in many areas. It has been reported from every state, and is a major pest in most of the northern states from

- Table 14 -

Human biting collections at the project site- 1977

Date		<u>Ae. cinereus</u>	<u>Ae. hendersoni</u>	<u>Ae. stimulans</u>	<u>Ae. triseriatus</u>	<u>Ae. trivittatus</u>	<u>Ae. vexans</u>	<u>An. punctipennis</u>	<u>An. quadrimaculatus</u>	<u>C. perturbans</u>	<u>C. pipiens</u>	<u>C. inornata</u>	<u>Ps. horrida</u>	<u>Ps. ferox</u>
July	17			3	4									
July	18			29	2							1		
July	19			36	10		5	2						
July	20	5	3	27	18		14	8						
July	21			5	2		7	1						
August	5	5	8	12	33		17	5						
August	7	2	9	10	33		16	10	3					
August	8			7	29		19	6	3		1		1	
August	10	2	1	7	30		11	5	2					
August	12		12	3	36		12	5	4					
August	15	5	21	2	44	1	5	16	10					
August	17	2	4		30		10	12	3					
August	19	1	5	1	18		10	6	6					
August	22		6		36		13	15	7					
August	24	2	1	1	20		7	13	8			1		
August	26		2		20		11	5	2					
August	29	3	1		23		13	10	3	1				4
August	31		1		16		2						1	4

- Table 15 -

Human biting collections at the project site- 1978

Date		<u>Ae. canadensis</u>	<u>Ae. cinereus</u>	<u>Ae. hendersoni</u>	<u>Ae. stimulans</u>	<u>Ae. sticticus</u>	<u>Ae. triseriatus</u>	<u>Ae. trivittatus</u>	<u>Ae. vexans</u>	<u>An. punctipennis</u>	<u>An. quadrimaculatus</u>	<u>C. perturbans</u>	<u>Ps. ferox</u>
June	19	1			142	3	2						
June	20				100	8	1		1	1			
June	27				46	3	2		1				
July	5				98				1	1			
July	6				96		6		5				
July	7			1	54		1		2				
July	12				39				3				
July	14		2		50	1	1	1	14	1			
July	15		1		25	5			4	1			1
July	17		3		82	8	3			1			
July	18		6		78	5			9				
July	24			3	59	3	3	1	13	1			
July	25				59	8	6		11				
July	28			2	49	11	3		26	1	1		
August	4	1			20	2			18	4	1	1	
August	6			1	29	6	4		40	2	3		
August	7				26		2	7	18			1	
August	9				22	1	3		12	1			1
August	11				8		4		19	1			

- Table 16 -

Mosquito larval collections at the project site- 1978

Date	<u>Ae. cinereus</u>	<u>Ae. stimulans</u>	<u>Ae. sticticus</u>	<u>Ae. vexans</u>	<u>An. punctipennis</u>	<u>C. pipiens</u>	<u>C. restuans</u>	<u>C. territans</u>	<u>C. inornata</u>
April 15	x	x	x						
April 21	x	x	x						
May 2		x	x						
May 16				x					
June 23							x	x	x
June 29					x		x	x	x
July 4								x	x
July 13					x	x		x	
July 19					x	x		x	
July 26						x	x	x	
August 2						x	x	x	

- Table 17 -

List of mosquito species collected at the project site

Mosquito Species	Biting Collection	Larval Collection
<u>Ae. canadensis</u>	x	
<u>Ae. cinereus</u>	x	x
<u>Ae. hendersoni</u>	x	
<u>Ae. stimulans</u>	x	x
<u>Ae. sticticus</u>	x	x
<u>Ae. triseriatus</u>	x	x
<u>Ae. trivittatus</u>	x	
<u>Ae. vexans</u>	x	x
<u>An. punctipennis</u>	x	x
<u>An. quadrimaculatus</u>	x	
<u>C. perturbans</u>	x	
<u>C. pipiens</u>		x
<u>C. restuans</u>		x
<u>C. territans</u>		x
<u>C. inornata</u>	x	x
* <u>Ps. horrida</u>	x	
<u>Ps. ferox</u>	x	
* new state record		

New England to the Pacific Coast. It is less abundant in the extreme south. This species has been found to be naturally or experimentally infected with several arboviruses included St. Louis encephalitis (SLE), eastern equine encephalitis (EEE), western equine encephalitis (WEE), and California encephalitis (CE) (Chamberlain, 1958; Davis, 1940; McLintock and Burton, 1967; Olson et al., 1961; Sudia et al., 1968 and 1971; Center for Disease Control Publ. no. 77-8140, 1977; and Wallis et al., 1960). It is also considered a potential vector of dog heartworm, Dirofilaria immitis (Bemrick and Sandholm, 1966; Ludlam et al., 1970; and Jankowski and Bickley, 1976).

Adults of this multivoltine mosquito species migrate long distances from their breeding places, 5 to 10 miles being rather common (Horsfall et al., 1973). They are vicious biters and are especially annoying at dusk and after dark.

Aedes sticticus: The adults and larvae of this mosquito species were found in large numbers at the project site during the mosquito season in 1978. They have never before been found in this project area and I believe this is a species that has recently moved into this sprayed woodlot. Larvae were collected in large numbers in floodwater pools, in mid to late spring. Adults were captured primarily in June and July.

Like Ae. vexans, this is also a floodwater mosquito species that occurs throughout most of the United States. It is an important pest mosquito with an extensive flight range (Twinn, 1931). The females are ferocious biters during the evening, and also during the day in

cloudy or shaded situations. They feed readily on livestock, particularly on horses and cattle, and man (Twinn, 1931). There is usually one brood annually. Adults may live as long as three months.

Aedes cinereus: Adult females and larvae of this mosquito species were collected in small numbers at the project site. Larvae were collected, in early spring, in the four shallow woodland pools and margins of the two semipermanent ponds at the project site. Adults were collected exclusively in the sprayed zone.

This mosquito species occurs sparsely throughout most of the United States. It is occasionally important as a pest in some of the northern states. The flight range is limited (Barr, 1958) and it is usually found in the woods near its larval habitats. There is usually one brood annually.

Anopheles punctipennis: A relatively large number of adults and larvae of this mosquito species was found in the project site. Larvae were collected in the semipermanent ponds in the wooded area. Adults were taken throughout the summer months. The adults apparently have a tendency to aggregate in the nonsprayed zones.

This is the most widely distributed anopheline in North America, being found from coast to coast from southern Canada to Mexico except in the rocky Mountains (Matheson, 1944). It is a rather vicious biter out-of-doors, but apparently does not enter homes as readily as does An. quadrimaculatus. This species is not known to be a natural vector of malaria although it may be infected in the laboratory. It is,

however, a potential vector of dog heartworm (Feng, 1930; Yen, 1938; and Phillips, 1939).

Culex spp.: Larvae of the three species of Culex; C. pipiens, C. restuans, and C. territans, were found in great numbers at the project site in the two semipermanent ponds as well as one produced mainly by sewage sprayed water. These species had not been found at the project site before, and it is likely that they have become established in this area due to the wastewater irrigation operations.

Mosquitoes of the Culex pipiens complex breed prolifically in rain barrels, tanks, tin cans, and practically all types of artificial containers. Heavy production of these mosquitoes is often found in water with high organic content, especially in storm-sewer catch basins and effluent drains from sewage disposal plants.

The females of the Culex pipiens complex show a preference for avian blood, but appear to accept mammalian hosts as well. They are considered one of the best vectors of St. Louis encephalitis virus in the United States. Feeding is usually restricted to hours of darkness, peaking during crepuscular periods and occurs inside or outside of dwellings. The overwintering stage is the adult female. These mosquitoes usually migrate only short distances.

Culex restuans: Ordinarily breeds in static water containing decaying grass or leaves. Favored breeding places are rain barrels, tin cans, woodland pools, and ditches. It appears early in the season and continues breeding throughout the summer. Although they have

preference to avian blood, this species is an occasional feeder on man, usually out-of-doors beginning at dusk. Both, C. restuans and C. pipiens are considered to be vectors of St. Louis encephalitis virus (CDC- Vector Topics, 1976).

Culex territans: Ordinarily breeds in semipermanent and permanent pools in streams, swamps, and ponds. The females are not known to bite man but have been observed feeding on cold-blooded vertebrates, particularly frogs (Carpenter and LaCasse, 1955). The adults are seldom taken in buildings but are encountered occasionally among vegetation and in shelters near their larval habitats. The females apparently pass the winter in hibernation in the cooler areas of the United States.

Culiceta inornata: This is another mosquito species which apparently has recently established in the project area. Large numbers of larvae were collected in the two semipermanent ponds in the wooded area.

This is a large mosquito reported from almost all states except in upper New England. It does not readily attack man, but it does feed on domestic animals and is sometimes a considerable annoyance to livestock (Yates, 1953). This mosquito has been found naturally infected with western equine encephalitis virus.

Ae. canadensis, Ae. trivittatus, An. quadrimaculatus, C. perturbans Ps. horrida, and Ps. ferox were also collected in the project site. However, they were taken in such small numbers that at this stage there appears to be no real potential health threat generated by them.

On the other hand, mosquito species like Ae. vexans, C. pipiens, C. inornata, and An. punctipennis which were not breeding in this area, but are now produced in large numbers, are known vectors of several arboviruses and other pathogens. The large numbers of these mosquitoes that could result from the numerous and varied breeding sites now available at the project site would greatly enhance the potential for outbreaks of these mosquito-borne diseases should the pathogens now be enzootic or later introduced into the area.

It is premature to predict with assurance the future effects of sewage water spray irrigation in the spray irrigation complex in terms of health hazards, but, present evidence indicates that spraying does produce an abundance of mosquitoes that may cause discomfort to neighboring communities. In the future there will likely be a need for mosquito control measures in these communities and, perhaps, restricted use of the recreation facilities now being contemplated for the project site.

- Appendix II -

Ae. triseriatus and Ae. hendersoni: Characterization
of larvae, larval hybrids, and differentiation
of the two species based on the surface
structure of the egg shell.

Appendix II

Ae. triseriatus and Ae. hendersoni: Characterization of larvae, larval hybrids, and differentiation of the two species based on the surface structure of the egg shell.

INTRODUCTION

Ae. hendersoni is a sibling species, closely related to Ae. triseriatus. The close similarities in adult morphology and breeding habitats of the two species has necessitated great caution in identifying specimens of each species. This is particularly important in studies concerned with mosquito-borne diseases.

Because of the relative importance of these two species in the study area and the possibility that hybridization could have important implications in evaluating their vector potentials, a study was initiated to identify and characterize populations of the two species in the project site, to determine if hybridization occurred, and if so, to what extent.

The generally accepted method to differentiate the two species is the examination of the 4th instar larvae. Since my research protocol called for the number of eggs laid by each species, necessitating the examination of large numbers of eggs, an investigation was made to determine if surface features also could be used to differentiate field collected eggs of these two species and their hybrids.

MATERIALS AND METHODS

In order to characterize the population of the two species in the project site, a number of larval morphological characters which have proven to be of value for comparative studies (Truman and Craig, 1968; and Grimstad et al., 1974) were examined. These characters were: 1) acus; 2) the lateral hair of segment X, located along the posterior margin of the saddle, 3) gill length and shape, 4) ventral brush characteristics (number of pairs and number of branches), 5) siphonal tuft (Figure 26). Gill length was categorized as unequal (UE), equal (EQ), or subequal (SE) (Figure 27A). Acus was categorized as well attached (WA), attached (A), lightly attached (LA), detached (D), or well detached (WD) (Figure 27B).

Fourth instar larvae reared from weekly ovitrap collections were used for this study and total of 50 to 100 larvae were examined each week. Results were tabulated and the percentage occurrence of each character in the population of the two species was calculated. When a certain character at one side of the larva was different from the other side (e.g. branches of siphonal tuft) both readings were recorded and averaged for each population.

To study the morphological features of the egg shells of the two species, both light and scanning electron microscope were used. The eggs examined in this study were obtained from two sources: (1) those laid by wild females in ovitraps placed in the project site, and (2) those obtained from colonies which originated from eggs collected at the same location. Eggs were stored for a minimum of seven days and then hatched, after which some were dried for examination in the

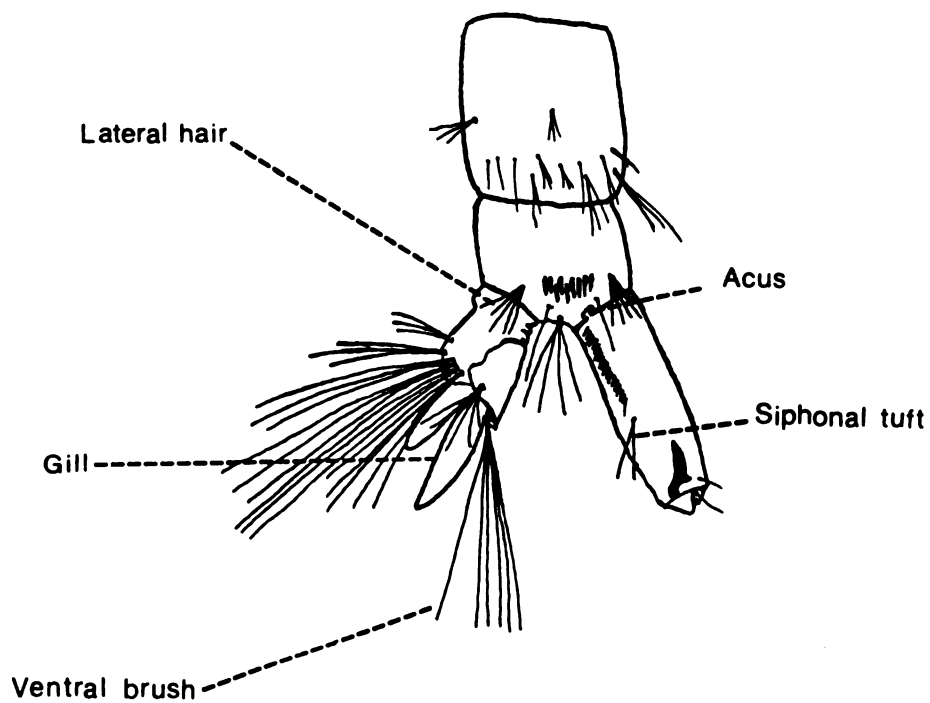


Figure 26. Terminal segments of an Aedes larva.

Figure 27:

- A) Gill characteristics used in separating the two species. UE (unequal); SE (subequal); EQ (equal).
- B) Position of acus used in separating the two species. WA (well attached); A (attached); LA (lightly attached); D (detached); WD (well detached).

scanning electron microscope. The remaining hatched eggs were cleared using the method of Craig (1955). This simply involved placing cuts of paper toweling with eggs attached to them in a watch glass with some crystals of potassium chlorate. Then concentrated hydrochloric acid was dropped on the crystals. This operation was done under the hood to remove the free chlorine produced in the resulting chemical reaction. After few minutes, eggs were removed, placed on microscope slides and examined with a light microscope at 100X.

For scanning electron microscopy, pieces of paper toweling with air dried eggs were fixed to specimen stubs with Television Tube Koat, a conductive carbon compound (GC Electronics, Illinois). The material was then coated with gold in a sputter coating apparatus and examined in a Supper Mini SEM (International Scientific Instruments, California).

In order to study the morphological characteristics in the F_1 larval hybrids, males and females of Ae. triseriatus and Ae. hendersoni were reared in separate cages. Ten to fifteen-day old females were mated by the forced copulation technique of McDaniel and Horsfall (1957). The males were very lightly anesthetized with CO_2 , decapitated, and held by the thorax, using a forceps. The females were also lightly anesthetized with CO_2 and held by the thorax with a suction tube. The vacuum was created by a water driven pump.

Copulation took place when the tips of the abdomen of the male and the female touched. Several hours after the copulation, the females were blood fed and placed in separate one pint cardboard ice cream containers, and a ten percent sugar solution was provided in each container. The bottoms of the containers were cut, so that a

150 ml beaker, lined with brown paper toweling, could be halfway passed through. Eggs were deposited on the paper toweling 6-7 days after the blood meal. They were kept moist for at least a week, after which they were hatched in separate rearing pans. The morphological characteristics of the F_1 larval hybrids were studied, as described above.

RESULTS AND DISCUSSION

Population of Ae. triseriatus, as reported by Grimstad et al. (1974), are characterized by usually having attached acus, 5-branched lateral hair, 2-branched siphonal tuft, unequal gill length, and 6 pairs of 3-to multiple-branched ventral brush hairs. However, in Ae. hendersoni populations, well detached acus, 2-and 3-branched lateral hairs, 3-branched siphonal tuft, equal gill length, and 5 pairs of 2-to 3-branched ventral brush hairs predominated. Their laboratory produced hybrids exhibited predominant percentages of several characteristics that were intermediate between those of the parent stock. Based on this criteria, of 1500 field collected larvae I examined, 1100 were included under Ae. triseriatus and the other 400 under Ae. hendersoni. No hybrids were found in the population examined.

Tables 18 and 19 list the percentage of larvae showing each condition of variation for the acus, lateral hair, siphonal tuft, gill length and ventral brush, for the field collected populations of Ae. triseriatus and Ae. hendersoni.

Of 1100 Ae. triseriatus larvae examined, the attached acus predominated (46.2%) followed by well attached acus (33.9%). A small

- Table 18 -

Summary of larval characteristics in Ae. triseriatus and
Ae. hendersoni

Population	No. larvae exam- ined	Lateral hair: no. branches									Acus*				
		2	3	4	5	6	7	8	9	10	WA	A	LA	D	WD
		(%)													
<u>Ae. triseriatus</u>	1100	-	0.3	5.4	25.3	35.1	26.2	6.3	1.2	0.2	33.9	46.2	19.2	0.7	-
<u>Ae. hendersoni</u>	400	16.1	76.3	7.6	-	-	-	-	-	-	-	-	-	-	100
F ₁ hybrid: lab- oratory rear- ed. **	150	-	8.3	49.6	32.3	9.8	-	-	-	-	12.5	20.3	60.9	4.7	1.6

* WA (well attached); A (attached); LA (lightly attached); D (detached); WD (well detached)

** Male Ae. triseriatus X female Ae. hendersoni

- Table 19 -

Summary of larval characteristics in Ae. triseriatus and
Ae. hendersoni

Population	No. larvae exam- ined	Siphonal tuft				Gill length*		Pairs of hairs		Number of branches	
		number of branches				EQ	SE (%)	5	6 (%)	2-3	3-multiple (%)
		1	2	3	4						
<u>Ae. triseriatus</u>	1100	1.7	96	2.3	-	-	-	-	100	-	100
<u>Ae. hendersoni</u>	400	-	4.3	58.1	34.2	100	-	100	-	77.9	-
F ₁ hybrid: lab- oratory rear- ed. **	150	-	38.3	59.6	2.2	11.8	70.6	48.5	51.5	68.4	31.6

* EQ (equal); SE (subequal); UE (unequal)

** Male Ae. triseriatus X female Ae. hendersoni

percentage (19.2%) of the population showed lightly attached acus. Also, 86.6% of the population examined had 5-7 branched lateral hair, but ranged from 3 to 10. Gill length was unequal in all specimens and the dorsal pair was longer than the ventral pair. Both pairs of gills tapered with lightly pointed ends. In addition, all Ae. triseriatus larvae examined had six pairs of ventral brush hairs that were always from 3-to multiple-branched. Ninety six percent of the larval population had 2-branched siphonal tuft with a small percentage showing 1 (1.7%) or 3 (2.3%) branches.

The larval population of Ae. hendersoni, on the other hand, exhibited predominantly 3-branched lateral hair, 3-4 branched siphonal tuft, and the acus was always well detached. There were 5 pairs of lateral brush hairs with almost 80% of these hairs 2-or 3-branched. Twenty two percent of the hairs, however, were 4-branched. The sausage shaped anal gills were long and equal in length.

Although no hybrids were found in the field population examined, 150 larvae of the hybrid F_1 crosses, made in the laboratory for the study of egg surface morphology, were examined. Since the gene flow between populations of Ae. triseriatus and Ae. hendersoni is partially blocked by the production of males with abnormal genitalia in the crosses of female Ae. triseriatus to male Ae. hendersoni (Truman and Craig, 1968) only larvae from the crosses of male Ae. triseriatus to female Ae. hendersoni were used for this study. Results of such crosses are also included in Tables 18 and 19.

The lightly attached acus, 4-or 5-branched lateral hair, 2-or

3-branched siphonal tuft predominated in larvae resulting from crosses between males of Ae. triseriatus and females of Ae. hendersoni. Gill length was mostly intermediate between the equal type in Ae. hendersoni and the unequal type in Ae. triseriatus. Unlike what has been reported by Grimstad et al. (1974), hybrids had almost a 1:1 ratio of 5 or 6 pairs of ventral brush hairs with 68.4% of them exhibited predominantly 2-3 branched hairs.

It seems that accurate separation can be made between populations of Ae. triseriatus and Ae. hendersoni, based on larval characters. However, this would be much more difficult when hybrids are present in the population. Crossing experiments between these two species have shown that some of the differences are possibly due to single genes and hybrids are likely to be phenotypically similar to one of the parent species and could be overlooked if one were searching for an intermediate form in the field (Truman and Craig, 1968). Establishing percentage frequencies for the above characteristics probably would enable one to detect possible hybridization in the field populations much easier. With known ranges for various characteristics established, hybrid larvae should also be easier to detect as individuals.

Eggs of some aedine mosquitoes are diagnostic and have been described and illustrated by Craig and Horsfall (1960), Horsfall and Craig (1965), Hinton and Service (1969) and Kalpage and Brust (1968). There also seems to be useful characters in the ornamentation of Ae. triseriatus and Ae. hendersoni eggs which can be used to separate the two species very easily.

Figure 28. Chorionic pattern of egg shells under light microscope (290X):

(A) Ae. triseriatus

(B) Ae. hendersoni- note the pattern of reticulation and the rosette cell (arrow).

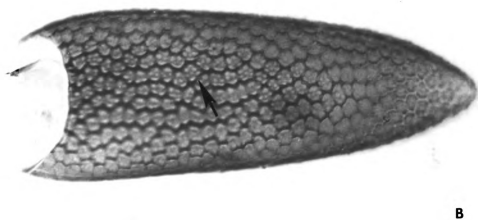
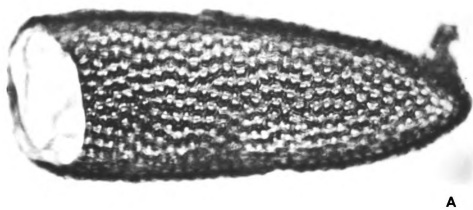


Figure 28

Figure 29. Surface sculpturing under SEM:

- (A) Ae. triseriatus (200X)
- (B) Ae. hendersoni (200X)
- (C) Ae. triseriatus (600X)
- (D) Ae. hendersoni (600X)
- (E) Ae. triseriatus (1200X)
- (F) Ae. hendersoni (1200X)- box shows the reticulation
around 1 hexagonal cell and the angular
projections of that cell.

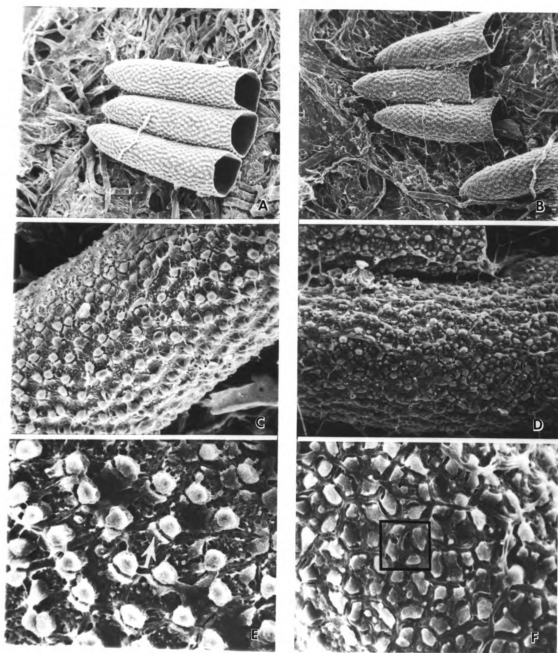


Figure 29

Eggs of Ae. hendersoni viewed through the light microscope have a reticulate pattern forming irregularly shaped cells on the surface. Each cell also appears to be further subdivided by several projections which give it a rosette appearance (Figures 28 B, arrow). In the SEM, the egg surface of Ae. hendersoni bears a dense cover of protrusions which, even at lower magnification, appear to be subdivided (Figure 29 B). At higher magnification, reticulations are visible and form irregular polygonic cells (Figure 29 F, box). These are often hexagonal in shape. Inside these cells there usually are 2 to 6 irregularly shaped projections which have uneven surfaces. Inner projections, when present, are smaller than the outer ones, which often bear minute protrusions at their corners.

Eggs of Ae. triseriatus have a reticulate pattern when viewed through the light microscope (Figure 28 A). The reticulations form irregularly shaped cells on the egg surface. In the SEM, eggs appear to be covered by numerous, somewhat uniformly spaced papillae (Figure 29 A). These papillae are spherical to ovoid in shape. At higher magnifications (Figure 29 E), it is evident that each papillae has a rough surface and most papillae are partially surrounded by a flattened, curved protrusions (Figure 29 E, arrow) whose surface appears similar to that of the papillae. Reticulations, although sometimes obscure, connect the papillae unless 2 of the papillae are closely situated.

- Appendix III -

Natural infection of the tree hole breeding mosquito
Aedes triseriatus (Diptera: Culicidae) with the
fungus Funicularius triseriatus.

Appendix III

Natural infection of the tree hole breeding mosquito
Aedes triseriatus (Diptera: Culicidae) with the
fungus Funicularius triseriatus.

INTRODUCTION

In the course of studying the population of Ae. triseriatus at the Michigan State University Water Quality Management Project site, a female mosquito of this species was found infected with a fungus. This female mosquito was being attracted to an ovitrap which was used to collect eggs of this mosquito species. She was attached, in a lifelike position, to the tongue depressor which was left in the ovitrap on which mosquitoes were to lay their eggs. The golden-colored hyphae projecting from the thorax and abdomen had fastened the insect to the tongue depressor (Figure 30 A). The identity of the fungus was a matter of interest as it represented a potential biocontrol agent.

Jenkins (1964) in his annotated list and bibliography of pathogens, parasites and predators reported 47 fungal species as pathogens of mosquitoes. The most widely known entomogenous fungi are found in the genera Coelomomyces, Entomophthora, Cordyceps, Aspergillus, Beauveria, and Metarhizium. Cordyceps has not been reported from mosquitoes. Roberts (1970) summarized some of the more recent information concerning Beauveria, Entomophthora, Metarhizium, and Coelomomyces and Chapman (1974) reviewed fungi parasitizing mosquitoes in his review

of biological control agents of mosquito larvae. Insect diseases caused specifically by Hyphomycetes were reviewed by Madelin (1963) who later summarized diseases on the basis of fungal classes (1966).

The purpose of this report is to document the natural occurrence of a Hyphomycete (Fungi imperfecti) on the tree hole breeding mosquito, Ae. triseriatus, as well as describing a new genus and species of fungus, infecting mosquitoes.

MATERIALS AND METHODS

Fungal culture: The fungus was isolated by streaking the infected mosquito across a petri dish of amended corn meal agar (1.7% corn meal agar, 0.3% sucrose, 0.2% glucose and 0.1% yeast extract). The fungus was maintained on PDA (Difco Laboratories, Detroit, MI) and transferred every two weeks. The fungus was grown on two additional media, YpSs (Benjamin, 1959) and 2% malt extract agar, to provide morphological information .

Light microscopy: Photographs of the infected mosquito were taken with Kodak Kodachrome 64 film. Hyphae were mounted in either water or 0.5% lactic acid and photographed with Kodak Panatomic X film using a Zeiss Photomicroscope II.

Scanning electron microscopy: The fungal hyphae and conidiophores were fixed in 5% gluteraldehyde in 0.1 M phosphate buffer for two hours on ice, washed twice in the same buffer, followed by post-fixation in 1% osmium tetroxide. Fixed tissues were dehydrated in a graded ethanol series and critical point dried. Samples were then

examined in a ISI Super-III SEM.

RESULTS AND DISCUSSION

The fungus was determined to belong in the class Hyphomycetes, however, it could not be placed in any of the common entomogenous genera. The conidiogenous cells were not true phialides as in Metarhizium and Hirsutella, nor were the conidia round as in Beauveria. In addition, the fungus could not be assigned to any of the saprophytic genera in the Hyphomycetes. This fungus has been designated as a new genus and species in the class Hyphomycetes.

FUNICULARIUS Baker and Zaim gen. nov.

The hyphomycete colonies had smooth, regularly septate hyphae with a conspicuous funiculose habit. The conidiophores were usually short, solitary on hyphae or mostly grouped on funicles. Conidia were thallic arthrospores, irregularly cylindrical to allantoid and smooth-walled.

Type species: Funicularius triseriatus

Etymology: from the conspicuous funiculose habit of the hyphae.

FUNICULARIUS TRISERIATUS Baker and Zaim sp. nov.

The hyphomycete colonies on PDA (Difco) were yellow-brown to golden in color. The reverse of the colonies was also yellow-brown. The colonies grew very rapidly at 25° C producing abundant aerial mycelium. The odor of the colonies was indistinct.

The vegetative hyphae was smooth and regularly septate with a conspicuous funiculose habit (Figure 30 B, C), The conidiophores were

Figure 30. Ae. triseriatus and the fungus F. triseriatus:

- (A) Ae. triseriatus with fungal hyphae protruding from thorax and abdomen (17X).
- (B) Light micrograph of conidia and conidiophores of F. triseriatus grouped on funicles (1100X).
- (C) Scanning electron micrograph (SEM) of conidia and conidiophores of F. triseriatus (1100X).
- (D) SEM of thallic-arthric conidia of F. triseriatus. (3000X).

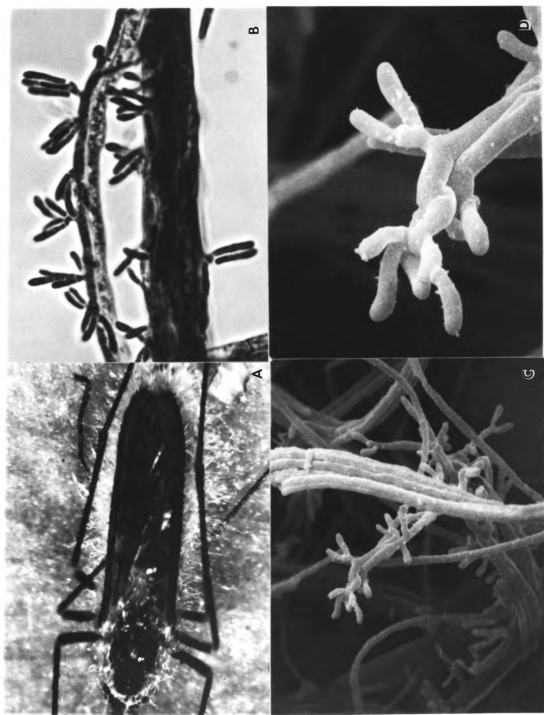


Figure 30

short conidiophores branches. Conidia were cylindrical to allantoid, 1.5-3.0 μm X 7.5-13.5 μm , smooth-walled and hyaline.

Type locality: East Lansing, Michigan

Etymology: species discovered on Ae. triseriatus

Type culture: Cultures have been deposited at the National Fungus Collection (Holotype # BPI 71907) and the USDA Culture Collection at Peoria, Illinois.

This is the first report of a fungus found on Ae. triseriatus in nature. As with many hyphomycetous species of fungi, it has not been proven as to whether this fungus is truly parasitic or just saprophytic. Further laboratory and field studies of Funicularius triseriatus may yield important information on this point as well as forming the basis for an analysis as to whether the fungus can be considered as a feasible biological control for Ae. triseriatus.

Appendix IV

Aedes triseriatus oviposition activity in 1976

- Appendix IV -
Aedes triseriatus oviposition activity in 1976

- Table A1 -
(Plot A)

[illegible]

Appendix IV—continued- Table A2 -
(Plot B)

Trap no.	Date									
	June		July				August			
	25-27	2-4	9-11	16-18	23-25	30-1	6-8	15-17	20-22	27-29
25	103				79			87		
26								63		
27										
28			52	70		44				
29										
30			21							
31										
32										
33										
34										
35										
36			136						83	
37										
38			100	23	37	23			107	125
39										
40										
41					194					
42										
43										
44				96						
45										
46						90		68	88	
47										19
48		62								

Appendix IV--continued

- Table A3 -
(Plot E)

[illegible]

[illegible]

Appendix IV--continued

- Table A5 -
(Plot G)

[illegible]

Appendix V

Aedes triseriatus oviposition activity in 1977

- Appendix V -
Aedes triseriatus oviposition activity in 1977

- Table B1 -
(Plot A)

[illegible]

Appendix V— continued

- Table B2 -
(Plot B)

Trap no.	Date												
	June				July				August			Sept.	
	8-10	15-17	22-24	29-1	9-11	13-15	20-22	27-29	3-5	10-12	17-19	24-26	31-2
25													
26													
27												2	
28													
29							11						
30			114									4	
31			121				30	29	43				
32			148					17					
33			121										
34			98										
35			97				45						
36													
37													5
38			95		49								
39													
40													
41													
42													
43		68											
44			34										
45													
46													
47			122										
48			30			95							

Appendix V-- continued

- Table B3 -
(Plot E)

[illegible]

Appendix V— continued

- Table B4 -
(Plot F)

Trap no.	Date												
	June				July				August			Sept.	
	8-10	15-17	22-24	29-1	9-11	13-15	20-22	27-29	3-5	10-12	17-19	24-26	31-2
73			149	14				60	13		11		
74			227	44							106		
75				115			20		7				
76								80					
77											45	50	
78				35						78			
79		70					59						
80			79		67								
81													
82													
83			48						75				
84													
85							63	56					
86									5		24		
87		108											4
88			132									3	2
89			60			54	87						
90					130	3							
91			10			88	84						
92			77										
93													
94			55					62				50	
95				161			137						
96					101	83		30	9				

Appendix V-- continued

- Table B5 -
(Plot G)

Trap no.	Date												
	June				July				August			Sept.	
	8-10	15-17	22-24	29-1	9-11	13-15	20-22	27-29	3-5	10-12	17-19	24-26	31-2
97							70						
98													
99						105							
100								25	8			20	
101												10	
102													
103			89				57						
104													
105										83			
106			140					59					45
107		96	289	154			43	202			38	34	
108						45	92	59					
109				104		3							
110			107				56		23	15			
111		45	80										
112									93				
113					60	34		19	22			14	
114				31			49						
115													
116					45								
117													
118			20		7			69		24			
119				199				75	55	60	30		
120						88		124				4	

Appendix VI

Daily temperatures (1977)

Appendix VI

Daily temperatures (1977)

Date		Maximum				Minimum			
		East Lansing	Block			East Lansing	Block		
			A	B	E		A	B	E
July	20	91	89	89	86	73	75	75	72
July	21	92	79	79	78	72	69	69	71
July	22	84	75	75	74	56	60	60	60
July	23	95	79	79	77	52	57	57	57
July	24	87	76	76	73	59	62	62	61
July	25	82	76	76	74	52	53	52	52
July	26	78	75	72	71	49	57	57	57
July	27	75	76	72	74	45	52	49	52
July	28	79	76	73	76	55	59	57	59
July	29	82	69	66	68	64	64	61	63
July	30	75	75	72	76	61	64	61	64
July	31	82	82	80	80	62	67	66	67
August	1	83	77	74	76	55	61	59	61
August	2	78	77	75	70	53	57	55	57
August	3	80	78	76	76	60	63	63	63
August	4	81	84	77	82	63	67	63	66
August	5	88	80	79	74	65	67	65	67
August	6	84	74	76	74	68	70	68	69
August	7	78	76	78	75	68	68	70	70
August	8	80	75	77	74	69	68	70	70
August	9	78	67	72	72	65	65	70	67
August	10	78	78	80	77	66	66	68	68
August	11	82	67	68	67	62	58	60	59
August	12	69	72	73	71	50	53	54	54
August	13	75	79	78	77	54	56	57	57
August	14	80	73	72	73	59	65	64	65
August	15	75	75	72	75	50	56	54	56
August	16	79	78	76	74	56	65	64	65
August	17	80	73	72	71	57	60	59	61
August	18	75	68	66	67	45	50	49	50
August	19	69	70	68	65	42	48	46	48
August	20	72	70	67	69	41	48	47	49
August	21	74	66	64	66	45	61	59	61
August	22	67	73	72	72	52	56	54	56
August	23	75	67	64	65	52	57	55	58
August	24	72	68	66	68	48	64	52	54
August	25	72	70	68	70	44	50	49	51
August	26	75	76	73	74	40	55	53	56

Daily temperatures—continued

Date	Maximum				Minimum			
	East Lansing	Block			East Lansing	Block		
		A	B	E		A	B	E
August 27	82	87	86	85	51	70	68	70
August 28	91	86	85	85	73	74	72	74
August 29	90	74	73	73	63	66	64	66
August 30	78	73	71	71	56	61	60	61
August 31	77	82	81	80	60	63	62	63
Sept. 1	87	75	74	74	63	68	68	70
Sept. 2	78	76	74	73	65	67	66	67
Sept. 3	78	70	68	71	58	61	60	62
Sept. 4	74	77	75	75	54	59	57	69
Sept. 5	79	71	69	71	57	64	62	64
Sept. 6	72	70	68	71	50	55	54	55
Sept. 7	76	74	72	70	54	59	58	60
Sept. 8	78	75	72	74	58	63	61	63
Sept. 9	78	78	75	71	59	64	61	63
Sept. 10	81	66	63	65	54	58	55	57
Sept. 11	66	70	65	68	46	52	49	51
Sept. 12	72	68	65	67	48	54	51	53
Sept. 13	70	60	58	61	53	56	54	57
Sept. 14	60	65	61	65	47	51	49	51
Sept. 15					49	54	51	54

Appendix VII

Aedes triseriatus oviposition behavior
in varying water-salt concentrations

- Appendix VII -
Aedes triseriatus oviposition behavior
in varying water-salt concentrations

Code *	Oviposition (in percentages)												Treatment total
	Repl. I	Repl. II	Repl. III	Repl. IV	Repl. V	Repl. VI	Repl. VII	Repl. VIII	Repl. IX	Repl. X			
N ₀ C ₀	0.71	9.76	6.9	13.0	20.5	17.07	8.76	20.19	8.67	11.0			116.56
N ₀ C ₁	21.04	0	0	17.1	29.6	0	12.29	4.26	13.39	15.7			113.38
N ₀ C ₂	38.06	0	7.4	22.4	8.6	9.78	12.23	24.11	5.78	24.1			152.46
N ₁ C ₀	11.11	29.27	0	14.5	30.5	20.00	21.79	7.2	22.85	14.5			171.72
N ₁ C ₁	5.44	48.78	81.4	12.5	0.8	13.26	19.07	19.67	4.92	7.0			212.84
N ₁ C ₂	2.6	2.44	2.6	4.4	4.9	14.02	4.54	2.61	9.85	16.8			64.76
N ₂ C ₀	11.11	0	0	4.6	2.2	20.98	11.43	2.09	5.12	5.8			63.37
N ₂ C ₁	8.75	0	0	2.3	2.4	1.3	6.25	9.92	2.82	1.8			35.64
N ₂ C ₂	1.18	9.76	1.7	9.2	0.5	3.59	3.63	9.92	26.59	3.3			69.37

* The code represents different concentrations of sodium chloride (C) and calcium nitrate (N) used in this study. C₀ to C₂ represent the three concentrations of NaCl and N₀ to N₂ represent the 3 concentrations of (NO₃)₂ Ca. Specific concentrations are included on page 36 . C₂ and N₂ represent the highest concentrations.

Appendix VIII

Larval development of Aedes triseriatus as
affected by water salinity

- Appendix VIII -
 Larval development of Aedes triseriatus as
 affected by water salinity

Code**	Larval mortality*				Treatment total
	Repl. I	Repl. II	Repl. III	Repl. IV	
C ₇ N ₅	5	0	4	0	9
C ₇ N ₄	5	0	1	3	9
C ₇ N ₃	1	0	0	4	5
C ₇ N ₂	4	1	3	1	9
C ₇ N ₁	5	4	0	2	11
C ₇ N ₀	2	2	2	4	10
C ₆ N ₅	0	2	0	1	3
C ₆ N ₄	0	3	0	1	4
C ₆ N ₃	2	2	1	0	5
C ₆ N ₂	0	2	0	2	4
C ₆ N ₁	0	2	2	2	6
C ₆ N ₀	0	6	1	2	9
C ₅ N ₅	0	0	0	4	4
C ₅ N ₄	0	1	0	1	2
C ₅ N ₃	1	4	0	3	8
C ₅ N ₂	2	3	2	4	11
C ₅ N ₁	1	7	0	2	10
C ₅ N ₀	0	1	0	3	4
C ₄ N ₅	0	2	1	0	3
C ₄ N ₄	0	2	1	3	6
C ₄ N ₃	1	0	3	2	6
C ₄ N ₂	0	1	1	1	3
C ₄ N ₁	1	3	1	0	5
C ₄ N ₀	3	1	2	3	9
C ₃ N ₅	0	0	1	0	1
C ₃ N ₄	0	0	1	1	2
C ₃ N ₃	0	2	1	4	7
C ₃ N ₂	1	3	1	2	7
C ₃ N ₁	1	2	0	1	4
C ₃ N ₀	1	1	2	0	4

Appendix VIII--Continued

Code**	Larval mortality*				Treatment total
	Repl. I	Repl. II	Repl. III	Repl. IV	
C ₂ N ₅	0	1	1	2	4
C ₂ N ₄	0	3	2	0	5
C ₂ N ₃	1	3	0	0	4
C ₂ N ₂	0	3	2	1	6
C ₂ N ₁	2	0	0	0	2
C ₂ N ₀	0	1	0	2	3
C ₁ N ₅	0	0	1	4	5
C ₁ N ₄	0	0	1	1	2
C ₁ N ₃	1	2	1	3	7
C ₁ N ₂	0	0	2	2	4
C ₁ N ₁	0	1	0	5	6
C ₁ N ₀	0	3	1	0	4

* 10 larvae per treatment replicate

** The code represents different concentrations of sodium chloride (C) and calcium nitrate (N) used in this study. C₁-C₇ represent the seven concentrations of NaCl and N₀-N₅ are the 5 concentrations of (NO₃)₂ Ca. Specific concentrations are included on page 37. C₇ and N₅ represent the highest concentrations of the chemicals.

LIST OF REFERENCES

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