

The Blood-Feeding Habits of *Aedes sollicitans* (Walker) in Relation to Eastern Equine Encephalitis Virus in Coastal Areas of New Jersey¹

III. Habitat Preference, Vertical Distribution, and Diel Periodicity of Host-Seeking Adults

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ABSTRACT: The relative abundance of host-seeking *Aedes sollicitans* (Walker) was compared among habitats that included a deciduous forest, a coniferous forest, a cedar swamp, and an abandoned field in southern New Jersey. Collections were made using dry ice baited CDC traps operated from dusk to dawn without a light source. Results indicated that significantly more mosquitoes were collected from the field; collections in wooded habitats and roadways were considerably lower. Fewest mosquitoes were collected in the cedar bog where eastern equine encephalitis (EEE) virus is believed to be most prevalent. The temporal and vertical distribution of host-seeking *Ae. sollicitans* were determined using dry ice baited CDC traps placed at 0.5, 2.5, and 6.0 m above ground level on the edge of an open field and a deciduous forest. Over the course of the investigation, 82% of the total collection was taken at the lowest level, 17% at the mid-level, and only 1% at the highest level. Host-seeking activity occurred in bimodal crepuscular peaks during the summer and early fall. Later in the season, host-seeking was reduced to a single peak prior to sunset, presumably the result of decreasing temperatures. Results suggest that habitat preference and vertical distribution strongly influence the mammalian blood-feeding habits of this species in nature. The reported paucity of avian blood meals in this mosquito may be due to a spatial disassociation between the mosquito and tree-roosting birds. The results are discussed in relation to *Ae. sollicitans* potential as a vector of EEE virus.

Keyword Index: *Aedes sollicitans*, host preference, vertical distribution, eastern equine encephalitis

INTRODUCTION

Blood-meal identification studies with *Aedes sollicitans* (Walker) based on precipitin tests of wild-caught adults indicate that the species is strongly oriented toward mammals and only rarely feeds on birds in nature (Thompson et al. 1963, Crans 1964, Edman and Downe 1964, Schaefer and Steelman 1969, Edman 1971, Crans et al. 1990). Crans et al. (1996), however,

showed that *Ae. sollicitans* is an opportunistic feeder and will readily accept a bird as a blood-meal host when given the opportunity.

Host availability can affect the range of hosts that are utilized by a particular mosquito species in nature and result in a feeding pattern that is determined by repeated contact with a particular host rather than fixed feeding behavior (Edman et al. 1972). Availability requires spatial and temporal synchrony between the

potential hosts and the host-seeking mosquitoes. Spatial relationships can occur within horizontal zones or habitats and in vertical strata or heights within those habitats. Temporal relationships may be either diel or seasonal. This paper reports the results of a study to investigate the habitat, vertical distribution, and diel rhythms of host-seeking *Ae. sollicitans* in relation to the availability of hosts. Particular attention has been focused on birds that roost in the forest canopy because of their role as reservoirs of eastern equine encephalitis (EEE) virus (Stamm et al. 1962, Stamm 1963).

MATERIALS AND METHODS

Habitat Distribution of Host-Seeking Adults

Studies were conducted in the southern-most portion of the Belleplain State Forest, Cape May County, New Jersey. The area represents a peninsula composed of pine-oak forest bordered on its southern extremes by deciduous, lowland tree species. The lowland forest adjoins a tidal salt marsh which extends south, east, and west of the study site. Large populations of *Ae. sollicitans* move into the area from adjacent marshland that has been diked for the production of salt hay.

Mosquitoes were collected using miniature CDC traps (Sudia and Chamberlain 1962), baited with approximately 3 kg of dry ice, and operated throughout the collection period without a light source. Nine collections were made at weekly intervals from mid-June to September 1983. On each collection date, nine traps were suspended at fixed locations at a height of 0.5 m and operated from one hour before sunset to one hour after dawn. Two intersecting roads served as transects for the experiment.

The habitats sampled in this study included: 1) a deciduous forest composed primarily of *Liquidambar styraciflua*, *Acer rubrum*, and *Nyssa sylvatica*; 2) a coniferous forest, dominated by *Pinus strobus*; 3) a dense cedar swamp where *Chamaecyparis thuyoides* was the main tree species; and 4) an upland field composed of numerous grasses and herbaceous plants. Four of the traps used in this study were placed along the shoulders of the intersecting roads to represent a ubiquitous habitat and determine if significant differences existed within the study area that could bias the comparison among habitats. A trap was also placed at the end of the transect on the edge of the nonbreeding *Spartina alterniflora* salt marsh that surrounded the upland peninsula to determine if proximity to the salt marsh biased the results.

Data were analyzed using an analysis of variance on the log transformed data, $\log(x + 1)$. One was added to each catch to avoid log transformations of 0. Log

transformation was utilized to meet the requirements of normality, additivity, and homoscedasticity in the test. Differences in the means were tested using Fischer's protected LSD method at $P < 0.05$.

Vertical Distribution and Diel Periodicity of Host-Seeking Adults

Aedes sollicitans were sampled weekly from late July until mid-October, 1982, on the edge of a 1 ha field in the Belleplain State Forest where large populations of *Ae. sollicitans* were observed to rest. The wooded border of the field was composed of *Myrica heterophylla*, *Prunus serotina*, *Viburnum dentatum*, *Rhus copallina*, *Sassafras albidum*, and *Diospyros virginiana*. Six miniature CDC traps baited with dry ice were operated each week. The traps were randomly assigned to three height levels (0.5, 2.5, and 6.0 m) in two replicate plots along the edge of the field and the deciduous forest. Traps were suspended from the branches of trees directly on the ecotone. A pulley system was used to hoist the traps operated at the 6.0 m level.

Each trap was baited with a 2.5 kg block of dry ice at the onset of the collection period, and fresh blocks were added over the 25-hour collection period as needed. At one-hour intervals, collection sleeves were retrieved and immediately replaced with empty duplicates. The mosquitoes were frozen on dry ice and placed in vials for later identification and sorting. Temperature and relative humidity were recorded for each hourly collection using a Bendix model 566 psychrometer. The first hour's collections were excluded from the analysis because of the initial disturbance and attraction of resting mosquitoes during the erection of the traps.

The exact time for beginning the hourly collections was determined by the time at which civil twilight occurred on that day. In this way, collection periods on different collection dates could be compared in relation to mosquito activity at sunrise and sunset.

A three way analysis of variance was performed to test the effects of height, time of day, and temperature upon trap catch. Because the length of scotophase increased during the course of this study, hourly samples could not be meaningfully compared by using recorded times. Rather, collections were grouped into diel periods (PM of day 1, sunset, night, sunrise, and AM of day 2), and a mean was taken for the hourly catch within each diel period. Similarly, samples were grouped into one of three temperature ranges, determined by the temperature at which the collection was taken. The groupings (10-15°, 16-20°, and 21-32°C) were based, in part, on the relative levels of activity for *Ae. sollicitans* as observed by Rudolfs (1923). Trap data were transformed to $\log_e(x+1)$ to approximate the conditions

of normality, additivity, and homoscedasticity required by the analysis. Catch was increased by 1 to avoid log transformation of 0. Means are reported as modified geometric means. Differences between means were tested using Fisher's protected LSD method at $P < 0.05$.

RESULTS

Habitat Distribution of Host-Seeking Adults

A total of 11,120 *Ae. sollicitans* was collected by the traps set in the various habitats found in the Belleplain State Forest. TABLE 1 lists trap catch by habitat and shows that the trap placed in the open field collected 6,579 specimens, or 59.2% of the total collection. Comparison of the mean catch among trap sites showed that the field contained significantly more mosquitoes than any of the road sites or wooded habitats. The collections from the traps placed along roadways showed that the difference did not appear to be biased by proximity to the breeding area or by mosquitoes moving into the study site from a single direction. Collections in the trap placed on the salt marsh did not vary significantly from any of the road traps, and the traps placed along the roads did not significantly differ from one another. The trap placed in the cedar swamp collected only 31 *Ae. sollicitans* during the course of the study. Data indicate that collections in the cedar swamp were significantly lower than any of the other habitats sampled in this study.

Vertical Distribution of Host-Seeking Adults

A total of 8,875 *Ae. sollicitans* was collected in 1,572 separate samples from the traps operated at defined levels along the ecotone of a field and a forest. A three-way analysis of variance indicated that the effects of height, time of day, temperature, and all interactions were significant ($P < 0.001$). Differences in catch among heights were great. Over the course of the investigation, 81.9% of the specimens were collected by the traps operated 0.5 m above ground level ($n = 7267$), 16.9% were collected at 2.5 m ($n = 1509$), and only 1.2% were collected in the traps hoisted 6.0 m into the canopy ($n = 103$). TABLE 2 compares the mean catches at the three elevations according to the temperature groupings observed by Rudolfs (1923). Temperature did not alter the elevation at which the mosquitoes were host-seeking; the lowest trap always collected significantly more mosquitoes. Under the coldest temperatures, however, the catch at 6.0 m was virtually eliminated.

Diel Rhythms of Host-Seeking Adults

When 24-hour host-seeking activity was averaged over the entire season, the pattern was bimodal with abrupt increases near sunset and sunrise. Figure 1 plots mean catch of *Ae. sollicitans* at the three heights sampled in this investigation and shows that the bimodal activity was most apparent in the trap operated closest to the ground. However, separate analyses of the 11 collection dates showed that only two actually exhibited this

TABLE 1. Collections of *Aedes sollicitans* from CDC traps placed at fixed locations at a coastal study site in New Jersey. Means with different letters are significantly different ($P < 0.05$).

Trap Site	Total catch	(%)	Mean Catch	
			Per Trap date	± S.D.
Field	6579	(59.2)	344.1	± 4.1 ^a
Road 1	1190	(10.7)	64.2	± 4.0 ^b
Coniferous Forest	737	(6.6)	57.4	± 2.8 ^b
Road 4	630	(5.7)	43.5	± 3.8 ^b
Salt Marsh	671	(6.0)	38.4	± 5.8 ^b
Road 2	160	(1.4)	22.1	± 3.2 ^b
Deciduous Forest	492	(4.4)	17.9	± 7.0 ^b
Road 3	630	(5.7)	14.7	± 12.0 ^b
Cedar Swamp	31	(0.3)	4.2	± 3.1 ^c

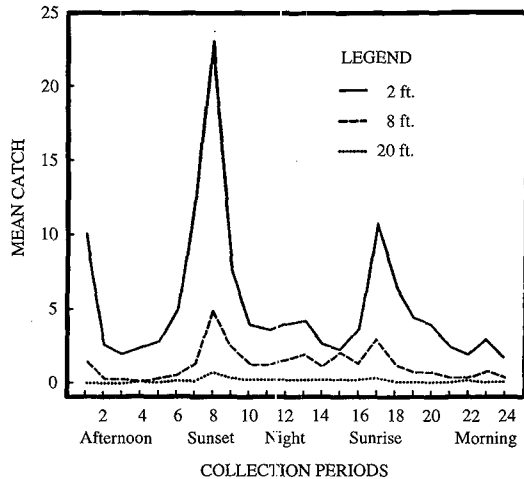


Figure 1. Mean catch of *Aedes sollicitans* taken at three heights over a 24-hour collection period.

generalized pattern. Most collection dates had only a single sunset or sunrise peak with a much smaller peak in the corresponding twilight period. Variability could not be associated with any single factor.

Crepuscular peaks in activity occurred at each height but relatively few mosquitoes flew at 2.5 m or higher. TABLE 3 lists mean trap catch at the three heights during the major diel periods recognized in this study. Data show that the activity peaks were most pronounced at the 0.5 m level (where most of the mosquitoes were trapped) but were also significant at height levels where relatively few specimens entered the traps.

The analysis of variance indicated that temperature had a significant effect on the activity of *Ae. sollicitans* ($P < 0.0001$) which becomes apparent when the data are viewed on a monthly basis over the course of the season. Figure 2 compares the monthly trap catches of *Ae. sollicitans* by diel period and indicates that a seasonal shift in activity occurred from summer to fall. Crepuscular activity peaks occurred during both sunset and sunrise during July, August, and September. Nighttime and early morning activity, however, appeared to diminish as the season advanced. The reduction of nocturnal and early morning activity as the season advanced may represent a seasonal trend that is based on

the effects of temperature. As the season progressed, nights became longer and the mosquitoes were exposed to colder temperatures for longer periods of time. Peak activity was observed during both of the twilight periods in August; but in October this changed to increased activity before sunset, reduced nocturnal activity, and very little activity at sunrise. During the month of October (when all collections fell under the coldest temperature grouping), activity after sunset was significantly reduced and the sunrise peak was virtually eliminated.

DISCUSSION

Data from this study suggest that *Ae. sollicitans* occurs in greatest numbers in open-field habitats. Few individuals appear to enter wooded habitats during the crepuscular and nocturnal host-seeking period; fewer still penetrate the cedar swamps that are so common in southern New Jersey. The results agree with the findings of Bidlingmayer (1971) who classified *Ae. sollicitans* as a field species based on suction trap collections in a field and a wooded swamp. The habitat preference of this species and its host utilization pattern appear to be related. Precipitin tests performed on wild-caught specimens show that *Ae. sollicitans* feeds primarily on

TABLE 2. The effect of temperature upon vertical distribution of *Aedes sollicitans* ($\bar{x} \pm s.d.$). Means with different letters within columns are significant $P (<0.05)$.

Height in Meters	Temperature					
	Warm*		Cool**		Cold***	
0.5	5.82	$\pm 1.33^a$	4.26	$\pm 2.90^a$	0.79	$\pm 1.25^a$
2.5	0.92	$\pm 0.76^b$	1.08	$\pm 1.59^b$	0.26	$\pm 0.60^b$
6.0	0.12	$\pm 0.22^c$	0.12	$\pm 0.38^c$	0.03	$\pm 0.15^c$

*21-32°C (n = 200)

**16-20°C (n = 178)

***10-15°C (n = 146)

TABLE 3. Mean catch (+ s.d.) of *Aedes sollicitans* taken within diel periods at three elevations. Means with different letters within columns are significantly different ($P < 0.05$).

Diel Periods	n	Height Levels in Meters					
		0.5		2.5		6.0	
PM of Day 1	124	3.93	$\pm 2.08^a$	0.43	$\pm 0.98^a$	0.05	$\pm 0.21^a$
Sunset	22	23.12	$\pm 2.98^b$	4.86	$\pm 2.84^b$	0.67	$\pm 0.87^b$
Night	194	3.17	$\pm 3.51^{ac}$	1.16	$\pm 1.80^c$	0.16	$\pm 0.46^a$
Sunrise	22	10.70	$\pm 4.62^d$	2.79	$\pm 3.38^b$	0.30	$\pm 0.62^{ab}$
AM of Day 2	162	3.09	$\pm 2.08^c$	0.42	$\pm 0.75^a$	0.01	$\pm 0.11^a$

large grazing mammals, rabbits, and a variety of other field-inhabiting mammals (Thompson et al. 1963, Edman and Downe 1964, Schaefer and Steelman 1969, Edman 1971, Crans et al. 1990). These hosts, most likely, represent the animals most available to *Ae. sollicitans* in the habitat most frequented by the species. Although some field-inhabiting birds are no doubt present at night, *Ae. sollicitans* appears to be spatially removed on a horizontal axis from the majority of birds that live in the forest at night.

Results of this study also indicate that *Ae. sollicitans* is relatively inactive during the day. The findings agree with the observations of Ebsary and Crans (1977) who based their study on attraction to a human host rather than traps baited with a carbon dioxide source. The mosquito exhibits marked crepuscular peaks in activity and demonstrates more host-seeking during the night

than the day. Crepuscular and nocturnal host-seeking behavior would be synchronized to the time when diurnal birds are most available and least defensive to mosquitoes. Data on the vertical distribution of *Ae. sollicitans*, however, indicate that the species seeks a host primarily at or near ground level. Significant differences in abundance between heights were observed during all diel periods, all temperature groupings, and throughout the season. As a result, *Ae. sollicitans* seeks a host at a time when birds are available but does so at ground level in open fields where deer and other mammals are the most abundant hosts. Although birds are undoubtedly nearby, the mosquito appears to be spatially removed on a vertical axis from the canopy where most passerine species spend the night.

Human cases of EBB and virus isolations from *Ae. sollicitans* have been reported only during the months of

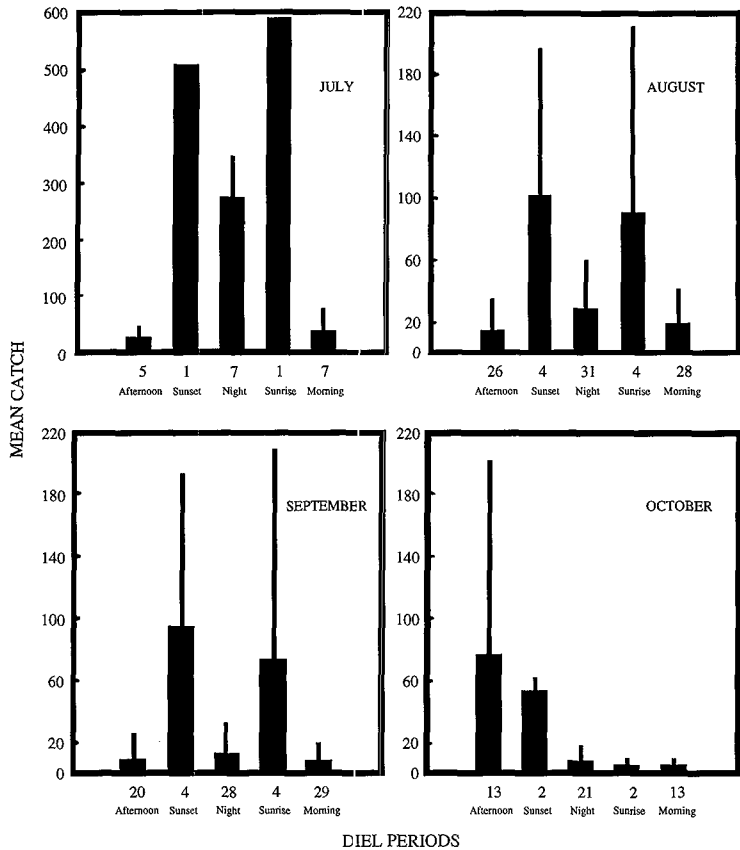


Figure 2. Mean hourly catch of *Aedes sollicitans* by diel period from July through October (Mean hourly catch per collection period $\pm$ s.d.). Numbers in parenthesis under X axis indicate number of hourly samples within each diel period for that month.

August, September, and early October in New Jersey (Hayes et al. 1962, Goldfield et al. 1968, Goldfield et al. 1969, Crans et al. 1986). During this period, *Ae. sollicitans* appears to be undergoing a subtle shift in host-seeking activity that is based primarily on decreasing nighttime temperatures. Rudolfs (1923) suggested that

Ae. sollicitans is relatively inactive at temperatures of 15°C or less. During the investigation in the Belleplain State Forest, temperatures of 15°C first occurred during the night in late summer. As the season progressed, temperatures at this level or lower occurred more frequently and extended over longer periods of the

night. Low temperatures appeared to gradually reduce the nocturnal activity of *Ae. sollicitans* and also lower the size of the activity peak in early morning. The trend was obvious throughout the month of September. By October, host-seeking at dawn was virtually eliminated and the peak at dusk shifted from sunset to early afternoon. Blaustein et al. (1980) made a similar observation in New Jersey when flight activity was being measured to determine the best timing for aerial applications for the control of *Ae. sollicitans*.

The combination of habitat preference, vertical distribution of host-seeking, and seasonal diel periodicity of *Ae. sollicitans* may be important factors limiting the species' potential as a vector of EEE virus. Forest-roosting birds are believed to function as the main disease reservoir (Stamm 1963) and two hypotheses have been proposed regarding the relationship of habitat and prevalence of EEE virus in birds and mosquitoes. Williams et al. (1972) suggested that EEE virus circulates primarily in birds deep within cedar bogs and wooded swamps. Morris et al. (1980) and Howard et al. (1983) felt that virus transmission to birds might be greatest on the edges of bogs, swamps, woodlands, or open fields. Data from the study conducted in the Belleplain State Forest of New Jersey suggest that in either case, *Ae. sollicitans* would have limited contact with the amplification source of EEE virus. If the virus circulates in birds in dense swamps and bogs, *Ae. sollicitans* would have little opportunity to make contact with the virus since it rarely enters this type of habitat. If virus occurs primarily in birds that inhabit ecotonal areas, *Ae. sollicitans* would be spatially removed from the reservoir source that roosted in the canopy surrounding the field habitat and be dependent upon field inhabiting birds as a virus source during enzootic and epizootic periods. Consequently, considerable virus activity might be required to result in the infection of this highly susceptible mosquito, even though the mosquito is abundant within the salt marsh/cedar swamp focus where EEE virus is most common.

Decreasing nighttime temperatures appear to increase the spatial disassociation between *Ae. sollicitans* and tree-roosting birds by reducing the numbers of mosquitoes that enter the canopy and shifting crepuscular and nocturnal host-seeking behavior to midday and late afternoon. Since low nighttime temperatures occur during late summer and fall when EEE virus is most abundant in the passerine population, opportunistic feeding would increase at ground level where mammals, rather than birds, would be the most likely hosts. Crans et al. (1990) suggested that nonpasserine bird species, particularly ciconiiforms, may have special epidemiological significance during periods of virus amplification.

The results of the studies conducted in the Belleplain State Forest agree with that observation and suggest that ground-dwelling birds rather than perching birds should be more fully investigated as a source for infection of *Ae. sollicitans* with EEE virus.

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Susceptibility of DDT, Dieldrin, and the Development of Malathion Resistance in *Anopheles culicifacies* (Diptera: Culicidae) in Ambala District of Haryana, India

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ABSTRACT: The susceptibility of the malaria vector *Anopheles culicifacies* to DDT, dieldrin, and malathion was determined in Nada Sahib of Ambala District, India. The vector population was resistant to DDT, dieldrin, and malathion. The mortality of *Anopheles culicifacies* to malathion ranged between 20 to 80% at different times. Malathion coverage during spray operations was between 62.1% in human dwellings and 91.6% in cattle sheds.

Keyword Index: Mosquitoes, insecticide resistance, malaria.

INTRODUCTION

The large scale use of insecticides for public health and agriculture, deforestation, extension of irrigation facilities to bring more areas under cultivation, and lack of water management have all changed the ecology of malaria vectors in India. *Anopheles culicifacies* is an important malaria vector in rural areas of India, and its resistance to DDT and dieldrin has been found to be widespread. This resistance may have resulted from a change in ecological conditions, and there is an urgent need for entomological studies in the malarious areas to assess vector susceptibility to different insecticides. *Anopheles culicifacies* has shown varying levels of resistance to DDT, dieldrin, and malathion. The resistance to malathion in *An. culicifacies* was identified in Gujarat State (Rajagopal 1977). Among the malaria vectors, *Anopheles stephensi* was first to have developed resistance to DDT in 1945 (Rajagopal et al. 1956). Subha Rao et al. (1984) identified the development of malathion resistance in *An. stephensi* in Haryana State. In western India, most parts of Gujarat, Maharashtra, and bordering districts of Madhya Pradesh, *An. culicifacies* has become resistant to malathion (Das et al. 1986, Vittal and Deshpande 1983). This study was undertaken to determine the level of susceptibility of *An. culicifacies* populations to diagnostic concentrations of DDT, dieldrin, and malathion.

MATERIALS AND METHODS

The field study was carried out in the Nada Sahib area of Ambala District of Haryana State, where during the last few years the incidence of malaria was extremely high. The susceptibility of the vector population was determined with the WHO Kit (WHO 1975). Gravid mosquitoes were exposed to malathion 5% impregnated papers. The exposure period was one hour, and mortality counts were made after a 24-hour holding period. The method reported by Brown and Pal (1971) was adopted in the study. Adult female mosquitoes were collected with aspirator tubes from the field and transferred to a plastic holding tube. Tests were carried out in different months within the study area. Three cycles (rounds) of malathion spray in the study area were carried out since 1983. Two rounds of malathion spray were undertaken in 1990 and 1992 due to nonavailability of insecticide.

RESULTS AND DISCUSSION

The malariometric indices of the study area are shown in TABLE 1. The annual blood examination rate was more than 10% in all years. The annual parasite incidence was highest at 6.1 in 1990. The slide positivity rates were highest in 1990 and 1991. Susceptibility tests were carried out by exposing field