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# AN INVESTIGATION ON THE LARVAL HABITAT OF FIVE SPECIES OF TREE-HOLE BREEDING MOSQUITOES (DIPTERA: CULICIDAE)

Lee Mitchell and C. Lee Rockett<sup>2</sup>

### ABSTRACT

A two-year study on the larval habitat of five species of tree-hole breeding mosquitoes was conducted in the vicinity of Bowling Green, Wood County, Ohio. Ninety-eight tree holes containing mosquito larvae were studied. Larval collections (27,741 specimens) were comprised of Aedes triseriatus (90.8%), Orthopodomyia signifera (3.3%), Aedes hendersoni (3.2%), Anopheles barberi (2.7%) and Orthopodomyia alba (< 0.1%). Chemical parameters (BOD, tannin-lignin, pH, and conductivity) of different tree holes were evaluated. Although different tree-hole species were commonly found in association with one another at breeding sites, ecological differences between species were found in spatial, trophic, and temporal niche dimensions.

Mosquitoes are often grouped as to the type of habitat occupied and given such names as floodwater, salt marsh, permanent water, or container-breeding mosquitoes. Defining a characteristic niche for each species is often difficult owing to frequent interspecific associations at breeding sites. Several ecologists have focused their attention on mosquitoes breeding in containers because these 'microhabitats' are relatively simple communities. Maguire et al. (1968) studied the control of community structure by mosquito larvae in the bracts of wild banana flowers. Istock et al. (1975) examined the ecology and evolution of the pitcher-plant mosquito, *Wyeomyia smithii* (Coquillett). The isolation of La Crosse virus (California encephalitis group) by Thompson et al. (1972) from a tree-hole breeding mosquitoe, *Aedes triseriatus* (Say), has created a renewed interest in the inhabitants and microhabitat of tree holes.

Tree holes are dynamic habitats of a temporary nature; wood decay may perforate the bottom of the cavity and subsequently drain it, the water may evaporate during drought, or the hole may become filled with wind-blown debris (twigs, leaves, and dirt) (Hanson and Hanson 1970). Numerous studies have been published on the biology and ecology of mosquitoes that breed in tree holes. Jenkins and Carpenter (1946) reviewed the biology, distribution and importance of the Nearctic tree-hole mosquitoes in relation to disease transmission. Lunt and Peters (1976) and Nielsen et al. (1968) reported on the distribution and bionomics of tree-hole mosquitoes in the Great Plains states and in the western United States, respectively. Several authors have focused their attention on the bionomics of specific tree-hole breeding mosquitoes (Hemmerlein and Crans 1968, Loor and DeFoliart 1970, Garcia and Ponting 1972).

Some specific objectives of our study were to determine: qualitative and quantitative species analysis of periodic tree hole samplings on a seasonal basis; associations of different tree-hole species; selected chemical analysis of the larval habitat; and criteria for the selection of oviposition sites by gravid females.

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### MATERIALS AND METHODS

Three primary collecting sites (designated as A, B, and C) were selected in Wood County, Ohio, for this study; specific geographical locations were given in Mitchell (1978). The vegetational composition of each site was distinct. Site A (2.6 ha) is characterized as an oak-hickory forest throughout, with shellbark hickory (Carya laciniosa), pignut hickory (C. glabra) and white oak (Quercus alba) as the most important canopy species. Site B (26.9 ha) is represented by two community types: swamp-forest, characterized by pin oak (Q. palustris) and swamp white oak  $(Q.\ bicolor)$ ; and sand-ridge, characterized by red oak  $(\widetilde{Q}.\ bicolor)$ ; rubra) and white oak (O. alba). White oak, red oak and common locust (Robinia pseudoacacia) are abundant at site C (6.1 ha). Three tree holes located in the City of Bowling Green (Wood County) were also examined during the study.

From 16 May through 14 November 1976, and 10 April through 26 November 1977, tree-hole breeding mosquitoes were collected at the study sites. Individual tree holes were examined approximately once every three weeks throughout the investigation. A 25 ml pipet (1.3 cm diam) with a rubber bulb was used to remove standing water (with immature mosquitoes) from the tree holes. All tree holes were filled with aged tap water after the collection process had been completed. Dry tree holes were occasionally flooded with aged tap water and rechecked at a later date in order to determine their mosquito-producing potential. Field samples were returned to the laboratory for qualitative and quantitative analysis.

The water chemistry of selected tree holes was also investigated. Specific conductance and tannin-lignin content were analyzed with a Hach<sup>®</sup> DR-EL/2 water test kit. A Hach<sup>®</sup> colorimeter was used for field pH tests. Biochemical oxygen demand (BOD) was analyzed with a Hach® BOD manometer apparatus.

#### RESULTS

Mosquito larvae were collected from 98 different tree holes (404 total samples) during the two-year study. Most of these trees were oaks (Quercus spp.) (57%), hickories (Carya spp.) (30%) and red maples (Acer rubrum) (7%). Tree holes containing larvae at site A were primarily hickories, whereas oaks were the common breeding sites at locations B and C.

The following species were identified from the 27,741 collected larval specimens: Aedes triseriatus (90.8%), Orthopodomyia signifera (Coquillett) (3.3%). Aedes hendersoni Cockerell (3.2%), Anopheles barberi Coquillett (2.7%) and Orthopodomvia alba Baker (< 0.1%) (Table 1). The collection of A. hendersoni (May 1976) and O. alba (August 1977) represented new distributional records for Wood County. Occasionally Culex pipiens L. and/or Culex restuans Theobald larvae were also present in the tree hole collections.

The tree-hole mosquitoes collected in this study showed a marked variation in terms of their seasonal abundance (Table 1). Aedes triseriatus was abundant from May through August (1976) and from April through August (1977). Other species followed in an orderly fashion: A. hendersoni, July-August (1976) and June-August (1977); A. barberi, July-October (1976 and 1977); and O. signifera, July-October (1976) and September-October (1977). Although few data were available for O. alba, this species seemed to follow the pattern of O. signifera. Orthopodomyia signifera and A. barberi were collected as late as November in both years.

Tree-hole mosquitoes were commonly found in association with one another at breeding sites. The data presented in Table 3 show that the most common species associations were A. triseriatus/A. barberi (54X) and A. triseriatus/A. hendersoni (52X). The A. barberi/A. hendersoni (20X) and A. barberi/O. signifera (19X) associations occurred less frequently.

This study provided no evidence for exclusive selection of a particular tree species by any of the tree-hole breeding mosquitoes. Tree-hole mosquitoes were collected most often from oaks and hickories, which were simply the most abundant trees with water-filled holes at the study sites (Table 4).

A summary of ecological data for the five species of tree-hole breeding mosquitoes is given in Table 2. Owing to its rare occurrence, the ecological data presented in Table 2 for O. alba are very limited and may not be an accurate representation of its habitat preference.

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Table 1. Larval collections from all locations.

1976					1977							Totals						
Species	May	June	July	Aug.	Sep.	Oct.	Nov.	•	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	1976	1977
Aedes hendersoni	1	0	55	21	3	0	0		26	0	75	533	144	14	4	0	80	796
Aedes triseriatus	1408	1245	2580	1306	47	24	3		4005	7544	2930	2752	759	586	0	0	6613	18576
Anopheles barberi	0	1	37	81	40	63	20		5	0	19	123	101	98	150	19	242	515
Ortĥopodomyia alba	0	0	0	0	0	0	0		0	0	0	0	2	8	3	0	0	13
Orthopodomyia signifera	0	0	58	229	65	115	19		0	0	0	0	0	167	238	15	486	420
Totals	1409	1246	2730	1637	155	202	42		4036	7544	3024	3408	1006	873	395	34	7421	20320

Table 2. Summary of ecological data for the five species of tree-hole breeding mosquitoes.

	A. hendersoni	A. triseriatus	A. barberi	O. alba	O. signifera	Total for all species
Relative frequency at tree holes	27/98 = 28%	96/98 = 98%	27/98 = 28%	1/98 = 1%	7/98 = 7%	
Diameter of tree hole opening (cm)	3.8 - 38.1 $\bar{x} = 13.5$	$\begin{array}{l} 2.5 - 40.6 \\ \bar{x} = 11.7 \end{array}$	5.1 - 33.0 $\bar{x} = 13.0$	$\begin{array}{l} 20.3 \\ \bar{x} = 20.3 \end{array}$	$6.4 - 27.9 \\ \bar{x} = 15.5$	2.5 - 40.6 $\bar{x} = 11.7$
Height of tree hole above ground level (cm)	0 - 147.3 $\bar{x} = 28.2$	0 - 147.3 $\bar{x} = 18.8$	$0 - 106.7$ $\bar{x} = 21.8$	$6.4 \\ \tilde{\mathbf{x}} = 6.4$	$0 - 50.8$ $\bar{x} = 17.8$	0 - 147.3 $\bar{x} = 19.6$
Location of tree hole	top = 67% $side = 33%$	top = 78% $side = 22%$	top = 67% side = 33%	top = 0 $side = 100%$	top = 43% side = 57%	top = 78% $side = 22%$
pH values	$5.0 - 8.0$ $\tilde{\mathbf{x}} = 6.6$	5.0 - 8.0 $\bar{x} = 6.6$	$5.0 - 8.0$ $\bar{x} = 6.5$	$7.0 - 8.0$ $\tilde{\mathbf{x}} = 7.6$	$6.0 - 8.0$ $\bar{x} = 6.7$	$5.0 - 8.0$ $\bar{x} = 6.6$
Specific conductance (micromhos/cm)	210 - 5,500 $\bar{x} = 1,485$	120 - 5,500 $\bar{x} = 993$	120 - 5,500 $\bar{x} = 993$	$5,500$ $\bar{x} = 5,500$	320 - 5,500 $\bar{x} = 1,273$	120 - 5,500 $\bar{x} = 993$
Tannin-lignin (mg/litre)	$6.0 - 450.0$ $\bar{x} = 177.0$	6.0 - 450.0 $\ddot{x} = 128.0$	$6.0 - 450.0$ $\tilde{x} = 128.0$	$450.0$ $\ddot{x} = 450.0$	$43.8 - 450.0$ $\bar{x} = 140.0$	6.0 - 450.0 $\bar{x} = 128.0$
BOD (mg/litre)	$58.0 - 248.0$ $\bar{x} = 125.8$	$   \begin{array}{r}     10.5 - 248.0 \\     \bar{x} = 94.7   \end{array} $	$   \begin{array}{r}     10.5 - 248.0 \\     \bar{x} = 97.0   \end{array} $	$\begin{array}{l} 248.0 \\ \bar{x} = 248.0 \end{array}$	58.0 - 248.0 $\bar{x} = 125.8$	$     \begin{array}{r}       10.5 - 248.0 \\       \bar{x} = 94.7     \end{array} $

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Table 3. Frequency of larval associations at tree hole breeding sites.<sup>a</sup>

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	A. hendersoni	A. triseriatus	A. barberî	O. alba
Aedes triseriatus	52	_	54	1
Anopheles barberi	20	54		4
Ortĥopodomyia alba	2	1	4	
Orthopodomyia signifera	8	12	19	3

aEach figure represents the number of times that one species was found with another.

	Aedes hendersoni	
Shellbark hickory (8) (Carya laciniosa)	Pignut hickory (3) (Carya glabra)	Cottonwood (1) (Populus deltoides)
Red oak (5) (Quercus rubra)	Red maple (2) (Acer rubrum)	Black gum (1) (Nyssa sylvatica)
White oak (4) (Quercus alba)	Stumps (2)	Box-elder (1) (Acer negundo)
	Aedes triseriatus	
Red oak (33) (Quercus rubra)	Red maple (7) (Acer rubrum)	Common lucust (1) (Robinia pseudoacacia)
White oak (19) (Quercus alba)	Swamp white oak (4) (Quercus bicolor)	Cottonwood (1) (Populus deltoides)
Shellbark hickory (14) (Carya laciniosa)	Stumps (2)	Black gum (1) (Nyssa sylvatica)
Pignut hickory (12) (Carya glabra)	Yellowbud hickory (2) (Carya cordiformis)	
	Anopheles barberi	
Red oak (11) (Quercus rubra)	Swamp white oak (2) (Quercus bicolor)	Black gum (1) (Nyssa sylvatica)
White oak (6) (Quercus alba)	Shellbark hickory (1) (Carya laciniosa)	Common locust (1) (Robinia pseudoacacia)
Red maple (3) (Acer rubrum)	Pignut hickory (1) (Carya glabra)	Box-elder (1) (Acer negundo)
	Orthopodomyia alba	
	White oak (1) (Quercus alba)	
	Orthopodomyia signifera	
White oak (3) (Quercus alba)	Red oak (3) (Quercus rubra)	Red maple (1) (Acer rubrum)

<sup>&</sup>lt;sup>a</sup>Number of trees of each species which contained mosquito larvae is given in parentheses.

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Aedes triseriatus was found to be the most widely distributed tree-hole mosquito in this study; 96 of the 98 different tree holes which periodically yielded larvae contained this species. The relative frequency for the various species found breeding in tree holes were as follows: A. triseriatus (98%), A. hendersoni (28%), A. barberi (28%), O. signifera (7%), and O. alba (1%).

The mean values for the diameter of the tree hole openings and the height above ground level were 11.7 cm and 19.6 cm, respectively. Distinct ovipositional preferences pertaining to the physical characteristics of tree holes were not noted for A. triseriatus owing to its high relative frequency. Aedes hendersoni and A. barberi were frequently collected from tree holes located higher than the mean height, whereas most of the O. signifera and all of the O. alba larvae were found in basal tree holes located less than 10.2 cm above ground level. None of the tree-hole species showed a preference for tree holes of a particular diameter. All five species were collected on occasion from side-opening tree holes; however, most tree hole openings in the study areas were exposed at the top (78%) rather than at the side (22%).

The pH range for all of the tree holes in this study was 5.0–8.0 ( $\bar{x}=6.6$ ). The mean values for other chemical parameters were determind from selected tree holes and were as follows: 993  $\mu$ mhos/cm (conductivity), 128.0 mg/litre (tannin-lignin) and 94.7 mg/litre (BOD). Orthopodomyia signifera, O. alba and A. hendersoni larvae were typically collected from tree holes having relatively high values for conductivity, BOD, and tannin-lignin content. Tree holes containing A. barberi and A. triseriatus were usually near the mean values calculated for all of the chemical parameters.

#### DISCUSSION

The frequent interspecific associations at tree holes is interesting because the possible role of competing species has figured prominently in speculations regarding discontinuities in the distribution of certain mosquitoes (Spielman and Feinsod 1979). Eventual exclusion of a species from its ecological niche by a competitively superior species with nearly identical requirements is a firmly established principle in ecology (Mayr 1963). Competitive displacement of one mosquito species by another has been shown to occur (Service 1966). Tree hole environments are far more heterogeneous than may appear at first sight and this no doubt helps to lessen interspecific competition between sympatric tree-hole breeding mosquitoes. As suggested by Pianka (1975), ecological differences in three niche "dimensions" (spatial, trophic, and temporal) should reduce competition and facilitate coexistence of a variety of species.

Physical differences in tree-hole breeding sites (size, elevation, and location of the opening) offer opportunities for niche segregation. Side-opening tree holes may often be selected by those species which are strongly photonegative, such as O. signifera. Conversely, the selection of a top-opening tree hole may be advantageous for greater net productivity since this type readily collects rain water and leaf debris, an important nutrient source (D. Fish, University of Notre Dame, pers. comm.). Some tree holes produce many more mosquitoes than others and this is due at least in part to the length of time and amount of water that may be retained. Conductivity, BOD, and tannin-lignin content seem to be more important than pH for site selection by oviposition females; only a slight variation from the mean pH value (6.6) was noted for all species.

Preferences for each tree-hole species became evident during the course of this study. *Aedes triseriatus* females oviposited at almost all of the available tree holes, the majority of which were located close to ground level. Although arboreal (canopy) tree holes do not seem to be common in our area, *A. hendersoni* larvae were collected from tree holes located higher than the mean height (19.6 cm). Studies conducted in Indiana (Sinsko and Grimstad 1977) and Wisconsin (Scholl and DeFoliart 1977) have indicated that *A. hendersoni* prefers ovipositional sites located 3–9 m above ground level, whereas *A. triseriatus* is much more prevalent at ground level. The occupation of a different spatial niche dimension by *A. hendersoni* may facilitate a lessening of interspecific competition with the more abundant sibling species, *A. triseriatus*. Breeding sites for both species of *Orthopodomyia* were usu-

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ally basal tree holes which had high conductivity, BOD, and tannin-lignin content. Specimens of O. signifera were rarely present in the tree hole collections, except for the frequent occurrence of this species with A. barberi at two very productive tree holes with a high organic content. Anopheles barberi females oviposited at a variety of tree holes.

On the basis of personal observations, A. barberi larvae compete very well for food resources with other larval species for at least two reasons: larvae of A. barberi fed on or just below the water surface, while other species in this study fed on bottom sediments or farther below the water surface; also fourth instar larvae are facultatively predaceous on early instar mosquito larvae of other species. Predation by A. barberi on mosquito larvae confirms the availability of an alternative trophic niche dimension.

All of the tree-hole mosquitoes showed a marked variation in terms of their seasonal abundance. An early population peak for A. triseriatus and late peaks for A. barberi. O. signifera, and O. alba were apparent. Aedes hendersoni were most abundant during midsummer. Seasonal population variations should facilitate a division of niche resources and would represent differences between species in a temporal niche dimension.

Because A. triseriatus has been recognized as the principal vector of La Crosse virus, this species is of particular interest to many public health and vector control agencies in the midwest. The control of A. triseriatus is difficult owing to the time and expense required to locate the breeding sites. The application of insecticides or the filling-in of tree holes with sand or other materials have questionable effectiveness. The use of residual insecticides may be unacceptable from an environmental perspective. The use of predaceous, non-anthropophilic tree-hole mosquitoes, such as Toxorhynchites, might be considered as a component in an integrated pest management program for A. triseriatus.

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