

tions on wolbachiae in mosquitoes. J. Invertebr. Pathol. 35:200-208.

Yen, J. H. 1975. Transovarial transmission of *Rickettsia*-like microorganisms in mosquitoes. Ann. N. Y. Acad. Sci. 266:152-161.

Yen, J. H. and A. R. Barr. 1973. The etiologic agent of cytoplasmic incompatibility in *Culex pipiens*. J. Invertebr. Pathol. 22:242-250.

TILLAGE—A NONCHEMICAL METHOD FOR THE CONTROL OF FLOODWATER MOSQUITOES

JOSEPH C. COONEY¹, EUGENE PICKARD¹, JOHN W. UPTON¹ AND BOBBY R. McDUFF¹

ABSTRACT. Studies were conducted both in the field and laboratory to investigate tillage as a means of controlling the production of floodwater mosquitoes, primarily *Aedes vexans*, in grassy depressions and river floodplains. Tractor plowing followed by disking produced control of mosquitoes ranging from 73 to 100 percent. This method of tillage displaced mos-

quito ova from the top 12.0 mm of the soil substrate to depths of 126 mm or more thereby trapping larvae in the soil and preventing their further development and emergence as adults. Laboratory studies confirmed that this action produced the control effect since essentially all eggs in all tests hatched, yet larval emergence decreased as soil cover over the eggs increased.

INTRODUCTION

The concept of integrated pest management (IPM) has recently received renewed attention and emphasis as the preferred approach to pest control. Federal agencies having pest control responsibilities have been especially involved due to the recent presidential directive requiring the development and implementation of IPM in control strategies.

The Tennessee Valley Authority (TVA) since its inception in 1933 has approached mosquito control in this fashion, coordinating control activities in a truly integrated manner in which the application of pesticides is used as a supplement to the various other elements of control. This integrated approach to mosquito control is described in the 1947 manual entitled, "Malaria Control on Impounded Waters" prepared and published jointly by TVA and the United

States Public Health Service. Most of the techniques and procedures described and utilized at that time are directly applicable today. Unfortunately, however, many control planners have elected, due to financial limitations, to forego integrated pest management for the sake of initially less costly and more efficient pesticide based programs.

Integrated pest management as the basis for mosquito control on the TVA system of multipurpose reservoirs includes the application of the following preimpoundage measures: reservoir basin clearing, shoreline modifications (cut and fill), operation of dewatering units, establishment of positive drainage systems, and development of a water level management schedule compatible with mosquito control. Postimpoundage measures include: mosquito population surveillance in critical areas, water level management, drainage maintenance, mechanical control of marginal vegetation, and finally larvicidal or adulticidal application.

Other mosquito abatement workers

¹ Tennessee Valley Authority, Office of Natural Resources, Fisheries and Aquatic Ecology Branch, Knoxville, TN 37902.

have utilized similar techniques to some extent and continue to investigate other potential non-pesticide methods. Rees and Winget (1969) demonstrated how proper shoreline modifications such as "cut and fill" could be used to effectively control mosquito production in shallow, salt-grass margins of lake marshes. Mulligan et al. (1979) reported on the effectiveness of vertical subsurface drainage as a means of eliminating mosquito breeding sites in flooded pastures. Small plot work in pastureland depressions by Breeland and Pickard (1964) suggested the novel idea that some type of tillage might be effective in controlling mosquito production in these types of situations. Owens et al. (1970) intensively studied control of floodwater mosquitoes through cultural practices and determined that certain types of tillage in small pastureland plots and playa lakes² of west Texas were effective for the control of *Aedes* and *Culex* species. They assumed that the control effect was produced by egg burial, crushing, or some other disturbance produced by the equipment. They also postulated that the eggs were "turned under" to such a depth that hatching was prevented or that young larvae from hatched eggs were trapped in the soil.

Studies were begun by the authors in the early 1970's to investigate tillage for control of floodwater mosquitoes (primarily *Aedes vexans* (Meigen) and *Psorophora columbiae*) (Dyar and Knab) in pastureland depressions and river floodplains and to evaluate its operational applicability for mosquito control. Studies also included an assessment of how the control effect was produced. Evaluation of field plots was deferred 2 or more years in several instances because it was

dependent on the frequency of river flooding which did not occur in every year. Consequently, the studies reported here extended over a 9-year period.

MATERIALS AND METHODS

PREPARATION AND TREATMENT OF TEST PLOTS. Small test plots ranging in size from 0.04 to 0.61 hectares were established in floodwater mosquito-producing pastureland depressions in northwest Alabama. A total of 14 test plots and a like number of controls were established by dividing equally 14 naturally occurring, pastureland depressions. The plots were bisected with a wooden and earth barrier to prevent migration of mosquito larvae. Prior to separating each plot into halves (a test area and an untreated check) their suitability for use had been predetermined by counting eggs from 6-inch square by 1-inch thick soil samples taken in each plot. Egg density values in Table 1 are an average of the samples collected per plot and the number of samples collected varied depending on the size of the plot. All plots generally contained a similar composition of mosquito eggs and plant species.

Two larger plots 1.2 and 1.4 hectares in size, one occurring in a river floodplain and the other in a large pastureland depression, were also evaluated. Untreated checks for these plots were established adjacent to, but separated from, the test plots. All test areas, both large and small, were plowed to a depth of 20–25 cm with a farm tractor equipped with a moldboard turning plow, and, with the exception of one plot, subsequently smoothed over with a disk harrow (Fig. 1).

Evaluation was based on a comparison of the number of larval mosquitoes occurring after flooding in both the treated and untreated check areas utilizing the standard dipper technique. Flooding of the plots was produced either by pumping water from a nearby creek or river, or by heavy rains and river flooding (Fig. 2).

LOCATION OF EGGS IN THE HABITAT.

² Playa lakes are hard clayey depressions occurring in the southwestern United States which are subject to inundation during heavy rains which subsequently became dry again. Intermittent cycles of wet and dry may occur several times each year.

Table 1. Percentage control of floodwater mosquito larvae (essentially all *Aedes vexans*) by tillage of the habitat, when compared with untreated check plots.

| Plot designation | Pre-treatment egg density | | | Larval density/flooding | | | Percentage of larval control |
|------------------|---------------------------|----------------------|------------------|-------------------------|----------------|------------------|------------------------------|
| | Date sampled | Portion to be tilled | Untilled portion | Date sampled | Tilled portion | Untilled portion | |
| Collier's #1 | 5/ 7/69 | 280.1** | 57.9** | 4/10/70 | 0.02 | 1.00 | 98.0 |
| Collier's #1 | 2/26/73 | 3.4 | 4.9 | 3/ 8/73 | 0.05 | 0.55 | 90.9 |
| Collier's #1 | 10/ 1/73 | 15.0 | 9.7 | 12/ 6/73 | 0.05 | 0.80 | 93.8 |
| Collier's #2 | 5/22/70 | 15.7 | 28.3 | 7/10/70 | 0.55 | 11.70 | 95.3 |
| Collier's #2 | 1/19/73 | 3.0 | 7.6 | 3/ 8/73 | 0.00 | 0.50 | 100.0 |
| Collier's #2 | 10/ 1/73 | 3.3 | 15.2 | 12/ 6/73 | 0.05 | 2.35 | 97.9 |
| Collier's #6 | 10/ 5/73 | 12.1 | 4.2 | 12/ 3/73 | 0.04 | 0.18 | 77.8 |
| Collier's #7 | 10/11/73 | 12.8 | 15.2 | 12/ 3/73 | 0.00 | 2.35 | 100.0 |
| Collier's #9 | 10/ 4/73 | 1.5 | 4.2 | 12/ 3/74 | 0.00 | 0.18 | 100.0 |
| Elkmont, AL | 4/15/71 | 83.0 | 53.7 | 5/14/71 | 5.36 | 19.79 | 72.9 |
| Elkmont, AL | 7/ 5/73 | 2.5 | 4.8 | 4/ 1/74 | 0.36 | 2.90 | 87.6 |
| Valdosta, AL* | 1/31/73 | 5.3 | 6.0 | 3/ 9/73 | 5.27 | 9.40 | 43.9 |
| McCall, Murphy | 11/13/72 | 9.9 | 9.9 | 5/13/73 | 0.31 | 8.40 | 96.3 |
| McCall, Murphy | 12/13/77 | 13.7 | 3.7 | 5/ 7/79 | 0.54 | 2.00 | 73.0 |
| McFarland-B | 11/ 6/73 | 2.4 | 5.4 | 3/22/74 | 0.00 | 2.75 | 100.0 |
| McFarland-C | 11/ 6/73 | 0.6 | 5.4 | 3/22/74 | 0.00 | 2.75 | 100.0 |
| Clark #1 | 2/26/73 | 8.1 | 4.4 | 3/25/74 | 0.14 | 2.28 | 93.9 |
| Clark #2 | 2/ 7/73 | 2.9 | 4.4 | 3/25/74 | 0.44 | 2.28 | 81.7 |
| Clark #7 | 6/11/73 | 0.2 | 0.3 | 9/21/73 | 0.34 | 1.92 | 82.3 |
| Clark #9 | 6/11/73 | 0.6 | 0.4 | 9/19/73 | 0.52 | 2.48 | 79.0 |
| Clark #10 | 6/11/73 | 0.2 | 0.0 | 9/20/73 | 1.24 | 11.56 | 89.3 |
| Clark #12 | 6/11/73 | 2.4 | — | 9/20/73 | 0.36 | 22.56 | 98.4 |

* Plowed only—flooded before disking could be conducted.

** Represents the average number of eggs per sample.



Fig. 1. Typical floodwater mosquito depression bisected with a wooden and earth barrier into a tilled and an untreated check portion.

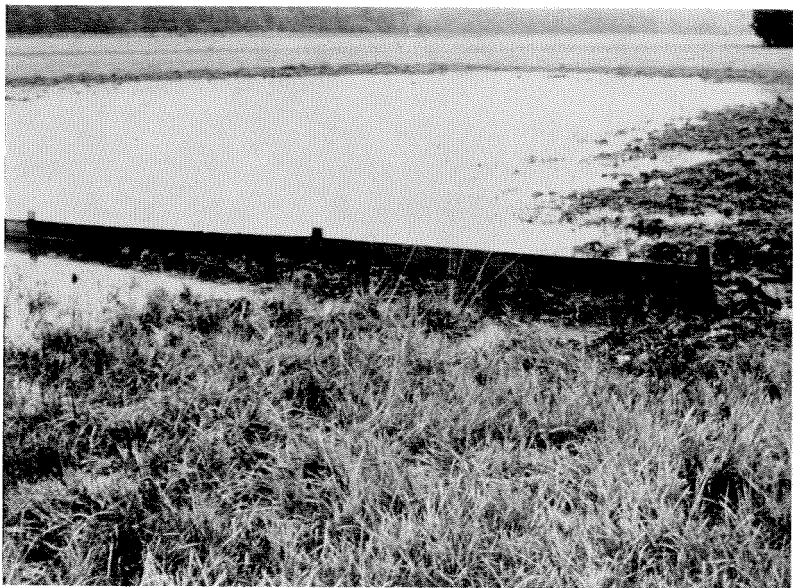


Fig. 2. Floodwater mosquito test plot showing extent of flooding and the difference in physical appearance of the 2 segments.

Cylindrical core soil samples, 8.3 cm diam were taken using a grass plugger to a maximum depth of 12.7 cm in known floodwater mosquito habitats, prior to tillage and immediately following tillage. The undisturbed cores were forced from the plugger into quart size cylindrical milk cartons whose diameter dimension coincided with that of the core samples for ease of handling and transport (Fig. 3). The samples were then transported to the laboratory where they were sectioned in 6 mm increments using the apparatus illustrated in Fig. 4. A hand-operated mechanical jack was calibrated so that with each stroke of the jack handle, the piston was forced up 6 mm into the carton from the bottom forcing a 6 mm thick increment of the soil core out of the top of the carton. The soil increments were then sliced from the core using an electric slicing knife. As the 6 mm thick segments were sliced from the core sample each was packaged for storage in a Whirl-pak® plastic bag. The incremental samples

were then processed for egg removal according to a modification of the Horsfall (1956) method. Eggs from each increment were counted and identified to species. Identifications were based on the taxonomic keys of Ross and Horsfall (1965).

LABORATORY EVALUATION OF DEPTH OF SOIL COVER ON LARVAL CONTROL. To determine the effect of soil cover depth on egg hatching and larval survival, known numbers of viable *Aedes vexans* eggs were placed on a 25 cm thick soil substrate in 250 ml glass beakers and covered with various depths of lightly-tamped, clay-loam soil ranging from 1.5 mm to 50 mm; controls were handled identically but the eggs were not covered. Six replicates of 50 eggs each were tested for each of the soil cover variations. The depths of soil cover tested were: 1.5 mm, 3.0 mm, 6.0 mm, 12.0 mm, 25.0 mm, and 50.0 mm. Six controls were also included.

Tap water was added to each beaker to stimulate egg hatching. The number of

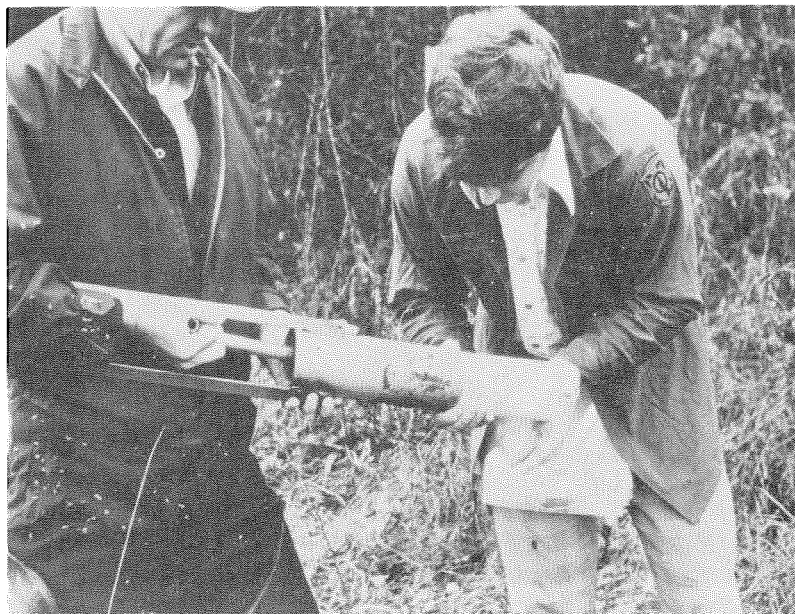


Fig. 3. Extraction of the core soil sample from the sampling tool and its insertion into the carrying and sectioning container.

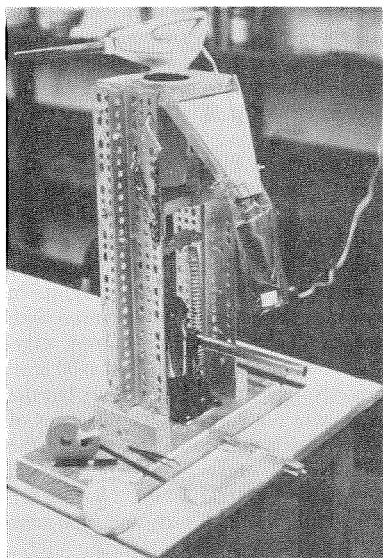


Fig. 4. Apparatus used for sectioning soil core samples.

larvae present was recorded and subsequently removed after 72 hours. The remaining soil and water residue was then processed according to a modification of the Horsfall procedure to remove the ova still remaining in the soil. The number of hatched and intact eggs remaining was recorded for each test vessel.

RESULTS AND DISCUSSION

Identification of eggs from plot samples and the identification of hatched larvae revealed that the mosquito population was essentially *Aedes vexans*, although in several test plots *Psorophora columbiae* and *Ps. cyanoescens* (Coquillett) comprised 5–10% of the population; *Aedes vexans* was still by far the dominant species in all test plots.

The percentage of mosquito control obtained in tilled test plots based on a comparison of larval production in the test plots with that in comparable controls

ranged from 100% to 73%. Table 1 data show that 100% control was obtained in 5 tests and in 8 tests control ranged from 90 to 98% so that in 13 of 21 tests, control exceeded 90%. In only 2 tests did control drop below 75%.

A total of 21 separate tests were performed although only 14 test plots were used, indicating that several plots were used for more than one test. The extended period of time over which these studies were conducted permitted the repeated use of a plot as long as a suitable time existed between tests to allow for oviposition by gravid females.

It was noted during the course of these studies in one plot which was not disked subsequent to plowing due to adverse weather conditions and untimely flooding, mosquito control was only 44%. These data demonstrate the need to complete the tillage cycle to produce control.

The level of mosquito control did not appear to be affected by the interval of time that elapsed between the tillage activity and flooding of the plot unless conditions occurred during the interval that were suitable for mosquito oviposition. In most tests this interval lasted from 1-4 months, although in one test it lasted 11 months and extended from May of one year to March of the following; nevertheless mosquito control was excellent at 98%. However, in another test involving a river floodplain in which an interval of 18 months had occurred between tillage and

flooding, and during which time conditions were suitable for limited oviposition, control was only 73%, the lowest value obtained. Apparently some oviposition had occurred in the test plot.

Based on core soil samples collected from known floodwater mosquito habitats it was determined that mosquito eggs occurred naturally in the top 25 to 31 mm of the substrate. Of the total number of positive samples recorded, 97.7% occurred in the top 25 mm of soil while only 2.3% occurred below this depth. Sixty-one percent of the eggs occurred in the top 6 mm of soil and 95% occurred in the top 75 mm (Table 2). After tillage of a floodwater mosquito habitat it was determined that the eggs were redistributed throughout the core samples as shown in Table 3. Eggs were recovered as deep as 126 mm down in the soil and only about 3% of the total number of eggs recovered occurred in the top 6.0 mm. Only about 12% of the total number of eggs recovered from all core samples occurred in the top 25 mm of soil, while about 23% of all of the eggs occurred between 63 and 75 mm down in the soil. It was at this depth also that the highest percentage of soil increments was positive. Of all of the positive samples recorded, only 14.9% occurred in the top 25 mm, while the remaining 85.1% were recovered at a depth of from 25 to 126 mm.

These data show that prior to tillage 97.7% of all positive samples occurred in

Table 2. Location of naturally occurring mosquito ova in relation to depth at which they occur in the soil of the habitat (essentially all *Aedes vexans*).

| Soil depth (mm) measured from surface down | Number of samples processed | Number of positive samples | Percent of positive samples | Average number of ova present/sample | Percent of ova of total |
|--|-----------------------------|----------------------------|-----------------------------|--------------------------------------|-------------------------|
| 0- 6.0 | 131 | 51 | 39 | 4.66 | 61.2 |
| 6.0- 12.0 | 131 | 38 | 29 | 1.70 | 22.4 |
| 12.0- 18.0 | 131 | 22 | 17 | 0.85 | 11.2 |
| 18.0- 25.0 | 131 | 16 | 12 | 0.34 | 4.4 |
| 25.0- 31.0 | 131 | 3 | 2 | 0.06 | 0.1 |
| 31.0-106.0 ^a | 114 | 0 | 0 | 0 | 0 |

^a No eggs were recovered below a depth of 31.0 mm although 1,048 additional 6 mm incremental samples were processed.

Table 3. Location of naturally occurring mosquito ova in relation to soil depth after tillage of the habitat (essentially all *Aedes vexans*).

| Soil depth (mm) measured from surface down | Number of samples processed | Number of positive samples | Percent of positive samples | Average number of ova present/sample | Percent of ova of total |
|--|-----------------------------------|----------------------------------|-----------------------------------|--|-------------------------------|
| 0 - 6.0 | 58 | 3 | 5.2 | 0.09 | 2.8 |
| 6.0- 12.0 | 58 | 3 | 5.2 | 0.07 | 2.3 |
| 12.0- 18.0 | 58 | 6 | 10.3 | 0.10 | 3.3 |
| 18.0- 25.0 | 58 | 4 | 6.9 | 0.07 | 2.8 |
| 25.0- 31.0 | 58 | 8 | 13.8 | 0.24 | 7.9 |
| 31.0- 37.0 | 58 | 8 | 13.7 | 0.21 | 6.8 |
| 37.0- 43.0 | 58 | 9 | 15.5 | 0.19 | 6.2 |
| 43.0- 50.0 | 58 | 6 | 10.3 | 0.17 | 5.6 |
| 50.0- 56.0 | 58 | 6 | 10.3 | 0.22 | 7.3 |
| 56.0- 62.0 | 58 | 4 | 6.9 | 0.13 | 3.4 |
| 62.0- 68.0 | 58 | 9 | 15.5 | 0.36 | 11.7 |
| 68.0- 75.0 | 56 | 9 | 16.1 | 0.36 | 11.3 |
| 75.0- 81.0 | 55 | 9 | 12.7 | 0.22 | 6.8 |
| 81.0- 87.0 | 51 | 6 | 11.8 | 0.14 | 3.9 |
| 87.0- 93.0 | 48 | 6 | 12.5 | 0.31 | 8.5 |
| 93.0-100.0 | 42 | 4 | 9.5 | 0.12 | 2.8 |
| 100.0-106.0 | 26 | 2 | 7.7 | 0.08 | 1.7 |
| 106.0-112.0 | 20 | 0 | 0.0 | 0.00 | 0.0 |
| 112.0-118.0 | 16 | 2 | 12.5 | 0.25 | 2.2 |
| 118.0-126.0 | 16 | 3 | 18.7 | 0.25 | 2.3 |

the top 25 mm of soil while only 14.9% occurred there after tillage reflecting a downward movement of eggs wrought by tillage.

In the laboratory tests in which mosquito eggs were covered with various depths of lightly tamped soil, larval recovery data indicated that control was better than 95% when eggs were covered with 12 mm of soil and 100% when they were covered with 25 mm or more of soil (Table 4). Larval mortality, expressed as a percentage of larval loss compared to the

expected larval production based on number of eggs present at the start of each test, was 20% in the controls during the course of these tests. Apparently soil cover depth did not influence hatching since the percentage of hatching in all tests ranged from about 98 to 99%; hatching in the controls was also 98%.

CONCLUSIONS

Tillage as described in these studies is a very effective nonchemical method of

Table 4. Effect of soil depth cover on the hatchability of *Aedes vexans* ova.

| Depth of cover (mm) | Number of eggs at start | Number of larvae present | Percent present | Percent control | Number of non-eclosed eggs remaining | Percent eclosion |
|------------------------|-------------------------------|--------------------------------|--------------------|--------------------|--|---------------------|
| 0 (control) | 300 | 240 | 80.0 | 20.0 | 6 | 98.0 |
| 1.5 | 300 | 202 | 67.3 | 32.7 | 7 | 97.7 |
| 3.0 | 300 | 183 | 61.0 | 39.0 | 5 | 98.3 |
| 6.0 | 300 | 153 | 51.0 | 49.0 | 6 | 98.0 |
| 12.0 | 300 | 13 | 4.3 | 95.7 | 2 | 99.3 |
| 25.0 | 300 | 0 | 0.0 | 100.0 | 5 | 98.3 |
| 50.0 | 300 | 0 | 0.0 | 100.0 | 5 | 98.3 |

Note: Tabular data are an average of 2 tests, totaling 6 trials of each cover depth.

controlling certain types of floodwater mosquitoes in grassy floodplains and pasture and crop land depressions. It can be economically employed in grassy river floodplains when conducted in conjunction with wildlife shoreline plantings. It has obvious benefit to the farmer in terms of personal, community, and livestock protection and can be readily incorporated into the annual tillage cycle. These depressions which are not normally included by the farmer in tillage because past experience has shown that they will be flooded during the growing season and nonproductive in terms of forage or row crop products, can be readily included with a minimum of effort producing a maximum return in mosquito control.

The obvious limitation of this control procedure is that it cannot be employed in forested floodplains or woodland pools. However, the most pestiferous and medically and economically important species of floodwater mosquitoes in the Tennessee Valley region breed in the grassy intermittently flooded areas and consequently are of greater concern to the farmer and mosquito control worker.

The mechanism of control is most probably related to displacement of the eggs down into the soil to such depth that larvae which hatch as a result of flooding are trapped in the soil and never develop or emerge as adults. Laboratory tests confirmed that this is probably the control mechanism since equal numbers of eggs hatched in all tests, yet larval appearance decreased as soil cover of the eggs increased.

This control procedure can be readily incorporated into most tillage programs by persons engaged in agricultural activities for very little additional effort, and could be easily implemented through the university extension system.

ACKNOWLEDGMENT

The authors wish to thank Thomas L. Hill for his help in preparing test plots and collecting data, and James L. Marsh for the development of the soil core sectioning apparatus. Thanks are also extended to William W. Barnes and John B. Moore for their critical review of the manuscript, and to Frances P. Kimbrough for her help in its preparation.

References Cited

- Breeland, S. G. and Eugene Pickard. 1964. Cultural attempts at controls of floodwater mosquitoes in small plots in the Tennessee Valley Region of North Alabama, TVA, Division of Health and Safety, Quarterly Progress Report.
- Carter, J. E. 1979. Integrated pest management directive to federal department and agencies heads. Presidential Memorandum, The White House, Washington, D.C.
- Horsfall, W. R. 1956. A method for making a survey of floodwater mosquitoes. *Mosq. News* 16:66-71.
- Mulligan, F. S., M. A. Hamza, and C. H. Schaefer. 1979. Vertical drainage as a method of source reduction for control of pasture mosquitoes. *Mosq. News* 39:605-610.
- Owens, J. C., C. R. Ward, E. W. Huddleston, and D. Ashdown. 1970. Nonchemical methods of mosquito control for playa lakes in West Texas. *Mosq. News* 30:571-579.
- Rees, D. M. and R. N. Winget. 1969. Mosquito control in Utah by shoreline modification. *Mosq. News* 29:368-370.
- Ross, H. H. and W. R. Horsfall. 1965. A synopsis of the mosquitoes of Illinois (Diptera, Culicidae). *Ill. Nat. Hist. Surv. Biol. Notes* No. 52, 50 pp.
- United States Public Health Service and Tennessee Valley Authority. 1947. Malaria control on impounded water. U.S. Government Printing Office, Washington D.C.