Mosquitoes (Diptera: Culicidae) Sampled from Treeholes and Proximate Artificial Containers in Central Missouri[1]

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ABSTRACT The immatures of eight mosquito species in six genera were collected from treeholes and artificial containers at three sites in central Missouri from May - October, 1986 - 1988. The species most frequently collected was *Aedes triseriatus* (Say), followed in descending order by *Anopheles barberi* (Coquillett), *Orthopodomyia signifera* (Coquillett), *Aedes hendersoni* (Cockerell), *Culex restuans* Theobald, *Culex territans* Walker, *Psorophora ferox* (Humboldt), and *Toxorhynchites rutilus septentrionalis* (Dyar & Knab). Larvae of *Ps. ferox* collected from a basal white oak treehole were the first record of this species from this habitat. Larvae of *Cx. restuans* and *Cx. territans* were collected only from artificial containers and not from treeholes. *Aedes triseriatus* was opportunistic with respect to tree species inhabited, with distributions roughly paralleling the relative abundance of available treeholes. Artificial containers, even when enriched with leaf litter, did not constitute an adequate mimic for treeholes when sampling *Ae. triseriatus* immatures.

KEY WORDS Diptera, Culicidae, treeholes, artificial containers.

Treeholes and artificial containers provide specialized ecological habitats for immature mosquitoes. Such habitats are attractive for study because they contain discrete communities with a relatively small number of species (Jenkins and Carpenter 1946, Fish and Carpenter 1982, Barton and Smith 1984, Chambers 1985, Laird 1988). Mosquitoes that exploit these breeding sites may transmit pathogens causing yellow fever, dengue, St. Louis encephalitis, La Crosse encephalitis and dog heartworm (Craig 1983, Resh and Grodhaus 1983). The biology and ecology of treehole and artificial container-breeding mosquitoes are relatively well understood in some areas of the midwestern U.S. (Mitchell and Rockett 1981, Copeland and Craig 1990), but have not been investigated previously in central Missouri.

The present study was designed to survey the species of mosquitoes breeding in treeholes and artificial containers in central Missouri. Our specific objectives were to determine the species of mosquitoes that develop in treeholes and proximate artificial containers and to identify the tree species with which each mosquito species was associated most commonly.

Materials and Methods

Study Sites. Three forested field sites (Hearnes, Hinkson Bottoms, and Ashland Wildlife Reserve) in Boone Co., MO (39°N), were used to study treehole

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and artificial container-breeding mosquitoes from May - October, 1986 - 1988. Totals of 30, 25 and 20 trees with water-filled treeholes were selected for routine sampling of mosquitoes at the Hearnes, Hinkson, and Ashland sites, respectively (Table 1). The treeholes used comprised those identified after inspection of trees at each site.

Table 1. Tree species at three sites containing treeholes sampled for immature mosquitoes in Boone County, MO, 1986 to 1988.

	Sample site (% of total)		
Species	Hearnes (n = 30)	Hinkson (n = 25)	Ashland (n = 20)
Yellow chestnut oak	53.3		
Green ash	16.6		
American elm	6.6	12.5	
Sugar maple	6.6		
Boxelder	3.3	20.8	
Hackberry	3.3		
Black walnut	3.3	8.3	
White oak	3.3	4.2	75.0
American basswood	3.3		
Silver maple		54.2	
Black oak			15.0
Flowering dogwood			10.0
Total	100.0	100.0	100.0

Hearnes forest consisted primarily of yellow chestnut oak, *Quercus muehleenbergii* (Engelm.), and green ash, *Fraxinus pennsylvanica* (Marsh). Less abundant trees included American elm, *Ulmus americana* (L.); sugar maple, *Acer saccharum* (Marsh); boxelder, *A. negundo* (L.); hackberry, *Celtis occidentalis* (L.); black walnut, *Juglans nigra* (L.); white oak, *Q. alba* (L.); and American basswood, *Tilia americana* (L.).

Hinkson Bottoms was dominated by a stand of silver maple, *A. saccharinum* (L.), and boxelder. Less abundant were American elm, black walnut, and white oak.

Ashland Wildlife Reserve was dominated by mature white oak. Less abundant were black oak, *Q. velutina* (Lam.), and flowering dogwood, *Cornus florida* (L.).

Sampling and Rearing Techniques. Mosquito larvae and pupae were sampled weekly from permanently-labeled treeholes and transported in 100-ml snap-cap plastic containers to the laboratory for rearing and identification. Three replicate samples per treehole or artificial container per sampling occasion were obtained using a 60-ml wide-mouth pipette. The water temperature of each treehole or artificial container was determined at the time of sampling with an electric thermometer. The pH and electrical conductivity of each water sample were determined in the laboratory, the former with a Fisher Acumet pH Meter (Model 144), and the latter with a Markson Model 4503 Selectro Mark Analyzer equipped with a conductivity meter.

Immature mosquitoes were reared at room temperature (ca. 22°C) in plastic rearing cups (4.5 cm diam × 3.8 cm deep) with 30 ml of water, plus 20 mg of dried yeast mixed with Purina Rabbit Chow (Ralston-Purina Co., St. Louis) (1:1 volume) and 0.5 g leaf litter (Chambers 1985). Pupae were transferred to plastic containers filled with distilled water (20 pupae/100 ml container) and held in a cage (30.5 cm^3) at ca. 25°C and 70-80% RH until adult mosquitoes emerged.

Artificial Containers. To compare the oviposition preferences of female mosquitoes for artificial containers versus treeholes, artificial containers were wired to trees at the same height as the treeholes. The artificial containers were 600-ml plastic cups painted dull black on the outside. Sampling of the plastic cups for immature mosquitoes was done exactly as for treeholes. At each of the sampling sites, leaf litter was added to half of the artificial containers. During dry periods, distilled water (300 ml) was added twice weekly to each treehole and artificial container.

Species Identificiation. Adult mosquitoes were identified using Darsie and Ward (1981) and Darsie (1986). The identification of a representative series of all species was confirmed by T. V. Gaffigan and R. E. Harbach (Walter Reed Biosystematics Unit, Washington, DC). Voucher specimens were deposited in the W. R. Enns Entomology Museum, University of Missouri, Columbia.

Results

Species Composition. Six species of mosquito in five genera were collected from water-filled treeholes at our three study sites in Boone Co., MO, from May to October, 1986 to 1988 (Table 2). A total of 38,911 mosquito larvae and pupae was collected from the 75 treeholes at the three sites during the three seasons: 16,070 (41%) from the Hearnes site, 10,329 (27%) from the Hinkson site, and 12,512 (32%) from the Ashland site. *Aedes triseriatus* (Say) was the dominant treehole species, representing 89% of mosquito immatures collected, followed in descending order by *Orthopodomyia signifera* (Coquillett) (6%) and *Anopheles barberi* (Coq.) (3%). *Aedes hendersoni* (Cockerell), *Toxorhynchites rutilus septentrionalis* (Dyar and Knab) and *Psorophora ferox* (Humboldt) together comprised about 2.0% of the immatures collected.

Seven mosquito species were collected from artificial containers proximate to treeholes at the three sites (Table 3). *Culex restuans* Theobald and *Cx. territans* Walker were collected only from the artificial containers. A total of 16,117 mosquito larvae and pupae was collected from the 75 artificial containers: 6,119 (38%) from Hearnes, 5,022 (31%) from Hinkson, and 4,976 (31%) from Ashland. As in collections from treeholes, *Ae. triseriatus* was the dominant species, representing 89% of mosquito immatures from artificial containers at all sites during the three seasons, followed by *Or. signifera* (6%) and *An. barberi* (3%). *Aedes hendersoni* and *Tx. r. septentrionalis* comprised only 0.2% of the artificial container-breeding mosquitoes sampled during this study. Most *Tx. r. septentrionalis* were collected from the Hinkson site, but none was collected from Hearnes. In contrast, *Ae. hendersoni* immatures were collected only from Hearnes during the 1986 and 1987 sampling seasons, and none was collected in 1988.

Table 2. Number and percent of mosquito larvae and pupae collected from tree holes at three study sites in Boone County, MO, May to October, 1986 to 1988.

	Study sites					
	Hearnes		Hinkson		Ashland	
Species	No. coll.	(%)	No. coll.	(%)	No. coll.	(%)
Aedes triseriatus	14,112	(87.8)	9,622	(93.2)	10,778	(86.1)
Aedes hendersoni	854	(5.3)	0		0	
Orthopodomyia signifera	623	(3.9)	407	(3.9)	1,325	(10.6)
Anopheles barberi	468	(2.9)	291	(2.8)	364	(2.9)
Toxorhynchites rutilus septentrionalis	13	(0.1)	9	(0.1)	43	(0.3)
Psorophora ferox	0		0		2	(0.1)
Total	16,070		10,329		12,512	

Table 3. Number and percent of mosquito larvae and pupae collected from artificial containers at three study sites in Boone County, MO, May to October, 1986 to 1988.

	Study sites					
	Hearnes		Hinkson		Ashland	
Species	No. coll.	(%)	No. coll.	(%)	No. coll.	(%)
Aedes triseriatus	5,527	(90.3)	4,578	(91.2)	4,317	(86.7)
Aedes hendersoni	47	(0.8)	0		0	
Orthopodomyia signifera	179	(2.9)	217	(4.3)	491	(9.9)
Anopheles barberi	215	(3.5)	173	(3.5)	119	(2.4)
Toxorhynchites rutilus septentrionalis	0		17	(0.3)	1	(0.1)
Culex restuans	107	(1.8)	37	(0.7)	48	(1.0)
Culex territans	44	(0.7)	0		0	
Total	6,119		5,022		4,976	

Culex restuans and *Cx. territans* comprised 0.5% of the artificial container mosquito larvae and pupae populations sampled. Most of the *Cx. restuans* (11%) were collected from the Hearnes site during the 1988 season, but none was collected in 1986. Immatures of *Cx. territans* were collected only from the Hearnes site during the 1987 and 1988 seasons.

Distribution of Species. *Aedes triseriatus* immatures were found in water-filled treeholes of all tree species sampled (Table 4), and were present throughout most of the three seasons sampled, except during October of each year. Most collections (expressed as percent of individuals of each mosquito species collected per site) were made from treeholes in yellow chestnut oak at the Hearnes site, silver maple at the Hinkson site, and white oak at the Ashland site, which were the most abundant tree species at each of the study locations (Table 1). The average numbers of *Ae. triseriatus* immatures from treeholes of each tree species sampled correlated positively ($P < 0.05$) by Kendall's coefficient of rank correlation (Sokal and Rohlf 1969) with the relative abundance of such trees at Hearnes (tau = 0.83) and Hinkson (tau = 1.0). The same trend for *Ae. triseriatus* was observed at Ashland (Table 4), but there were too few species of trees sampled to calculate a significant correlation. Average populations of *Ae. triseriatus* immatures were larger (22.2 per sample) in treeholes versus those in artificial containers (7.9 per sample) during each of the three seasons.

Orthopodomyia signifera immatures were collected from water-filled treeholes in American elm, green ash, hackberry, white oak, and yellow chestnut oak at the Hearnes site; at the Hinkson site, they were collected from American elm, silver maple, and white oak, but at the Ashland site, they were collected only from white oak. Average numbers of *Or. signifera* immatures in treeholes were larger than were those in artificial containers at Hearnes and Ashland in 1986 and 1987.

Anopheles barberi was collected at all sites and from a wide range of tree species during this study, but its distribution was scattered and its immatures occurred in large numbers only in white oak treeholes (Table 4). Larvae and pupae of *An. barberi* were collected from artificial containers, but abundance was greater in treeholes.

Aedes hendersoni larvae and pupae were collected only at the Hearnes site during the 1986 and 1987 seasons from treeholes in yellow chestnut oak and sugar maple; fewer were collected from black walnut, white oak, and artificial containers.

Toxorhynchites rutilus septentrionalis immatures were collected from all sites during all years of the study and were present in treeholes in green ash, yellow chestnut oak, boxelder, silver maple, and white oak.

Psorophora ferox immatures were collected only once from a basal water-filled white oak treehole at the Ashland site in 1988.

Culex restuans and *Cx. territans* larvae and pupae were collected only from artificial containers. Immatures of *Cx. restuans* were collected from all sites, but those of *Cx. territans* were collected only from Hearnes.

Ecological Observations. The average water temperature in shaded treeholes (19.2°C) was lower than in shaded artificial containers (22.2°C) at all sites. Similarly, sunlit treehole water (21.7°C) was on the average cooler than water in sunlit artificial containers (24.9°C) at all sites. During the 1988 season, more larvae and pupae of *Ae. triseriatus* were collected at the three sites from treeholes exposed to the sun (avg. = 26.2 immatures per sample) than from shaded treeholes (avg. = 18.1 immatures per sample). Similarly, more *Ae. triseriatus* immatures were collected from sunlit artificial containers (avg. = 26.1 per sample) than from shaded artificial containers (avg. = 18.1 per sample). The temperature differentials between the sunlit and shaded habitats may have contributed to these population differences.

The height of the treeholes and proximate artificial containers sampled during this study ranged from 0 to 5 m above ground level. *Ae. triseriatus* was collected from treeholes and artificial containers at all heights sampled at each site, followed in descending frequency by *An. barberi, Ae. hendesoni,* and *Or. signifera.* Immatures of *Tx. r. septentrionalis* were not collected at heights > 3 m, and those of *Cx. restuans, Cx. territans,* and *Ps. ferox* were collected only from heights < 1 m.

Table 4. Tree species inhabited (expressed as % of sample of each mosquito species per site) by larval and pupal treehole-breeding mosquitoes at three study sites in Boone county, MO, 1986 to 1988.

	Tree species* at 3 sites with holes containing mosquito immatures (%)																
	Hearnes									Hinkson					Ashland		
Species	BA	AE	BW	BX	GA	HB	SU	WO	YC	AE	BW	BX	SM	WO	BO	FD	WO
A. triseriatus	4.7	6.6	3.4	2.4	21.1	2.5	11.2	5.3	42.8	14.0	11.8	16.5	48.5	9.2	17.0	10.3	72.7
A. hendersoni			4.8				28.8	4.6	61.8								
O. signifera		38.6			4.9	2.8		31.9	21.8	31.2			24.6	44.2			100
An. barberi		1.3	10.3	5.6	11.3	4.5	7.7	43.6	15.8	13.5		11.5	22.2	52.8			100
T. rutilus septentrionalis					30.8				69.2			55.6	44.4				100
P. ferox																	100

* Abbreviations for tree species are: AE (American elm), BA (American basswood), BO (black oak), BW (black walnut), BX (boxelder), FD (flowering dogwood), GA (green ash), HB (hackberry), SM (silver maple), SU (sugar maple), WO (white oak), and YC (yellow chestnut oak).

Ae. triseriatus immatures were collected from water with the widest pH and conductivity ranges recorded during this study. This species occurred in treehole and artificial container water with pH values ranging from 7.0 to 8.9 and 6.0 to 8.2, respectively. These data agree with previous studies (Thomas 1964, Petersen and Chapman 1969). Conductivity varied from 430 to 2,852 microhms in the treehole water, and from 156 to 1163 microhms in the artificial container water.

More *Ae. triseriatus* larvae and pupae were collected from artificial container water enriched with leaf litter (avg. = 10.9 per sample) than from unenriched water (avg. = 4.7 per sample) at all sites during the study. There was no significant correlation ($P > 0.05$; Kendall's coefficient of rank correlation) between the average number of immature *Ae. triseriatus* collected from treeholes and artificial containers at Hearnes (tau = 0.4), Hinkson (tau = 0.4), or Ashland (tau = 0.2).

Discussion

Our present data suggest that *Ae. triseriatus* is opportunistic with respect to tree species inhabited, with distributions roughly paralleling the relative abundance of available treeholes. In contrast, *Or. signifera* exhibited a preference for treeholes in American elm and white oak, and *An. barberi* preferred treeholes in white oak.

More larvae and pupae of *Ae. triseriatus* were collected from treeholes than from artificial containers at each sampling site during this study, possibly because of the higher concentrations of nutrients available in treehole water (Fish and Carpenter 1982, Kitching 1983, Walker and Merritt 1988). Tree hole water (Petersen and Chapman 1969) and stemflow (Eaton et al. 1973, Carpenter 1982) have been found to provide nutrients (nitrogen, phosphorus, and dissolved carbon) essential for microbial grown on leaves. Recently, Walker and Merritt (1988) showed that larvae of *Ae. triseriatus* exploit a variety of food resources in treeholes (e.g., filter-feeding on bacteria in treehole water and browsing on leaf and bark surfaces).

Factors such as food supply (in treehole water and artificial container water enriched with leaf litter), water temperature, height of treeholes or artificial containers, pH, and conductivity may have been important in the selection of oviposition sites by female mosquitoes or in the survival of their progeny. Mosquito larvae feed on leaf litter by removing the nutrient-rich microbials from this substrate (Wallace and Merritt 1980, Fish and Carpenter 1982, Walker and Merritt 1988). Despite the benefit of enrichment of artificial containers with leaf litter, the lower numbers of *Ae. triseriatus* immatures collected from artificial containers and those from treeholes indicate that the type of container used in the present study constitutes an inadequate mimic for treeholes when monitoring *Ae. triseriatus*.

Larger populations of treehole and artificial container-breeing mosquitoes were recorded in 1986 and 1987, whereas smaller numbers were collected in 1988, probably because of drought during which the average rainfall was about 50 mm compared to 116 mm and 86 mm in 1986 and 1987, respectively (National Oceanic and Atmospheric Administration 1986, 1987, 1988). The 1988 summer drought may have affected treehole and artificial container-breeding mosquitoes intolerant to dry conditions, such as *An. barberi* and *Or. signifera*. *Ae. triseriatus*, whose eggs are drought-resistant, can remain viable for months (Chambers 1985, Bradshaw and Holzapfel 1988).

Immature *Or. signifera, An. barberi,* and *Tx. r. septentrionalis* were collected from treehole and artificial container water characterized by high pH (usually above 8) and conductivity (usually above 1500 microhoms). Fewer *Ae. hendersoni* and more *Ae. triseriatus* immatures per sample occurred in treeholes and artificial containers at lower heights, indicating possible preferences of these species. *Ae. hendersoni* females have been noted to oviposit in higher treeholes (Loor and DeFoliart 1970, Scholl and DeFoliart 1977, Sinsko and Grimstad 1977) and occurred more frequently in biting or sweeping collections from the forest canopy (Novak et al. 1981). If artifical containers were used to monitor populations of *Ae. triseriatus,* it would be important to place such containers at lower heights for greatest sampling efficiency.

The larvae of *Or. signifera* were most abundant during the 1986 and 1987 seasons. In contrast, few larvae of this species were collected from treeholes and artificial containers during the 1988 season, probably because of the aforementioned drought. These results agree with those from a recent study that reported *Or. signifera* larvae to be drought-susceptible (Bradshaw and Holzapfel 1988).

The present study provided the first record of *Psorophora ferox* larvae collected from a treehole. The eggs of this species are usually deposited on moist soil under leaves, and the larvae are found in temporary woodland pools lined with leaves (Horsfall 1955, Siverly 1972, Wood et al. 1979).

Culex restuans and *Cx. territans* are rarely collected from basal treeholes, various artificial containers, and rock pools (Bradshaw and Holzapfel 1983, Chambers 1985). Their usual breeding habitats consist of ditches and temporary pools with decaying vegetation, and swampy marshes (Carpenter and LaCasse 1955, Wood et al. 1979). Their low abundance during the present study probably resulted from infrequent oviposition in the treeholes and artificial containers sampled.

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