

**Bionomics of *Ochlerotatus triseriatus* Say (Diptera: Culicidae) and
Aedes albopictus Skuse (Diptera: Culicidae) in emerging La Crosse
virus foci in Virginia**

Christopher M. Barker

Thesis submitted to the faculty of Virginia Polytechnic Institute and State
University in partial fulfillment of the requirements for the degree of:

MASTER OF SCIENCE
in
ENTOMOLOGY

Advisory Committee:

Dr. Sally Paulson, Chair
Dr. Carlyle Brewster
Dr. Charles Apperson
Dr. Shirley Luckhart

July 10, 2001
Blacksburg, Virginia

Keywords: *Ochlerotatus triseriatus*, *Aedes albopictus*, La Crosse virus, geospatial
modeling

Copyright 2001, Christopher M. Barker

Bionomics of *Ochlerotatus triseriatus* Say (Diptera: Culicidae) and *Aedes albopictus* Skuse (Diptera: Culicidae) in emerging La Crosse virus foci in Virginia

Christopher M. Barker

(ABSTRACT)

Recently, the number of human cases of La Crosse encephalitis (LACE), an illness caused by mosquito-borne La Crosse (LAC) virus, has increased in southwestern Virginia, resulting in a need for better understanding of the virus cycle and the biology of its vectors in the region. This project examined the spatial and temporal distributions of the primary vector of LAC virus, *Ochlerotatus triseriatus*, and a potential secondary vector, *Aedes albopictus*. Ovitraping surveys were conducted in 1998 and 1999 to determine distributions and oviposition habitat preferences of the two species in southwestern Virginia. For virus assay, adult mosquitoes were collected at a tire dump and a human case site during 1998 and 1999, and ovitrap samples were taken from a human case site in 2000.

In a separate study, a landcover map of Wise County was created by supervised classification of Landsat Enhanced Thematic Mapper imagery, and maps indicating posterior probabilities of high mosquito abundance were created by combining ovitrap survey-derived, landcover-based prior and conditional probabilities for high and low mosquito abundance using remote sensing techniques and Bayesian decision-making rules.

Both *Oc. triseriatus* and *Ae. albopictus* were collected from all ovitrap sites surveyed in Wise, Scott, and Lee Counties during 1998. Numbers of *Oc. triseriatus* remained high from late June through late August, while *Ae. albopictus* numbers increased gradually through June and July, reaching a peak in late August and declining thereafter. Overall, *Oc. triseriatus* accounted for 90.1% of eggs collected during this period, and *Ae. albopictus* made up the remaining 9.9%. Abundance of the two species differed among the sites, and in Wise County, relative *Ae. albopictus* abundance was highest in sites with traps placed in open residential areas. Lowest numbers of both species were found in densely forested areas. Ovitraping at a human LACE case site during 1998 and 1999 revealed that *Aedes albopictus* was well-established and overwintering in the area. An oviposition comparison between yard and adjacent forest at the Duncan Gap human LACE case site in 1999 showed that *Ae. albopictus* preferentially oviposited in the yard surrounding the home over adjacent forested areas, but *Oc. triseriatus* showed no preference. LAC virus was isolated from 1 larval and 1 adult collection of *Oc. triseriatus* females from the Duncan Gap human case site, indicating the occurrence of transovarial transmission at this site.

The supervised landcover classification for Wise County yielded a landcover map with an overall accuracy of 98% based on comparison of output classification with user-defined ground truth data. Posterior probability maps for *Oc. triseriatus* and *Ae. albopictus* abundance reflected seasonal and spatial fluctuations in mosquito abundance with an accuracy of 55-79% for *Oc. triseriatus* (Kappa=0.00-0.53) and 70-94% for *Ae. albopictus* (Kappa=0.00-0.49) when model output was compared with results of an ovitraping survey. Other accuracy measures were also considered, and suggestions were offered for improvement of the model.

Acknowledgments

I would first like to thank my advisory committee for their excellent guidance during my graduate study. In particular, I owe a debt of gratitude to my major advisor, Dr. Sally Paulson, who has spent numerous hours with me discussing research and demonstrating laboratory techniques, and her contributions during the preparation of this thesis have been invaluable. She also has encouraged me to frequently participate in scientific meetings, and I will always be grateful for those experiences. I am also very much indebted to Dr. Carlyle Brewster, who has never seemed too busy to discuss research or anything else, and he has spent untold hours working side-by-side with me to develop the model used in this project. Without him, much of my project would have been impossible, and I should also thank his wife, Charlene, for allowing me to borrow him on the many evenings when he stayed late to help with research-related issues. I would also like to thank my other advisory committee members, Dr. Charles Apperson and Dr. Shirley Luckhart, who also provided excellent advice during the course of my project. Throughout the project, I have appreciated Dr. Apperson's ever-insightful comments on my research, and I thank him for the many times that he forced me to think by asking questions that I had not thought of. I also thank Dr. Luckhart, who always took time out of her very hectic schedule to sit and talk with me when I needed assistance.

I would like to especially thank Mary Rhoades for her cheerful assistance with my research, including counting mosquito eggs, maintaining cell culture chambers, entering data, and numerous other contributions for which I will always be appreciative. Thank you also to Kathy Shelor and Sarah Kenley for their frequent assistance with almost anything I needed.

Thank you to the faculty members of the department who have provided excellent instruction in my coursework, and I am grateful for the interest that the faculty members take in the graduate students. I thank the faculty members for their openness toward students and me in particular, both inside and outside the classroom.

I have thoroughly enjoyed knowing the graduate students in the department, and I look forward to continuing the friendships we have made. In particular, I thank Jessica, Anne, Bryan, Ken, Lois, Riella, Jen, Sarah, Ksenia, Adam, Joan, Sean, Jarrod, Tree, Mike, and Hope for your friendship and support.

I am also very appreciative of my family, particularly my parents, who always supported me, even when they couldn't understand why anyone would want to study "bugs."

Table of Contents

Chapter 1

Introduction and Review of Literature.....1

Chapter 2

Habitat Preferences and Phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in Southwestern Virginia22

Chapter 3

Geospatial and statistical modeling of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) distribution in an emerging focus of La Crosse virus.....40

Chapter 4

Summary.....65

List of Figures

Chapter 1 Figure	Page No.
1.1 Virginia counties with confirmed human LACE cases, 1994-1998.....	1
Chapter 2 Figures	
2.1 Virginia counties with confirmed human LACE cases, 1994-1998.....	23
2.2 Ovitraping sites in Wise, Scott, and Lee Counties, 1998.....	24
2.3 Map showing relative abundance of <i>Oc. triseriatus</i> and <i>Ae. albopictus</i> by site for Wise, Scott, and Lee Counties, 1998.....	27
2.4 Mean weekly egg counts per ovitrap-day - Wise County, 1998.....	28
2.5. Relative <i>Ae. albopictus</i> abundance and total eggs per ovitrap-week by site – Wise County, 1998.....	29
2.6a. Back-transformed mean egg numbers per ovitrap-day – Rocky Fork, 1998.....	30
2.6b. Back-transformed mean egg numbers per ovitrap-day – Rocky Fork, 1999.....	30
2.7. Geometric means of <i>Oc. triseriatus</i> and <i>Ae. albopictus</i> eggs per ovitrap day for yard versus forest comparison – Duncan Gap, 1999.....	31
Chapter 3 Figures	
3.1. Virginia counties with confirmed human LACE cases, 1994-1998.....	41
3.2. Composite image showing ovitrap collection sites – Wise County, 2000.....	44
3.3. Computer windows showing feature mapping for supervised landcover classification.....	45
3.4. Buffer zones surrounding ovitrap sites used for error assessment.....	47
3.5a. Back-transformed mean <i>Oc. triseriatus</i> counts per ovitrap day by landcover type – Wise County, 2000.....	50
3.5b. Back-transformed mean <i>Ae. albopictus</i> counts per ovitrap day by landcover type – Wise County, 2000.....	50
3.6. Back-transformed mean <i>Oc. triseriatus</i> and <i>Ae. albopictus</i> counts per ovitrap day – Wise County, 2000.....	51

Chapter 3 Figures (cont.)	Page No.
3.7. Landcover map of Wise County resulting from supervised maximum likelihood classification of Landsat ETM+ imagery.....	52
3.8. Maps of Wise County showing posterior probability of high <i>Oc. triseriatus</i> abundance....	54
3.9. Maps of Wise County showing posterior probability of high <i>Ae. albopictus</i> abundance....	55

List of Tables

Chapter 2 Table	Page No.
2.1. Mosquito species tested for virus – Wise County, 1998-2000.....	32
Chapter 3 Tables	
3.1. Error matrix and accuracy measures used for assessing predictive capabilities of the Bayesian model.....	48
3.2. Results of GLM ANOVA and Fisher’s LSD tests – Wise County, 2000.....	49
3.3. Error matrix for supervised maximum likelihood landcover classification of Wise County Landsat ETM+ imagery.....	52
3.4. Sampling intensity by landcover type and actual prevalence of landcover type from landcover classification.....	53

Chapter 1

Introduction and Review of Literature

INTRODUCTION

La Crosse (LAC) virus is a spherical, enveloped RNA virus in the California serogroup within the genus *Bunyavirus* (Family: BUNYAVIRIDAE). This virus causes La Crosse encephalitis (LACE) in humans, which is the most common and important endemic mosquito-borne disease in the USA (Rust *et al.* 1999), with cases distributed mainly in the eastern and midwestern states. It affects mainly school-aged children (<15 yrs. old), causing a febrile illness usually accompanied by headache and vomiting. In more severe cases, patients often have seizures, which may recur for some time after the acute period of illness (McJunkin *et al.* 1998).

From 1975 through 1993, only one case of LACE was reported from the state of Virginia. However, between 1994 and 1998, CDC recorded a total of 13 cases of LACE from areas in southwest Virginia in the Appalachian Mountains (Fig. 1.1). In 1997, 5 cases were reported from Wise County, comprising disease incidence of approximately 13 per 100,000. Three of the 5 cases were from a small cluster of homes in a single community within the county. Because of these recent LACE cases, southwest Virginia is emerging as a significant focus of LAC virus activity and the need has arisen for studying the LAC virus cycle there and the biology of its vectors.

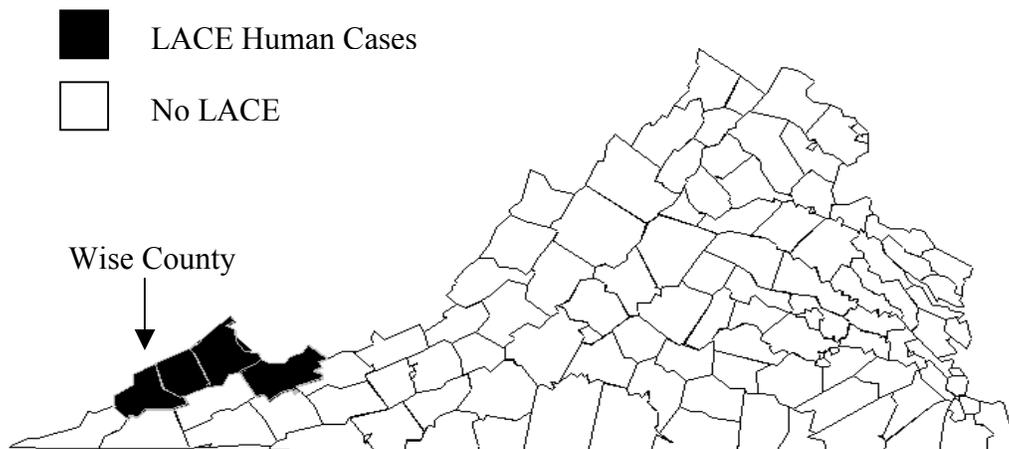


Fig. 1.1. Virginia counties reporting LACE cases (1994-1998).

REVIEW OF LITERATURE

La Crosse virus in humans

Discovery of La Crosse virus

In 1960, a 4-year old girl died with meningoencephalitis in La Crosse, Wisconsin. Routine virus testing did not detect the cause of her illness, so samples of brain and spinal cord tissues were frozen until May, 1964 when the tissues were tested again for virus by injection into suckling mice (Thompson et al. 1965). After multiple passages in mice with increasing pathogenesis, additional tests were used to further characterize the isolate. The isolate was infective following filtration and in the presence of penicillin and streptomycin, ruling out bacteria as causative agents. Following neutralization and complement fixation (CF) tests that implicated a California group or closely related virus, the isolate was sent to CDC for confirmation. The CDC confirmed that the virus was closely related but not identical to known California group viruses, including a strain of California encephalitis virus, first isolated by Hammon and Reeves in 1943 from *Aedes melanimon* (then called *dorsalis*) collected in Kern County, California (Hammon et al. 1952). The newly discovered virus from Wisconsin would soon become known as La Crosse virus.

Humans are dead-end hosts for LAC virus, being susceptible to infection, but incapable of developing a sufficient viremia to permit passage of the virus from a human to a susceptible mosquito. LAC virus is an endemic disease, meaning that exposure risk is relatively constant from year to year. Thus, LAC virus-endemic areas are characterized by additively increasing seroprevalence with age in humans (Szumlas et al. 1996a). Clinical disease is apparent usually only in children under 15 years of age, and even in children, the proportion of inapparent cases is high (Kappus et al. 1983).

Clinical studies

Several studies have been completed detailing clinical findings for La Crosse encephalitis cases in various regions where LAC virus is endemic. Balfour et al. (1973) found that La Crosse virus was the cause of illness for at least 61 of 66 children in Minnesota who had been diagnosed with California encephalitis between 1967 and 1972. These children were aged 6 months to 17 years, and all were from rural or suburban areas. All survived the illness, but abnormal electroencephalograms (EEGs) were noted in a number of cases and sequelae were experienced

by 5 of 33 patients for whom follow-up data were obtained. Four of the five had personality or behavioral problems such as emotional instability, irritability, and inattentiveness at school.

Rie et al. (1973) compared 29 children who had confirmed cases of California encephalitis in Ohio to 29 age and sex-matched control children to look for differences in intellectual ability and visual-motor coordination. Results were inconclusive, with no uniform effects of the illness identified in comparisons of cases with controls. Pre- and post-illness IQ levels were compared for 9 of the case children, again showing no uniform change. A group of six children exhibiting focal neurologic signs during illness was shown to have a greater discrepancy between nonverbal and verbal IQ scores and slightly poorer visual-motor coordination than other study groups.

In a study of 15 children in Ohio (Cramblett et al. 1966), LACE cases occurred between June and October, with most cases occurring during August and September. Most clinical presentations involved common symptoms of febrile illness, such as headache, fever, vomiting, and lethargy, and in approximately 60% of cases, seizures, irritability, and disorientation were apparent. 5 patients were comatose during the illness, but none experienced paralysis. Eight of nine EEGs obtained during acute illness and 6 of 9 obtained during convalescence showed abnormalities indicative of generalized cerebral dysfunction. Similar clinical presentations were seen in LACE patients in a newly emerging focus in Tennessee during 1997, except that the majority of cases (7/10) occurred during September (Jones et al. 1999).

La Crosse virus in mosquitoes

Vector competence

Following the reported isolation of La Crosse virus from the fatal human case in 1965, *Oc. triseriatus* was implicated as the primary vector of La Crosse virus in many areas based on: 1) presence of *Oc. triseriatus* in areas where LACE cases had occurred (Berry et al. 1975, Balfour et al. 1976), 2) numerous field isolations of LAC virus from *Oc. triseriatus* (Sudia et al. 1971, Thompson et al. 1972, Berry et al. 1974, Pantuwatana et al. 1974, Balfour et al. 1975), 3) associations of *Oc. triseriatus* with vertebrate hosts which had shown evidence of infection with LAC virus (Moulton and Thompson 1971; Gauld et al. 1974, 1975), 4) experimental infection of *Oc. triseriatus* by feeding on a viremic vertebrate host (Sudia et al. 1971, Watts et al. 1972), and 5) demonstration of LAC virus transmission by bite from *Oc. triseriatus* to a susceptible vertebrate host (Sudia et al. 1971; Watts et al. 1972, 1973; Patrican et al. 1985). Thompson et al. (1972) collected and tested 182,437 mosquitoes for virus over a period of 5 years from areas in

southwest Wisconsin and neighboring states where human cases or seroconversions in wild animals or sentinel rabbits had occurred. 8 isolates were obtained: 5 from *Oc. triseriatus* and 1 from each of 3 other species. Sudia et al. (1971) reported 100% infection rates in *Oc. triseriatus* seven days after feeding on a viremic host, and similarly high infection rates were maintained through 42 days of incubation. Transmission rates for *Oc. triseriatus* were 50, 79, and 82% after 21, 28, and 35 days of incubation, respectively.

Several additional mosquito species were tested by Watts et al. (1973a) to determine susceptibility and oral transmission rates for LAC virus. *Oc. canadensis*, *Ae. vexans*, *Ae. aegypti*, and *Culiseta inornata* were capable of LAC virus transmission, but at considerably lower rates than *Oc. triseriatus*, adding to evidence that *Oc. triseriatus* is the primary vector of LAC virus. *Oc. canadensis* has since been implicated by Berry et al. (1986) as a secondary vector of LAC virus in Ohio, based on virus isolations from field-collected mosquitoes, an experimental infection rate of 75%, and an oral transmission rate of 54% from infected individuals. Nasci et al. (2000) also reported isolations of LAC virus from host-seeking *Oc. canadensis* in two West Virginia study sites at rates similar to those found in *Oc. triseriatus*. Freier and Beier (1984) found that *Oc. atropalpus* also is experimentally susceptible to LAC virus and capable of oral and transovarial transmission rates similar to those for *Oc. triseriatus*.

Following reports of oral infection and transmission rates for *Oc. triseriatus*, many workers began to examine causes for variation in these rates. Comparisons were made between strains of *Oc. triseriatus* based on geographic origin by Grimstad et al. (1977). Strains from LAC virus-endemic areas in the midwestern U.S. were compared with strains from other areas where LAC virus was not present, and strains from LAC virus-endemic areas were orally infected and transmitted virus at lower rates than strains from non-endemic regions. The authors hypothesized that this possibly was due to evolution of mosquito resistance to LAC virus in endemic areas.

Grimstad and Haramis (1984) examined effects of larval diet on LAC virus transmission, finding that small *Oc. triseriatus* adults reared from nutritionally deprived larvae transmitted the virus at a higher rate than larger adults reared on more adequate diets. Because larval nutritional resources are often less than optimal in nature, these results indicated that previously reported transmission rates for mosquitoes reared in the laboratory on optimal larval diets might have been underestimated compared to naturally occurring rates. Paulson and Hawley (1991) tested

disseminated infection and oral transmission rates in *Oc. triseriatus* adult females field-collected as pupae to determine whether previously reported size-related variation in transmission rates from lab-reared mosquitoes (Grimstad and Haramis 1984) would exist in mosquitoes reared under natural conditions. Indeed, the smallest mosquitoes developed disseminated infections and orally transmitted virus at higher rates than the larger mosquitoes tested. The F₁ progeny of the largest and smallest adult populations showed no difference in susceptibility to infection or oral transmission rates, indicating that differences in the field-collected parental groups had been primarily a result of larval rearing conditions.

Another factor affecting infection and transmission rates for LAC virus in *Oc. triseriatus* is infective dose of virus received when a susceptible uninfected mosquito feeds on a viremic mammalian host (Patrican et al. 1985). It was shown that oral infection and transmission rates increased as infective dose of LAC virus increased, and that a certain threshold viremia titer must be exceeded before >50% of bloodfed *Oc. triseriatus* will be infected and capable of oral transmission.

Vertical transmission

Vertical transmission of LAC virus was first demonstrated by Watts and others (1973b) by showing infection in progeny of infected female *Oc. triseriatus* that had been experimentally infected. These infected offspring were then shown to effectively transmit LAC virus to suckling mice, and the authors suggested that this evidence plus isolations of LAC virus from field-collected larvae (Pantuwatana et al. 1974) indicated that transovarial transmission to overwintering eggs could be the means for maintenance of the virus during winter. Watts et al. (1974) found further evidence that transovarial transmission is the overwintering mechanism for LAC virus by isolating the virus from field-collected *Oc. triseriatus* larvae collected during the spring of 1973 in Wisconsin. Similar isolations of LAC virus from apparently overwintered eggs and larvae of *Oc. triseriatus* were also obtained by Balfour et al. (1975) in Minnesota and Beaty and Thompson (1975) in Wisconsin. Beaty and Thompson (1975) found low LAC virus infection rates (0.6%) in adults reared from overwintered *Oc. triseriatus* larvae from 4 enzootic study areas. In one of these study areas, treeholes were covered with screen early in the season to ensure that all collected mosquitoes originated from overwintered eggs, and isolates were obtained from these treeholes throughout the season from *Oc. triseriatus*, showing that hatching of overwintered eggs provided an early season and continuing summer season source of LAC

virus in this forested area. Similar rates of infection in overwintered *Oc. triseriatus* eggs (0.29-0.59%) were found by Lisitza et al. (1977).

Miller et al. (1979) found that no infection of *Oc. triseriatus* eggs occurred during the first ovarian cycle following oral infection of females. Thus, an orally-infected female must survive at least 13 days (Miller et al. 1979) and take 2 bloodmeals to successfully transmit virus to her offspring, underscoring the importance of continuous transovarial transmission for virus maintenance. Landry and DeFoliart (1987) also found that only 15.9% of parous *Oc. triseriatus* females in Wisconsin had completed two or more ovarian cycles, further indicating that infection of mosquitoes by viremic vertebrate amplifiers plays a relatively minor role in LAC virus maintenance.

Rates of transovarial transmission of LAC virus by *Oc. triseriatus* have been tested by Miller et al. (1977), who found that nearly all (98%) transovarially infected females transmitted virus to their progeny, and approximately 71% of offspring from these females was infected. Based on these rates, the authors suggested that the virus would eventually dilute itself out in the absence of occasional horizontal amplification. However, this study did show that the virus could persist in *Oc. triseriatus* for at least 8 generations without horizontal amplification in a vertebrate host. Miller et al. (1982) demonstrated variation in the ability of three geographic strains of *Oc. triseriatus* to transovarially transmit LAC virus. These strains were from Massachusetts, Connecticut, and Wisconsin, and had filial infection rates of 26%, 13%, and 50%. Similar differences in filial infection rates were also reported for geographic strains of *Oc. triseriatus* from Florida (33%) and Wisconsin (45%), although transovarial transmission rates did not differ between these strains (Woodring et al. 1998). The variation in filial infection rates among geographic *Oc. triseriatus* strains implied that even though LAC virus oral infection and transmission rates for mosquitoes in a given area may be high, reduced transovarial transmission rates might limit the prevalence of the virus in that area, since transovarial transmission is the primary means for LAC virus maintenance.

Horizontal transmission within mosquitoes

In addition to vertebrate amplification, another mode of horizontal LAC virus transmission was revealed when venereal transmission of LAC virus from transovarially infected males to previously uninfected females was discovered by Thompson and Beaty (1977). Since females and males had shown similar infection rates resulting from transovarial transmission of LAC

virus (Miller et al. 1977), venereal transmission was suggested as an important supplement to vertical viral transmission, even though rates of disseminated infection were low for venereally infected females (3.4%). In an additional study by Thompson (1979), venereal infection rates in female *Oc. triseriatus* were found to increase substantially when the female engorged blood before mating with a LAC virus-infected male (49% versus 4% when mating took place prior to engorgement). Salivary transmission also occurred at higher rates in females engorged before mating (35%) compared to those engorged after mating (2%). Transovarial transmission of LAC virus by venereally infected female *Oc. triseriatus* was also demonstrated in this study at a rate of 64% of eggs from second or later ovarian cycles. Kramer and Thompson (1983) found 26.3% venereal infection rates for *Oc. triseriatus* females that were bloodfed prior to mating with males intrathoracically inoculated with an unpassed field strain of LAC virus.

La Crosse virus in mammalian hosts

A serosurvey of small mammals on a Wisconsin farm where a case of LACE had occurred (Moulton and Thompson 1971) found that antibody prevalence rates to LAC virus were quite high, particularly in eastern chipmunks (*Tamias striatus*) and tree squirrels (*Sciurus niger* and *Sciurus carolinensis*), which had seroprevalence rates of 53 and 39%, respectively. Cottontail rabbits (*Sylvilagus floridanus*) and flying squirrels (*Glaucomys volans*) also possessed LAC virus antibody at respective rates of 15 and 5%. White-footed mice (*Peromyscus leucopus*) were not found with LAC virus antibody. These seroprevalence rates agreed well with the hypothesis that *Oc. triseriatus* was the primary vector of LAC virus, since chipmunks and tree squirrels had greater ecological overlap with this mosquito than the other mammals sampled. Tree squirrels and mice share habitat with *Oc. triseriatus*, but they are nocturnal and therefore rarely come into contact with day-biting mosquitoes. In an extensive study on vertebrate host preferences for a variety of mosquito species in Wisconsin (Wright and DeFoliart 1970), *Oc. triseriatus* engorged on gray squirrels and chipmunks at rates higher than those for the other mosquito species studied. Nasci (1985) also found that *Oc. triseriatus* fed predominantly on gray squirrels in a LAC virus enzootic area in Illinois. Conversely, blood-engorged *Oc. triseriatus* in a LAC virus-endemic area of North Carolina were found by Szumlas et al. (1996b) to feed predominantly on dogs (40%) and turtles (22%), which are non-amplifier hosts for LAC virus, and rabbits (27%), which may be capable of developing viremias sufficient to infect susceptible *Oc. triseriatus* (Yuill

1983). Only 7.5% of bloodfed *Oc. triseriatus* had fed on eastern chipmunks, and 2.1% had fed on humans (Szumlas et al. 1996b).

In a study in southwestern Wisconsin (Gauld et al. 1974), *Oc. triseriatus* abundance was found to be temporally associated with LAC virus antibody prevalence rates in chipmunks, and in one study area, seroprevalence rates in adult and spring-born juvenile chipmunks increased from 46% in late July to 100% by late September. LAC virus transmission was focal and varied widely among sites, with late September seroprevalence rates in adult and spring-born juvenile chipmunks reported as 0, 30, 11, 55, and 100% from five respective study sites. LAC virus was isolated from 7 chipmunks from two study sites during July and August (Gauld et al. 1975), further implicating chipmunks as important amplifier hosts in the LAC virus cycle. Isolates of LAC virus also have been obtained from gray squirrels and chipmunks by Ksiazek and Yuill (1977), who found that both species develop sufficient viremias to infect susceptible *Oc. triseriatus*.

Patrican et al. (1985) determined that infective viremias in chipmunks are sufficiently high to ensure oral infection and subsequent transmission by susceptible bloodfeeding *Oc. triseriatus* for only about 1 day per infective bite delivered to the chipmunk. Similarly, Pantuwatana et al. (1972) had found that high titer viremias were of short duration in chipmunks and squirrels, indicating the significance of transovarial transmission for LAC virus maintenance in mosquitoes.

Host suitability studies for LAC virus have also been conducted for other mammalian species such as whitetail deer (*Odocoileus virginianus*) (Issel et al. 1972, Osorio et al. 1996) and several species of domestic animals (Godsey et al. 1988) including dogs, cats, cows, horses, pigs, and sheep. So far, these species have not been implicated as important amplifiers of La Crosse virus based on results from experimental infection studies.

Biology of *Ochlerotatus triseriatus*

Ochlerotatus triseriatus is common throughout much of the eastern United States, especially in areas where deciduous forests predominate. This species breeds in treeholes or artificial containers such as tires (Craig 1983) and is common near areas of human habitation (DeFoliart and Lisitza 1980). Studies on dispersal of *Oc. triseriatus* differ in their conclusions, with some finding that *Oc. triseriatus* move only within the woodland larval habitat (Sinsko and Craig 1979) and others determining that they are willing to traverse large open areas (Mather and

DeFoliart 1984). Apparently, fencerows or other areas of dense vegetation linking woodland habitats provide a corridor for dispersal of *Oc. triseriatus* (Nasci 1982). Oviposition activity seems to be aggregated in certain areas within larger woodlots or other habitats, but studies to date have failed to correlate *Oc. triseriatus* oviposition with specific habitat variables (Beier et al. 1982, Kitron et al. 1989, Beehler and DeFoliart 1990). Kitron et al. (1989) suggest that deposition of a large number of eggs per oviposition event (29-47 in their study) would be enough to confound an otherwise random egg dispersion pattern within a woodlot. They further hypothesize, but could not prove, that water levels in the ovitraps, locations of the ovitraps on individual trees, types of trees to which the ovitraps were attached, specific areas within the woods, weather conditions, and shading of ovitraps may have contributed to the non-random oviposition patterns observed. *Oc. triseriatus* prefers to oviposit at or within approximately 1 m of ground level (Loor and DeFoliart 1970, Scholl and DeFoliart 1977, Aziz and Hayes 1987), in basal treeholes or other containers, preferably in shaded areas (Nasci 1988).

Periods of highest *Oc. triseriatus* oviposition activity occur during early to mid-summer (Scholl and DeFoliart 1977, Beehler and DeFoliart 1990, Szumlas et al. 1996c) with high levels of oviposition activity often sustained for approximately two months (Scholl and DeFoliart 1977, Nasci et al. 2000). Abundance levels within a season are quite variable, and are affected by rainfall, temperature, and other environmental factors, which serve to limit available larval development sites, alter development times, and affect survivorship (Teng and Apperson 2000).

Eggs of *Oc. triseriatus* are photoperiod-sensitive (Shroyer and Craig 1980), and as daylength shortens in late summer at middle and northern temperate latitudes, eggs enter diapause, which is not terminated until the following spring. Termination of diapause is thought to be triggered by a prolonged exposure to low temperatures (Shroyer and Craig 1983). It is in the diapausing eggs of *Oc. triseriatus* that La Crosse virus survives the winter. Larval diapause also occurs in *Oc. triseriatus*, but as an overwintering form, it occurs mainly in areas where winter freezing temperatures are uncommon (Sims 1982). Most of these areas are outside of the principal range for LAC virus activity, and thus larval diapause is not considered to be important in overwintering of LAC virus.

Adult female *Oc. triseriatus* feed during daylight hours and at dusk. Loor and DeFoliart (1970) observed feeding in early morning (5-9 am) and in mid- to late-afternoon (2-6 pm) in Wisconsin, but little biting activity was observed at dusk. Aziz and Hayes (1987) reported

similar patterns in Texas, except that peak biting activity was recorded at dusk, with smaller peaks in biting activity in early morning and mid-afternoon. Oviposition by *Oc. triseriatus* occurs during all periods of day and night, but activity peaks during the evening crepuscular period (Trexler et al. 1997).

Aedes albopictus

Another mosquito, *Aedes albopictus* (Skuse), has spread throughout the eastern United States in recent years, and is now sympatric with *Oc. triseriatus* in most of the USA east of the Mississippi River, including many areas endemic for LAC virus. Like *Oc. triseriatus*, this mosquito breeds in containers and treeholes and commonly inhabits areas near human dwellings. Oviposition by *Ae. albopictus* occurs almost entirely during daylight hours, particularly during mid-afternoon (Trexler et al. 1997).

Although earlier small-scale introductions that apparently failed to establish have been noted (Eads 1972, Reiter and Darsie 1984), breeding populations of *Ae. albopictus* were first found in the United States in 1985 near Houston, Texas (Sprenger and Wuithiranyagool 1986), probably introduced via shipments of used tires from Japan (Hawley et al. 1987). The vehicle for its spread likely has been used tires or other portable water-collecting containers, since *Ae. albopictus* is not given to long-range flight or wind-aided dispersal (Bonnet and Worcester 1946, Hawley 1988).

Ae. albopictus overwinters in a diapausing egg stage, but this species differs from *Oc. triseriatus* because the adult is the photoperiodically sensitive stage. Exposure to short daylengths causes production of diapause eggs by adult females (Hawley 1988), and these diapause eggs survive harsh winters at higher rates than non-diapause eggs (Hawley et al. 1989). However, eggs of *Oc. triseriatus* showed greater overwintering survival than *Ae. albopictus* (Hawley et al. 1989). The ability of *Ae. albopictus* to survive temperate winters and become established in these regions (Swanson et al. 2000) has certainly aided its spread in the eastern U.S.

Ae. albopictus is a competent experimental vector of LAC virus, with infection and oral transmission rates equal to or higher than those for *Oc. triseriatus* (Grimstad et al. 1989, Cully et al. 1992). The rates for transovarial transmission of LAC in *Ae. albopictus* are lower than those for *Oc. triseriatus* (Tesh and Gubler 1975). However, two isolates of LAC virus recently have been obtained from *Ae. albopictus* collected as eggs from LAC virus-endemic areas of North

Carolina and Tennessee (Gerhardt et al. 2001), indicating that natural transovarial transmission of the virus is occurring in these regions.

Cully et al. (1991) and Niebylski et al. (1994) have reported evidence from mosquito bloodmeals showing that wild *Ae. albopictus* feed on eastern chipmunks, implying that horizontal transmission of LAC virus between this species and mammalian amplifiers may also be possible in a natural setting. Niebylski et al. (1994) also reports a number of other potential hosts, including humans, but most are non-amplifier hosts, which means that many bites by LAC virus-infected *Ae. albopictus* are probably “wasted” in terms of virus transmission. Studies in Thailand (Sullivan et al. 1971) indicated that *Ae. albopictus* fed on a wide variety of hosts, including humans, pigs, buffaloes, dogs, and chickens, with humans ranking as the most attractive host.

Competitive Interaction between *Oc. triseriatus* and *Ae. albopictus*

Ae. albopictus initiates larval development later in the season than *Oc. triseriatus*, so numbers of *Oc. triseriatus* may be higher in the spring (Teng and Apperson 2000). However, this difference is made up by *Ae. albopictus*, which has shorter larval development periods (fewer degree-days to complete larval development), so that *Ae. albopictus* population growth exceeds that of *Oc. triseriatus* by late season (Teng and Apperson 2000). Previous workers have suggested that these newly sympatric species will compete for larval habitat and food resources, with the two species coexisting in natural treehole habitats and *Ae. albopictus* eventually displacing *Oc. triseriatus* in tire habitats (Livdahl and Willey 1991). Likewise, Swanson et al. (2000) hypothesize that *Oc. triseriatus* will prevail in treeholes and artificial containers within wooded areas, but that *Ae. albopictus* will be the dominant competitor in artificial containers in open and residential areas. Novak et al. (1993) has indicated that when larval diet is limited, *Ae. albopictus* will outcompete *Oc. triseriatus*, but both species are capable of completing larval development in mixed-species environments.

Remote sensing and GIS to predict vector populations and abundance

Because both *Oc. triseriatus* and *Ae. albopictus* are associated with characteristic habitats (Nasci et al. 2000), remote sensing techniques can be used to map landscapes and to identify habitats that are associated with breeding of these species. In recent years, geographic information systems and remote sensing have proven useful in showing relationships between environmental variables and disease vector abundance (Wood et al. 1992, Beck et al. 1994,

Roberts et al. 1996, Cross et al. 1996). Because both vegetation type and vector abundance are influenced by environmental factors such as rainfall, elevation, and temperature, it seems logical that a relationship would exist between vector population levels and landcover type. Beck et al. (1994) demonstrated that *Anopheles albimanus* abundance near villages in Mexico could be predicted as either high or low based on the landcover composition surrounding the villages. In particular, proportions of two landcover types used as breeding sites by the mosquitoes were indicative of vector abundance. Similarly, Moncayo and others (2000) analyzed the relationship between eastern equine encephalomyelitis virus vector abundance and Landsat TM-derived landscape proportions surrounding human and horse case sites. Wetlands were determined to be the most important landscape class element, accounting for up to 72.5% of observed variation in host-seeking vector mosquito populations. Roberts et al. (1996) also showed that landcover information derived from multispectral satellite data successfully predicted areas of high and low mosquito abundance.

Use of Bayesian procedures to predict species presence or absence

Bayesian modeling procedures can be used to link survey data with satellite imagery to produce distribution maps for a given species. Using Bayesian statistics, predictive relationships can be built between species and their environment. Bayesian procedures have been used mainly to model bird and mammal distributions (Aspinall 1991, Aspinall and Veitch 1993, Hepinstall and Sader 1997, Tucker et al. 1997). Bayesian modeling procedures were used by Aspinall (1991) to predict the distribution of Red deer in Scotland based on accumulated frost, altitude, and landcover. Aspinall and Veitch (1993) modeled distributions of wading birds in Scotland based on bird survey data, satellite imagery, and a digital elevation model (DEM). Conditional probabilities of bird presence and absence were calculated for spectral values in the satellite image bands and DEM, and these were used to create a map showing bird distribution as a probability of occurrence. Similar methods were used by Hepinstall and Sader (1997) in Maine. Tucker et al. (1997) used Bayesian procedures to integrate data from ornithological literature on habitat preferences and life history for 3 species of birds with landcover and physiographic data to produce species distribution maps in England. Bayesian procedures use survey data or expert knowledge to calculate prior probabilities of finding a species at any point within the landscape, regardless of habitat. The model also requires that conditional probabilities be assigned to habitat variables, given the presence or absence of the species. Conditional probabilities are then

used to modify prior probabilities, so that the end result is a map showing the distribution of the species as a probability of occurrence (Aspinall and Veitch 1993). This approach also has been used to model insect populations (Wood et al. 1992). In this study, Bayesian procedures were used effectively to predict high and low mosquito-producing rice fields in California based on remotely sensed early-season canopy development in the rice fields and proximity to mosquito bloodmeal sources (pastures with livestock). Rice fields predicted to produce high mosquito numbers were identified with 85% accuracy, indicating that Bayesian modeling could be useful for directing vector control efforts or for warning citizens about potential disease risk.

References Cited

- Aspinall, R. J. 1991. Use of an inductive modeling procedure based on Bayes Theorem for analysis of pattern in spatial data. *Computer Modeling in the Environmental Sciences* (D.G. Farmer and M.J. Rycroft, editors), Institute of Mathematics and its Applications Conference Series No. 28, Oxford University Press, Oxford, pp. 325-339.
- Aspinall, R. and N. Veitch. 1993. Habitat mapping from satellite imagery and wildlife survey data using a Bayesian modeling procedure in a GIS. *Photogrammetric Engineering and Remote Sensing*. 59(4): 537-543.
- Aziz, N. and J. Hayes. 1987. Oviposition and biting patterns of *Aedes triseriatus* in the flood plains of Fort Bend County, Texas. *J. Am. Mosq. Control Assoc.* 3(3): 397-399.
- Balfour, H. H., Jr., R. A. Siem, H. Bauer, and P. G. Quie. 1973. California arbovirus (La Crosse) infections I. Clinical and laboratory findings in 66 children with meningoencephalitis. *Pediatrics*. 52(5): 680-691.
- Balfour, H. H., Jr., C. K. Edelman, F. E. Cook, W. I. Barton, A. W. Buzicky, R. A. Siem, and H. Bauer. 1975. Isolates of California encephalitis (La Crosse) virus from field-collected eggs and larvae of *Aedes triseriatus*: Identification of the overwintering site of California encephalitis. *J. Infect. Dis.* 131(6): 712-716.
- Balfour, H. H., Jr., C. K. Edelman, H. Bauer, and R. A. Siem. 1976. California arbovirus (La Crosse) infections. III. Epidemiology of California encephalitis in Minnesota. *J. Infect. Dis.* 133(3): 293-301.
- Beaty, B. J. and W. H. Thompson. 1975. Emergence of La Crosse virus from endemic foci. Fluorescent antibody studies of overwintered *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 24(4): 685-691.
- Beck, L. R., M. H. Rodriguez, S. W. Dister, A. D. Rodriguez, E. Rejmankova, A. Ulloa, R. A. Meza, D. R. Roberts, J. F. Paris, M. A. Spanner, R. K. Washino, C. Hacker, and L. J. Legters. 1994. Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. *Am. J. Trop. Med. Hyg.* 51(3): 271-280.
- Beehler, J. W. and G. R. DeFoliart. 1990. Spatial distribution of *Aedes triseriatus* eggs in a site endemic for La Crosse virus. *J. Am. Mosq. Control Assoc.* 6(2): 254-257.
- Beier, J. C., W. J. Berry, and G. B. Craig, Jr. 1982. Horizontal distribution of adult *Aedes triseriatus* (Diptera: Culicidae) in relation to habitat structure, oviposition, and other mosquito species. *J. Med. Entomol.* 19(3): 239-247.
- Berry, R. L., B. J. LaLonde, H. W. Stegmiller, M. A. Parsons, and G. T. Bear. 1974. Isolation of La Crosse virus (California encephalitis group) from field collected *Aedes triseriatus* (Say) larvae in Ohio (Diptera: Culicidae). *Mosquito News*. 34(4): 454-457.

- Berry, R. L., M. A. Parsons, B. J. LaLonde, H. W. Stegmiller, J. Lebio, M. Jalil, and R. A. Masterson. 1975. Studies on the epidemiology of California encephalitis in an endemic area in Ohio in 1971. *Am. J. Trop. Med. Hyg.* 24(6): 992-998.
- Berry, R. L., M. A. Parsons, B. J. LaLonde-Weigert, J. Lebio, H. Stegmiller, and G. T. Bear. 1986. *Aedes canadensis*, a vector of La Crosse virus (California serogroup) in Ohio. *J. Am. Mosq. Control Assoc.* 2(1): 73-78.
- Bonnet, D. D. and D. J. Worcester. 1946. The dispersal of *Aedes albopictus* in the territory of Hawaii. *Am. J. Trop. Med.* 26(4): 465-476.
- Craig, G. B., Jr. 1983. Biology of *Aedes triseriatus*: some factors affecting control, pp. 329-341. In C. H. Calisher and W. H. Thompson [eds.], *California Serogroup Viruses*. Liss, New York.
- Cramblett, H. G., H. Stegmiller, and C. Spencer. 1966. California Encephalitis Virus Infections in Children: Clinical and Laboratory Studies. *JAMA.* 198(2): 128-132.
- Cross, E. R., W. W. Newcomb, and C. J. Tucker. 1996. Use of weather data and remote sensing to predict the geographical and seasonal distribution of *Phlebotomus papatasi* in southwest Asia. *Am. J. Trop. Med. Hyg.* 54(5): 530-536.
- Cully, J. F., Jr., P. B. Heard, D. M. Wesson, and G. B. Craig, Jr. 1991. Antibodies to La Crosse virus in eastern chipmunks in Indiana near an *Aedes albopictus* population. *J. Am. Mosq. Control Assoc.* 7(4): 651-653.
- Cully, J. F., Jr., T. G. Streit, and P. B. Heard. 1992. Transmission of La Crosse virus by four strains of *Aedes albopictus* to and from the eastern chipmunk (*Tamias striatus*). *J. Am. Mosq. Control Assoc.* 8(3): 237-240.
- DeFoliart, G. R. and M. A. Lisitza. 1980. Activity by *Aedes triseriatus* in open terrain. *Mosquito News.* 40(4): 650-652.
- Eads, R. B. 1972. Recovery of *Aedes albopictus* from used tires shipped to United States ports. *Mosquito News.* 32(1): 113-114.
- Freier, J. E. and J. C. Beier. 1984. Oral and transovarial transmission of La Crosse virus by *Aedes atropalpus*. *Am. J. Trop. Med. Hyg.* 33(4): 708-714.
- Gauld, L. W., R. P. Hanson, W. H. Thompson, and S. K. Sinha. 1974. Observations on a natural cycle of La Crosse virus (California group) in southwestern Wisconsin. *Am. J. Trop. Med. Hyg.* 23(5): 983-992.
- Gauld, L. W., T. M. Yuill, R. P. Hanson, and S. K. Sinha. 1975. Isolation of La Crosse virus (California encephalitis group) from the chipmunk (*Tamias striatus*), an amplifier host. *Am. J. Trop. Med. Hyg.* 24(6): 999-1005.

- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. The first isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases* (in press).
- Godsey, M. S., Jr., F. Amoo, T. M. Yuill, and G. R. DeFoliart. 1988. California serogroup virus infections in Wisconsin domestic animals. *Am. J. Trop. Med. Hyg.* 39(4): 409-416.
- Grimstad, P. R., G. B. Craig, Jr., Q. E. Ross, and T. M. Yuill. 1977. *Aedes triseriatus* and La Crosse virus: Geographic variation in vector susceptibility and ability to transmit. *Am. J. Trop. Med. Hyg.* 26(5): 990-996.
- Grimstad, P. R. and L. D. Haramis. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus III. Enhanced oral transmission by nutrition-deprived mosquitoes. *J. Med. Entomol.* 21(3): 249-256.
- Grimstad, P. R., J. F. Kobayashi, M. Zhang, and G. B. Craig, Jr. 1989. Recently introduced *Aedes albopictus* in the United States: Potential vector of La Crosse virus (Bunyaviridae: California Serogroup). *J. Am. Mosq. Control Assoc.* 5(3): 422-427.
- Hammon, W. M., W. C. Reeves, and G. Sather. 1952. California encephalitis virus, a newly described agent. II. Isolations and attempts to identify and characterize the agent. *J. Immunol.* 69(5): 493-510.
- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: Probable Introduction in Used Tires from Northern Asia. *Science.* 236: 1114-1116.
- Hawley, W. A. 1988. The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 4(Suppl.1): 1-40.
- Hawley, W. A., C. B. Pumpuni, R. H. Brady, and G. B. Craig, Jr. 1989. Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs in Indiana. *J. Med. Entomol.* 26(2): 122-129.
- Hepinstall, J. A. and S. A. Sader. 1997. Using Bayesian statistics, thematic mapper satellite imagery, and breeding bird survey data to model bird species probability of occurrence in Maine. *Photogrammetric Engineering and Remote Sensing.* 63(10): 1231-1237.
- Issel, C. J., D. O. Trainer, and W. H. Thompson. 1972. Experimental studies with white-tailed deer and four California group arboviruses (La Crosse, Trivittatus, Snowshoe Hare, and Jamestown Canyon). *Am. J. Trop. Med. Hyg.* 21(6): 979-984.
- Jones, T. F., A. S. Craig, R. S. Nasci, L. E. R. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly recognized focus of La Crosse encephalitis in Tennessee. *Clin. Infect. Dis.* 28: 93-97.

- Kappus, K. D., T. P. Monath, R. M. Kaminski, and C. H. Calisher. 1983. Reported encephalitis associated with California serogroup virus infections in the United States, 1963-1981, pp. 31-41. *In* C. H. Calisher and W. H. Thompson [eds.], *California Serogroup Viruses*. Liss, New York.
- Kitron, U. D., D. W. Webb, and R. J. Novak. 1989. Oviposition behavior of *Aedes triseriatus* (Diptera: Culicidae): prevalence, intensity, and aggregation of eggs in oviposition traps. *J. Med. Entomol.* 26(5): 462-467.
- Kramer, L. D. and W. H. Thompson. 1983. Quantitation of La Crosse virus in venereally infected *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 32(5): 1140-1146.
- Ksiazek, T. G. and T. M. Yuill. 1977. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciurus carolinensis*) and chipmunks (*Tamias striatus*). *Am. J. Trop. Med. Hyg.* 26(4): 815-821.
- Landry, S. V. and G. R. DeFoliart. 1987. Parity rates of *Aedes triseriatus* (Diptera: Culicidae) collected in a female-retaining ovitrap. *J. Med. Entomol.* 24(3): 282-285.
- Lisitza, M. A., G. R. DeFoliart, T. M. Yuill, and M. G. Karandinos. 1977. Prevalence rates of La Crosse virus (California encephalitis group) in larvae from overwintered eggs of *Aedes triseriatus*. *Mosquito News.* 37(4): 745-750.
- Livdahl, T. P. and M. S. Willey. 1991. Prospects for an invasion: Competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science.* 253: 189-191.
- Loor, K. A. and G. R. DeFoliart. 1970. Field observations on the biology of *Aedes triseriatus*. *Mosquito News.* 30(1): 60-64.
- Mather, T. N. and G. R. DeFoliart. 1984. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. *J. Med. Entomol.* 21(4): 384-391.
- McJunkin, J. E., R. R. Khan, and T. F. Tsai. 1998. Emerging Infectious Diseases: California-La Crosse Encephalitis. *Infect. Dis. Clin. North Am.* 12(1): 83-93.
- Miller, B. R., G. R. DeFoliart, and T. M. Yuill. 1977. Vertical transmission of La Crosse virus (California encephalitis group): Transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 14(4): 437-440.
- Miller, B. R., G. R. DeFoliart, and T. M. Yuill. 1979. *Aedes triseriatus* and La Crosse virus: Lack of infection in eggs of the first ovarian cycle following oral infection of females. *Am. J. Trop. Med. Hyg.* 28(5): 897-901.

- Miller, B. R., B. J. Beaty, and L. H. Lorenz. 1982. Variation of La Crosse virus filial infection rates in geographic strains of *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 19(2): 213-214.
- Moncayo, A. C., J. D. Edman, and J. T. Finn. 2000. Application of geographic information technology in determining risk of eastern equine encephalomyelitis virus transmission. *J. Am. Mosq. Control Assoc.* 16(1): 28-35.
- Moulton, D. W. and W. H. Thompson. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin: Some ecological considerations. *Am. J. Trop. Med. Hyg.* 20(3): 474-482.
- Nasci, R. S. 1982. Activity of gravid *Aedes triseriatus* in wooded fencerows. *Mosquito News.* 42(3): 408-412.
- Nasci, R. S. 1985. Local variation in blood feeding by *Aedes triseriatus* and *Aedes hendersoni* (Diptera: Culicidae). *J. Med. Entomol.* 22(6): 619-623.
- Nasci, R. S. 1988. Biology of *Aedes triseriatus* (Diptera: Culicidae) developing in tires in Louisiana. *J. Med. Entomol.* 25(5): 402-405.
- Nasci, R. S., C. G. Moore, B. J. Biggerstaff, N. A. Panella, H. Q. Liu, N. Karabatsos, B. S. Davis, and E. S. Brannon. 2000. La Crosse encephalitis virus habitat associations in Nicholas County, West Virginia. *J. Med. Entomol.* 37(4): 559-570.
- Niebylski, M. L., H. M. Savage, R. S. Nasci, and G. B. Craig, Jr. 1994. Blood hosts of *Aedes albopictus* in the United States. *J. Am. Mosq. Control Assoc.* 10(3): 447-450.
- Novak, M. G., L. G. Higley, C. A. Christianssen, and W. A. Rowley. 1993. Evaluating larval competition between *Aedes albopictus* and *A. triseriatus* (Diptera: Culicidae) through replacement series experiments. *Environ. Entomol.* 22(2): 311-318.
- Osorio, J. E., M. S. Godsey, G. R. DeFoliart, and T. M. Yuill. 1996. La Crosse viremias in white-tailed deer and chipmunks exposed by injection or mosquito bite. *Am. J. Trop. Med. Hyg.* 54(4): 338-342.
- Pantuwatana, S., W. H. Thompson, D. M. Watts, and R. P. Hanson. 1972. Experimental infection of chipmunks and squirrels with La Crosse and Trivittatus viruses and biological transmission of La Crosse virus by *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 21(4): 476-481.
- Pantuwatana, S., W. H. Thompson, D. M. Watts, T. M. Yuill, and R. P. Hanson. 1974. Isolation of La Crosse virus from field collected *Aedes triseriatus* larvae. *Am. J. Trop. Med. Hyg.* 23(2): 246-250.

- Patrican, L. A., G. R. DeFoliart, and T. M. Yuill. 1985. Oral infection and transmission of La Crosse virus by an enzootic strain of *Aedes triseriatus* feeding on chipmunks with a range of viremia levels. *Am. J. Trop. Med. Hyg.* 34(5): 992-998.
- Paulson, S. L. and W. A. Hawley. 1991. Effect of body size on the vector competence of field and laboratory populations of *Aedes triseriatus* for La Crosse virus. *J. Am. Mosq. Control Assoc.* 7(2): 170-175.
- Reiter, P. and R. F. Darsie, Jr. 1984. *Aedes albopictus* in Memphis, Tennessee (USA): an achievement of modern transportation?. *Mosquito News.* 44(3): 396-399.
- Rie, H. E., M. D. Hilty, and H. G. Cramblett. 1973. Intelligence and coordination following California encephalitis. *Am. J. Dis. Child.* 125: 824-827.
- Roberts, D. R., J. F. Paris, S. Manguin, R. E. Harbach, R. Woodruff, E. Rejmankova, J. Polanco, B. Wullschleger, and L. J. Legters. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. *Am. J. Trop. Med. Hyg.* 54(3): 304-308.
- Rust, R. S., W. H. Thompson, C. G. Matthews, B. J. Beaty, and R. W. Chun. 1999. La Crosse and other forms of California encephalitis. *J. Child Neurol.* 14(1): 1-14.
- Scholl, P. J. and G. R. DeFoliart. 1977. *Aedes triseriatus* and *Aedes hendersoni*: Vertical and temporal distribution as measured by oviposition. *Environ. Entomol.* 6(3): 355-358.
- Shroyer, D. A. and G. B. Craig, Jr. 1980. Egg hatchability and diapause in *Aedes triseriatus* (Diptera: Culicidae): Temperature- and photoperiod-induced latencies. *Ann. Entomol. Soc. Am.* 73(1): 39-43.
- Shroyer, D. A. and G. B. Craig, Jr. 1983. Egg diapause in *Aedes triseriatus* (Diptera: Culicidae): Geographic variation in photoperiodic response and factors influencing diapause termination. *J. Med. Entomol.* 20(6): 601-607.
- Sims, S. R. 1982. Larval diapause in the eastern tree-hole mosquito, *Aedes triseriatus*: Latitudinal variation in induction and intensity. *Ann. Entomol. Soc. Am.* 75(2): 195-200.
- Sinsko, M. J. and G. B. Craig, Jr. 1979. Dynamics of an isolated population of *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 15(2): 89-98.
- Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J. Am. Mosq. Control Assoc.* 2(2): 217-219.
- Sudia, W. D., V. F. Newhouse, C. H. Calisher, and R. W. Chamberlain. 1971. California Group arboviruses: Isolations from mosquitoes in North America. *Mosquito News.* 31(4): 576-600.

- Sullivan, M. F., D. J. Gould, and S. Maneechai. 1971. Observations on the host range and feeding preferences of *Aedes albopictus* (Skuse). *J. Med. Entomol.* 8(6): 713-716.
- Swanson, J., M. Lancaster, J. Anderson, M. Crandell, L. Haramis, P. Grimstad, and U. Kitron. 2000. Overwintering and Establishment of *Aedes albopictus* (Diptera: Culicidae) in an Urban La Crosse Virus Enzootic Site in Illinois. *J. Med. Entomol.* 37(3): 454-460.
- Szumlas, D. E., C. S. Apperson, P. C. Hartig, D. B. Franczy, and N. Karabatsos. 1996a. Seroepidemiology of La Crosse virus infection in humans in western North Carolina. *Am. J. Trop. Med. Hyg.* 54(4): 332-337.
- Szumlas, D. E., C. S. Apperson, E. E. Powell, P. Hartig, D. B. Franczy, and N. Karabatsos. 1996b. Relative abundance and species composition of mosquito populations (Diptera: Culicidae) in a La Crosse virus-endemic area in western North Carolina. *J. Med. Entomol.* 33(4): 598-607.
- Szumlas, D. E., C. S. Apperson, and E. E. Powell. 1996c. Seasonal occurrence and abundance of *Aedes triseriatus* and other mosquitoes in a La Crosse virus-endemic area in western North Carolina. *J. Am. Mosq. Control Assoc.* 12(2): 184-193.
- Teng, H. and C. S. Apperson. 2000. Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: Effects of density, food, and competition on response to temperature. *J. Med. Entomol.* 37(1): 40-52.
- Tesh, R. B. and D. J. Gubler. 1975. Laboratory studies of transovarial transmission of La Crosse and other arboviruses by *Aedes albopictus* and *Culex fatigans*. *Am. J. Trop. Med. Hyg.* 24(5): 876-880.
- Thompson, W. H. 1979. Higher venereal infection and transmission rates with La Crosse virus in *Aedes triseriatus* engorged before mating. *Am. J. Trop. Med. Hyg.* 28(5): 890-896.
- Thompson, W. H., B. Kalfayan, and R. O. Anslow. 1965. Isolation of California encephalitis group virus from a fatal human illness. *Am. J. Epidem.* 81(2): 245-253.
- Thompson, W. H., R. O. Anslow, R. P. Hanson, and G. R. DeFoliart. 1972. La Crosse virus isolations from mosquitoes in Wisconsin, 1964-68. *Am. J. Trop. Med. Hyg.* 21(1): 90-96.
- Thompson, W. H. and B. J. Beaty. 1977. Venereal Transmission of La Crosse (California Encephalitis) Arbovirus in *Aedes triseriatus* Mosquitoes. *Science.* 196: 530-531.
- Trexler, J. D., C. S. Apperson, and C. Schal. 1997. Diel oviposition patterns of *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) in the laboratory and the field. *J. Vector Ecol.* 22(1): 64-70.

- Tucker, K., S. P. Rushton, R. A. Sanderson, E. B. Martin, and J. Blaiklock. 1997. Modelling bird distributions – a combined GIS and Bayesian rule-based approach. *Landscape Ecology*. 12(2): 77-93.
- Watts, D. M., C. D. Morris, R. E. Wright, G. R. DeFoliart, and R. P. Hanson. 1972. Transmission of La Crosse virus (California encephalitis group) by the mosquito *Aedes triseriatus*. *J. Med. Entomol.* 9(2): 125-127.
- Watts, D. M., P. R. Grimstad, G. R. DeFoliart, T. M. Yuill, and R. P. Hanson. 1973a. Laboratory transmission of La Crosse encephalitis virus by several species of mosquitoes. *J. Med. Entomol.* 10(6): 583-586.
- Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill, and W. H. Thompson. 1973b. Transovarial transmission of La Crosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. *Science*. 182: 1140-1141.
- Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart, and R. P. Hanson. 1974. Overwintering of La Crosse virus in *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 23(4): 694-700.
- Wood, B. L., L. R. Beck, R. K. Washino, K. A. Hibbard, and J. S. Salute. 1992. Estimating high mosquito-producing rice fields using spectral and spatial data. *Int. J. Remote Sensing*. 13(15): 2813-2826.
- Woodring, J., L. J. Chandler, C. T. Oray, M. M. McGaw, C. D. Blair, and B. J. Beaty. 1998. Short Report: Diapause, transovarial transmission, and filial infection rates in geographic strains of La Crosse virus-infected *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 58(5): 587-588.
- Wright, R. E. and G. R. DeFoliart. 1970. Associations of Wisconsin mosquitoes and woodland vertebrate hosts. *Ann. Entomol. Soc. Am.* 63(3): 777-786.
- Yuill, T. M. 1983. The role of mammals in the maintenance and dissemination of La Crosse virus, pp. 77-87. *In* C. H. Calisher and W. H. Thompson [eds.], *California Serogroup Viruses*. Liss, New York.

Chapter 2

Habitat Preferences and Phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in Southwestern Virginia

INTRODUCTION

La Crosse encephalitis (LACE) is the most common and important endemic mosquito-borne disease in the USA (Rust *et al.* 1999), with cases distributed mainly in the eastern and midwestern states. It affects mainly school-aged children causing a febrile illness usually accompanied with headache and vomiting. Symptoms indicating central nervous system involvement include seizures, disorientation, and aseptic meningitis (McJunkin *et al.* 2001). Although death from LACE is rare (<1% of cases), neurologic deficits and electroencephalogram (EEG) abnormalities can persist for months after discharge from the hospital (McJunkin *et al.* 2001). The most serious consequence of LACE is the development of epilepsy in nearly 25% of cases (Rust *et al.* 1999).

The principal vector of La Crosse (LAC) virus is *Ochlerotatus triseriatus* (Say), the eastern treehole mosquito (Berry *et al.* 1974, Pantuwatana *et al.* 1974, Watts *et al.* 1974, Balfour *et al.* 1975, Beaty and Thompson 1975). As its common name indicates, this mosquito develops in treeholes, which are very common in eastern deciduous forests. As a result, *Oc. triseriatus* is encountered commonly throughout the forested areas of the eastern and midwestern United States.

In the natural cycle of LAC virus, the virus is amplified in a mammalian reservoir host such as the eastern chipmunk (*Tamias striatus*) (Gauld *et al.* 1975) or the gray squirrel (*Sciurus carolinensis*) (Moulton and Thompson 1971, Ksiazek and Yuill 1977). Infected female *Oc. triseriatus* also are capable of transmitting LAC virus transovarially to their progeny (Watts *et al.* 1973, Miller *et al.* 1977). LAC virus overwinters in transovarially infected eggs, and infected larvae hatch the following spring (Lisitza *et al.* 1977). Miller *et al.* (1977) showed that LAC virus could persist for at least 4 years in the absence of horizontal amplification in vertebrate reservoir hosts. Therefore, transovarial transmission and the overwintering of LAC virus-infected eggs are critical for virus maintenance in nature (Miller *et al.* 1979).

Humans are dead-end hosts for LAC virus, being susceptible to infection, but incapable of developing a sufficient viremia to permit passage of the virus from a human to a susceptible

mosquito. As humans have populated forested areas, contact with *Oc. triseriatus* has increased. Another factor that increases *Oc. triseriatus*-human contact is that *Oc. triseriatus* also will breed in artificial containers near homes, particularly if the containers are found in shaded areas (Nasci 1988).

Another mosquito that has spread throughout the eastern United States is *Aedes albopictus* (Skuse). Like *Oc. triseriatus*, this mosquito breeds in containers and commonly inhabits areas near human dwellings. Breeding populations of *Ae. albopictus* were first found in the United States in 1985 near Houston, Texas (Sprenger and Wuithiranyagool 1986), probably introduced via shipments of used tires from Japan (Hawley et al. 1987). Its range now extends through most of the USA east of the Mississippi River, including areas endemic for LAC virus. *Ae. albopictus* is a competent experimental vector of LAC virus, with infection and oral transmission rates equal to or higher than those for *Oc. triseriatus* (Grimstad et al. 1989, Cully et al. 1992). The rates for transovarial transmission of LAC virus in *Ae. albopictus*, however, are lower than those for *Oc. triseriatus* (Tesh and Gubler 1975).

From 1975 through 1993, only one case of LACE was reported from the state of Virginia. However, between 1994 and 1998, CDC recorded a total of 13 cases of LACE from areas in southwest Virginia in the Appalachian Mountains (Fig. 2.1). In 1997, 5 cases were reported

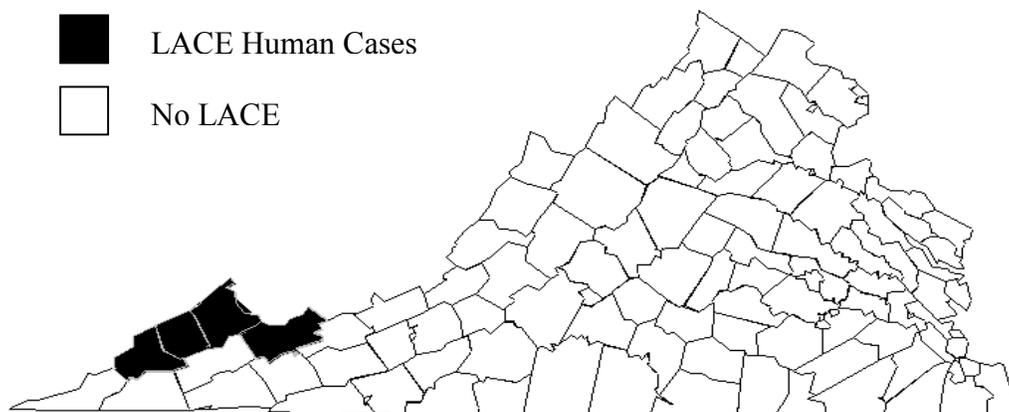


Fig. 2.1. Virginia counties with confirmed human LACE cases, 1994-1998.

from Wise County, comprising disease incidence of approximately 13 per 100,000. Three of the 5 cases were from a small cluster of homes in a single community within the county. Because of

these recent LACE cases, southwest Virginia is emerging as a significant focus of LAC virus activity and the need has arisen for studying the LAC virus cycle there and the biology of its vectors. The objective of this study was to determine the spatial and seasonal distributions of *Ochlerotatus triseriatus* and *Aedes albopictus* as measured by oviposition activity in southwestern Virginia. Additionally, mosquitoes from two sites in Wise County were collected and tested for LAC virus infection.

MATERIALS AND METHODS

Study Areas

This study was conducted in Wise, Scott, and Lee Counties, which form the western tip of Virginia (Fig. 2.2) in the Appalachian Mountains. Landcover in the area consists largely of

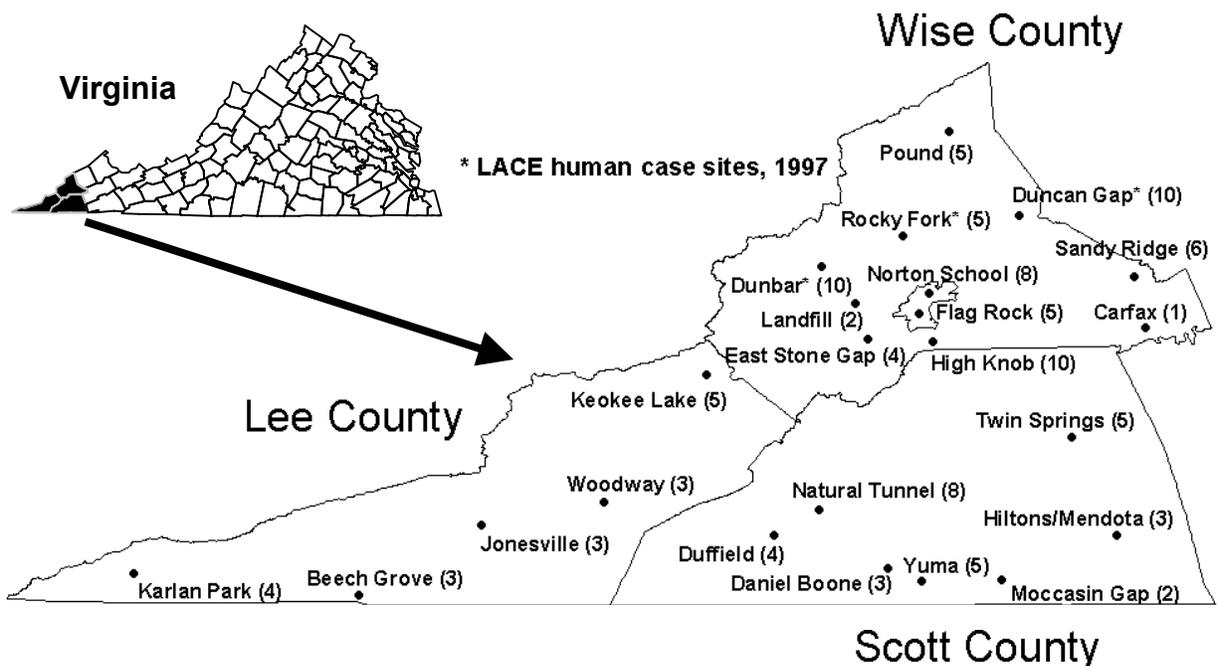


Fig. 2.2. 1998 collection sites showing numbers of ovitraps per site in parentheses.

Appalachian mixed hardwoods that include oak-hickory, maple-beech-birch and white pine-hemlock forest types (Johnson 1992). Forest stands are interrupted by small towns and large cleared areas resulting from strip mining for coal or occasional pastureland. Elevations range from approximately 400 m to 1287 m, with most residential areas between 400m and 800 m. Wise County was chosen for study because of recent human La Crosse virus cases, and two adjacent counties, Lee and Scott, were chosen because they, along with Wise County and the city

of Norton, make up the Lenowisco Health district, facilitating cooperation among the county health departments.

Mosquito Sampling

The ovitrap design consisted of a 450 ml black plastic cup nailed to a tree or post, with drain holes approximately halfway up each side. Strips of seed germination paper, approximately 5 cm wide, served as the oviposition substrate, or ovistrips (Steinly et al. 1991). The ovistrips were collected and replaced each week, and remaining water and organic matter were replaced with fresh water to bring the level up to the drain holes. After collection, the eggs were brought to the lab and examined under a dissecting microscope to count and identify to species (Linley 1989a,b). Eggs from the year 2000 collections at Duncan Gap were stored in an insectary at 24°C, 80% RH and 16:8 (L:D) photoperiod. Eggs were hatched and reared to adults according to the methods of Munstermann and Wasmuth (1985). Adult mosquitoes were sorted by species and sex and stored at -70°C for later virus assay.

Adult mosquitoes were collected approximately every two weeks from June through October 1998 and weekly from May 28 through October 1, 1999 in the shrubby areas bordering the Wise County Landfill's tire dump and in forested areas surrounding the human case site in Duncan Gap. Adult host-seeking mosquitoes were collected using a flashlight-type aspirator. For a 30-minute collection period, all mosquitoes approaching or landing on the human collector were aspirated. Live specimens were transported to the lab in cages constructed from 3.6 L plastic buckets (Munstermann and Wasmuth 1985) with a wet paper towel draped over the top to increase humidity. Mosquitoes were frozen live at -70°C and were later sorted and identified by species and sex over wet ice, then stored at -70°C until virus testing was performed.

Virus Assay of Mosquitoes

Mosquitoes were pooled in groups of 50 or fewer specimens according to species and sex. Pools were prepared for assay according to the methods of Nasci et al. (2000) and tested for virus by plaque assay on Vero cells (Beaty et al. 1995) using methyl cellulose (0.8% w/v) as the overlay and staining with crystal violet in buffered formalin (0.5g CV/500mL BF). Virus isolates were confirmed as LAC virus by RT-PCR (Kuno et al. 1996).

Mosquito Distribution Survey

To determine the distribution and relative oviposition activity of *Oc. triseriatus* and *Ae. albopictus*, ovitraps were placed at eleven sites in Wise County, 5 sites in Lee County and 7 sites

in Scott County. Three of the sites in Wise County were in proximity to LACE cases from 1997. The number of ovitraps per site varied according to the nature of the habitat and ranged from 1 to 10 (Fig. 2.2). Collections were made every 7 to 12 days from June 8 – October 3, 1998.

Wise County Collections in 1999 and 2000

In 1999, ovitraps were set out at 2 of the sites, Duncan Gap and Rocky Fork, from the previous year. The Duncan Gap site was associated with 3 LACE cases and the Rocky Fork site was associated with one case, all from 1997. Rocky Fork, an open area without adjacent forest, was selected because it had a high proportion of *Ae. albopictus* present during the previous season. At Duncan Gap, casual observations from the previous year suggested that *Ae. albopictus* preferentially oviposited in ovitraps located in the yard as compared to the forested area surrounding the home. To compare the oviposition preferences of the 2 species, 5 ovitraps were placed in the yard immediately surrounding the home and 5 ovitraps were placed in the adjacent forest. Ovitrips were collected and replaced weekly. Our intent was to hatch the eggs and rear them for virus isolation, but after an insectary failure, the eggs were found to be inviable. In 2000, eggs were again collected weekly from 10 ovitraps at the Duncan Gap site. These eggs were reared to adults and tested for the presence of LAC virus. Eggs tested for virus were collected during the periods from June 20-July 18, 2000 and August 15-September 18, 2000.

Statistical Analysis

Differences between the mean number of eggs per species collected between years for the Rocky Fork site were compared by *t*-test (GraphPad Software, Inc. 1994). Prior to analysis, egg counts were subjected to a square-root transformation [$\sqrt{(y+0.1)}$] to normalize variances. Differences between the mean number of eggs per species collected between habitat types for the Duncan Gap site were analyzed using a generalized linear models analysis of variance (ANOVA). Prior to analysis of egg counts, logarithmic transformations $\{\log[(y+0.01)*100]\}$ were performed because square-root transformations of the counts did not provide sufficient normalization of the variances.

RESULTS

Mosquito Distribution and Phenology

In 1998, a total of 114 ovitraps distributed among 23 sites in southwestern Virginia were sampled repeatedly throughout the season (Fig. 2.2). *Ochlerotatus triseriatus* and *Aedes*

albopictus were found at each of the sites surveyed. *Oc. triseriatus* was present at all study sites and abundant in most of the areas studied in southwest Virginia (Fig. 2.3). *Ae. albopictus* was

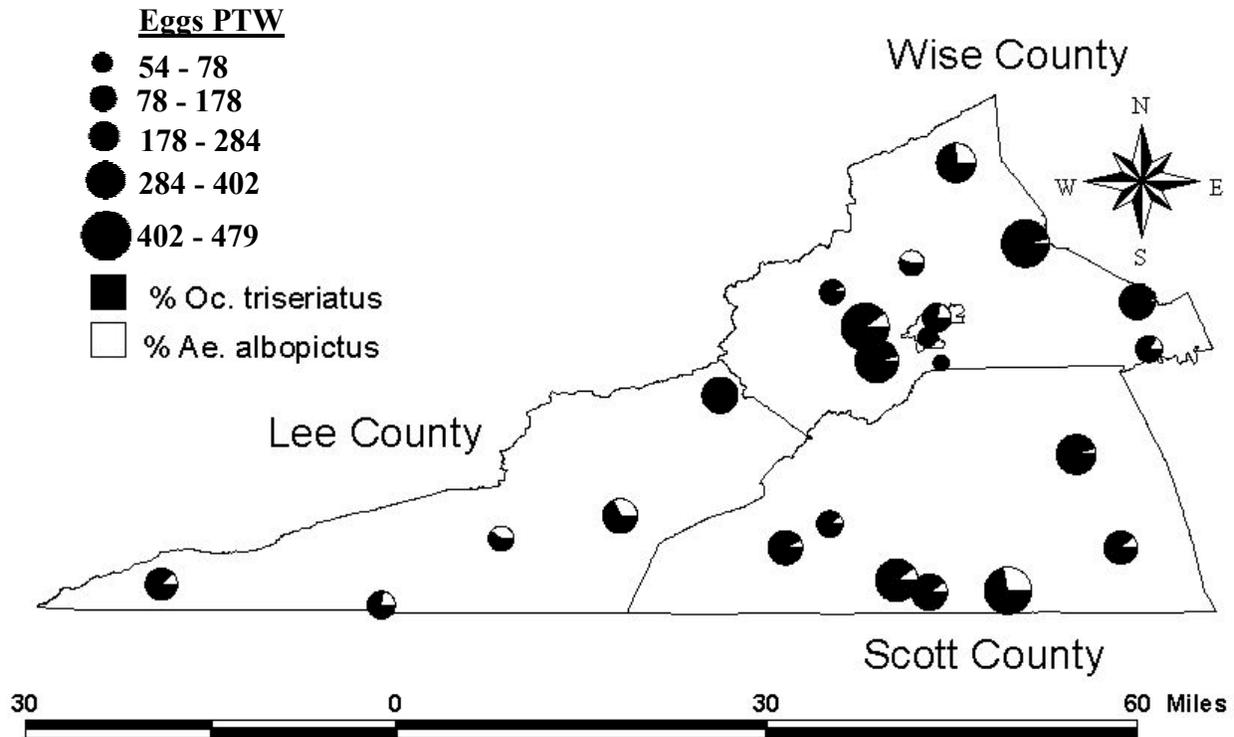


Fig. 2.3. Percentages of *Oc. triseriatus* and *Ae. albopictus* by site for ovitrap collections between June 8 and October 3, 1998. PTW = per trap week. Size of circle indicates mean total egg count per ovitrap-week for the collection season.

also present at every site, but its relative abundance was generally lower than that of *Oc. triseriatus*. *Oc. triseriatus* comprised 90.1% of all mosquitoes collected during this period, with *Ae. albopictus* comprising the remaining 9.9%.

In Wise County, the oviposition activity of *Oc. triseriatus* increased steadily through June (Fig. 2.4), peaking in late June/early July (43.7 eggs/trap-day) and again with three peaks of similar intensity at the end of July through late August (46.0, 44.0, and 46.4 eggs/trap-day, respectively); oviposition then declined through the last collection date in early October. Oviposition by *Ae. albopictus* gradually increased to a peak (9.6 eggs/trap-day) in late August and declined thereafter.

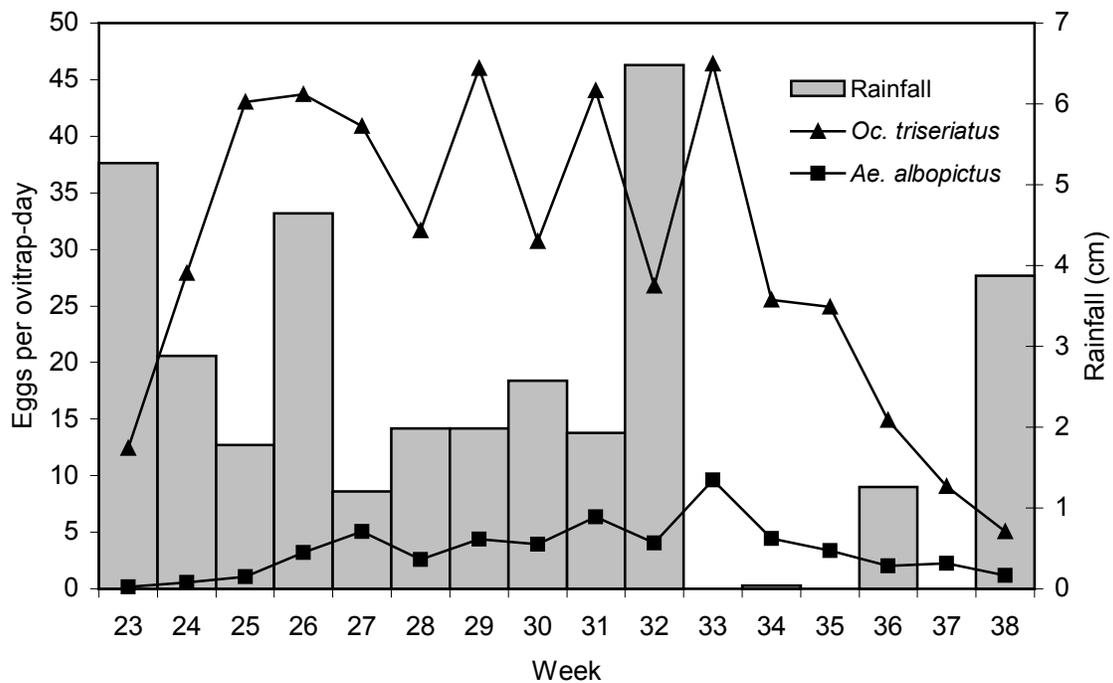


Fig. 2.4. Mean weekly egg counts per ovitrap-day for all Wise County ovitrap collections between June 8 and October 3, 1998.

Oviposition habitat preferences

The relative oviposition activity of the mosquito species varied among the Wise County sites in 1998 (Fig. 2.5). Although statistical comparisons could not be made due to differing sample sizes and variances, ovitraps from open, unforested sites had a higher proportion (25.9-44.2%) and greater intensity (62.0-86.7 eggs/trap-week) of *Ae. albopictus* eggs than those from densely forested surroundings (0.1-0.3%, 0.1-0.2 eggs/trap-week). Locations in which ovitraps were near edges of forested areas, such as forest surrounding a residential area, had intermediate *Ae. albopictus* egg numbers (4.6-36.5 eggs/trap-week) and relative abundance (1.7-20.5%). Numbers of *Oc. triseriatus* were lowest (53.7-77.9 eggs/trap-week) in densely forested sites, but no clear pattern was evident for the other 2 categories (131.7-458.4 eggs/trap-week in forest edge; 78.3-248.2 eggs/trap-week in open areas).

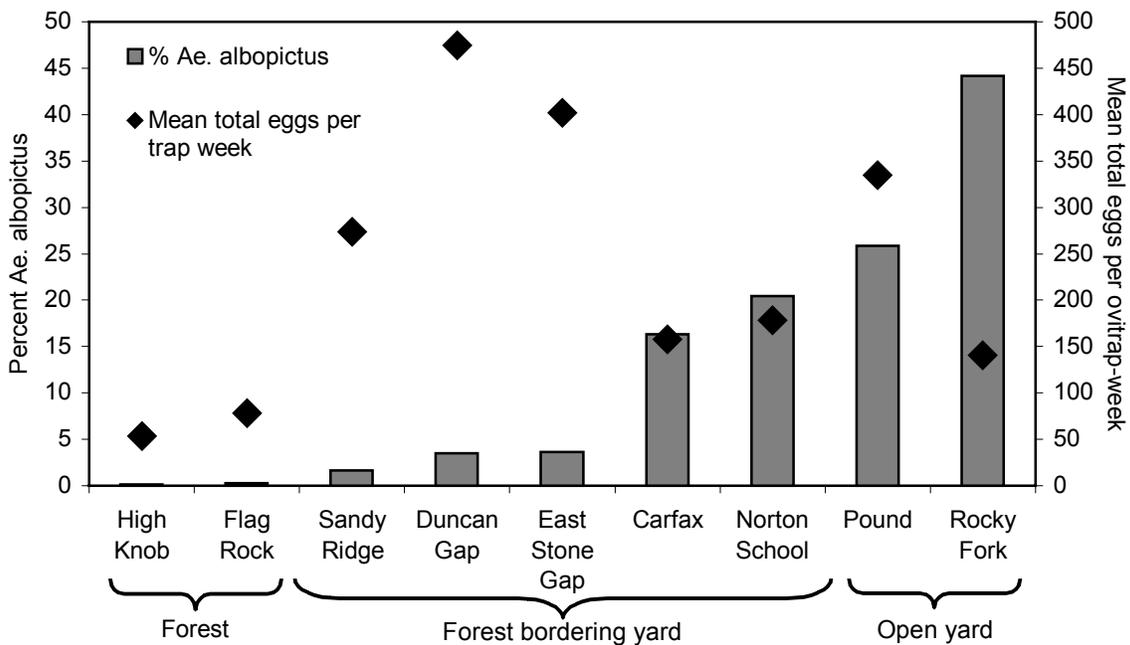


Fig. 2.5. Seasonal averages of relative *Ae. albopictus* egg abundance and total eggs per ovitrap-week for both species for Wise County collection sites showing differences in species composition among oviposition habitats. Collections were made weekly from June 8 – October 3, 1998. Dunbar and Landfill sites were excluded from the figure because they failed to fit into one of the three site openness categories.

sizes and variances, ovitraps from open, unforested sites had a higher proportion (25.9-44.2%) and greater intensity (62.0-86.7 eggs/trap-week) of *Ae. albopictus* eggs than those from densely forested surroundings (0.1-0.3%, 0.1-0.2 eggs/trap-week). Locations in which ovitraps were near edges of forested areas, such as forest surrounding a residential area, had intermediate *Ae. albopictus* egg numbers (4.6-36.5 eggs/trap-week) and relative abundance (1.7-20.5%). Numbers of *Oc. triseriatus* were lowest (53.7-77.9 eggs/trap-week) in densely forested sites, but no clear pattern was evident for the other 2 categories (131.7-458.4 eggs/trap-week in forest edge; 78.3-248.2 eggs/trap-week in open areas).

Of the 1998 Wise County collections, the highest prevalence of *Ae. albopictus* was found at the Rocky Fork site. However, this site was only intermediate in total mosquito abundance, as measured by the mean number of eggs per ovitrap (Fig. 2.5). The temporal distribution and relative abundance of *Ae. albopictus* and *Oc. triseriatus* were equivalent (Fig. 2.6a) ($P > 0.4$). Ovitrap data from 1999 showed a similar pattern (Fig. 2.6b) ($P > 0.5$). The mean density of

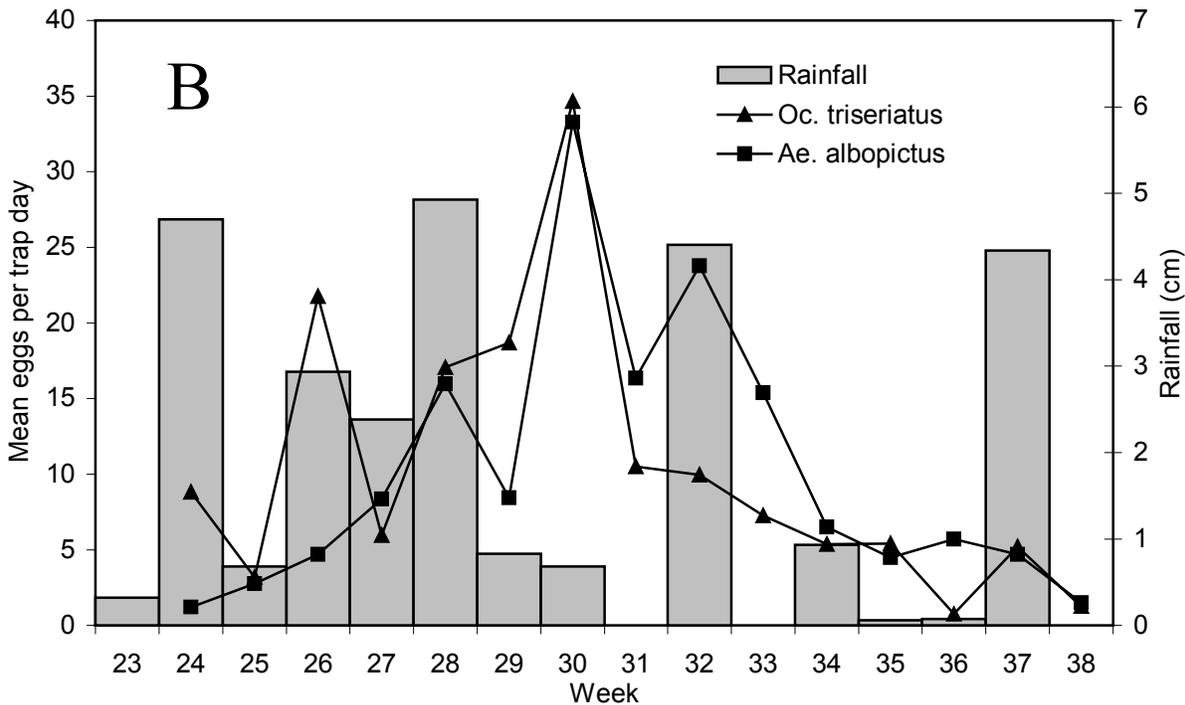
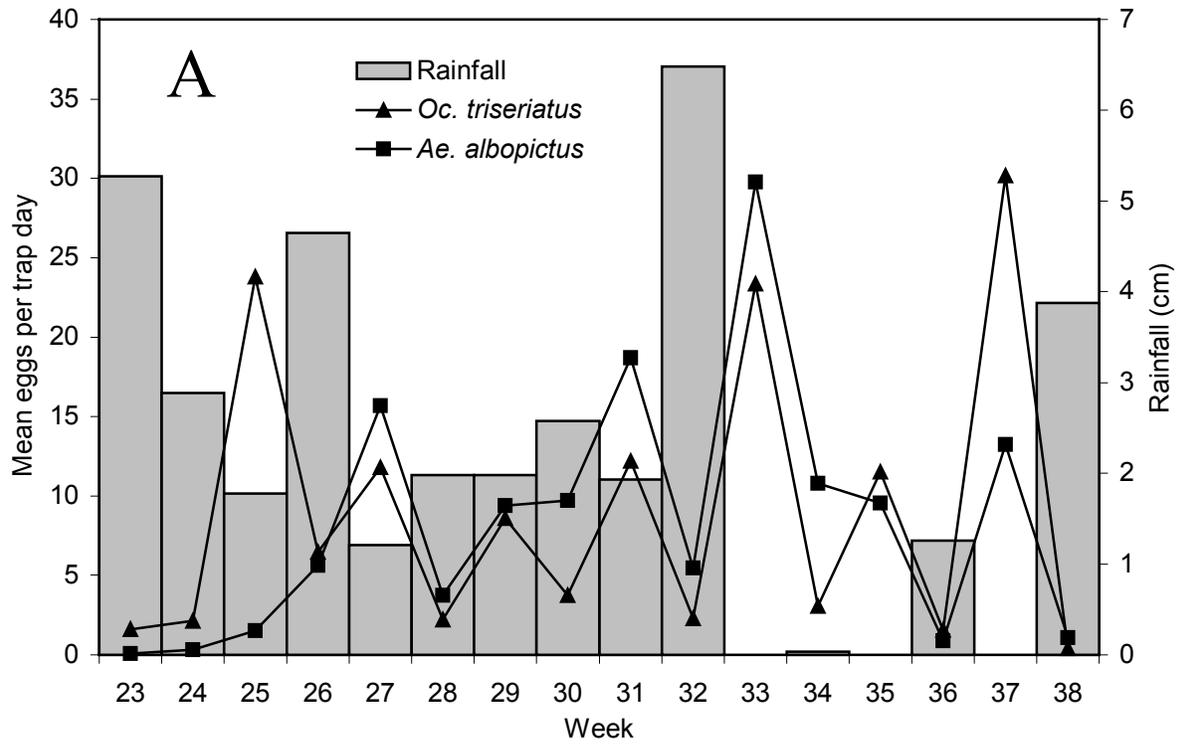


Fig. 2.6. Mean egg numbers per trap day for ovitrap collections from Rocky Fork human LACE case site. Eggs were collected weekly between June 8 and October 3, 1998 (A), and again the following year between June 18 and October 1, 1999 (B). Numbers displayed in figure are back-transformed from means of $\sqrt{(Y+0.1)}$ transformed data used for statistical comparisons.

eggs per ovitrap and relative abundance for each species did not change significantly between 1998 and 1999 (Figs. 2.6a,b) ($P > 0.2$).

The Duncan Gap site showed the highest mean total of mosquitoes per ovitrap for the Wise county sites but the relative abundance of *Ae. albopictus* was intermediate (Fig. 2.5). In 1999, equal numbers of ovitraps were placed in the yard and in the surrounding forest. Analysis of oviposition preferences revealed that *Ae. albopictus* laid a significantly higher number of eggs in the yard versus the forest ovitraps (Fig. 2.7) ($F = 22.69$, $df = 1$, $P = 0.001$). The difference

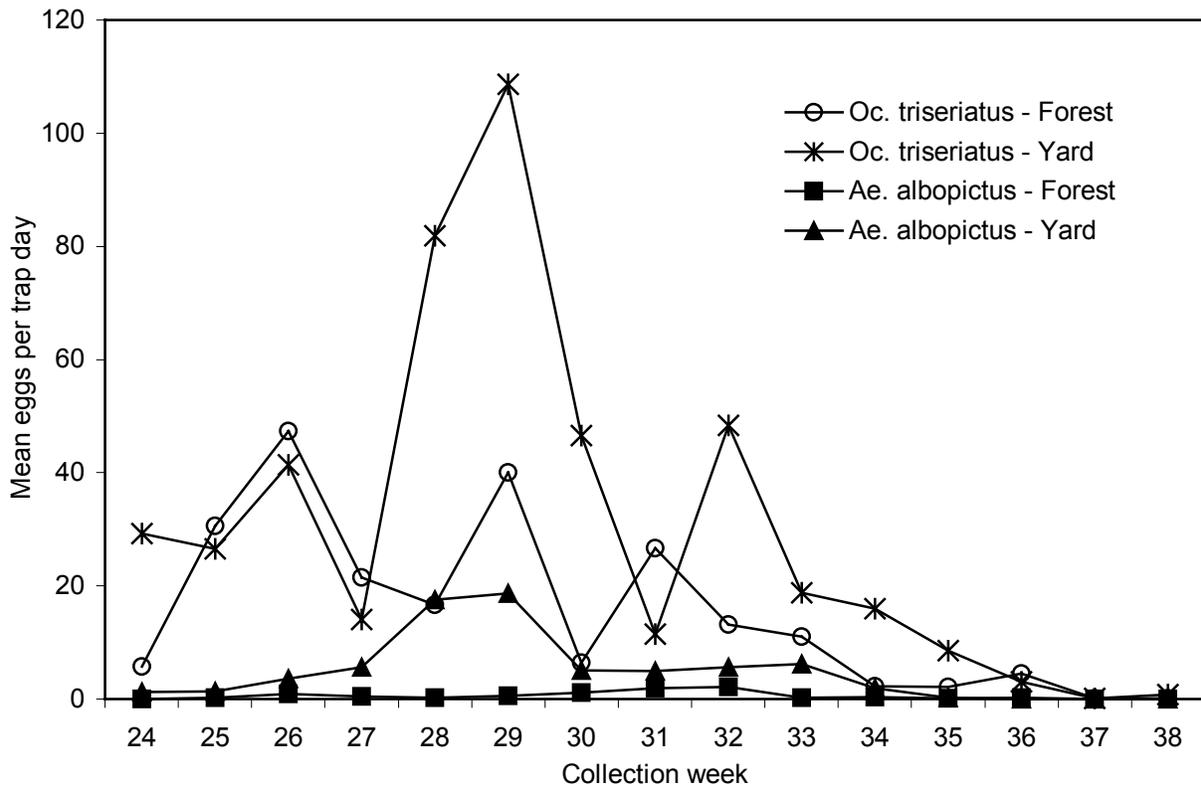


Fig. 2.7. Geometric means of *Aedes albopictus* and *Ochlerotatus triseriatus* egg counts per ovitrap day at the Duncan Gap human LACE case site showing numbers for yard and forest. Eggs were collected weekly from June 18 – October 1, 1999.

between the two habitat types was greatest during late July and early August, when *Ae. albopictus* populations were highest. *Oc. triseriatus* showed no preference between yard and forest over the collection period (Fig. 2.7) ($F = 1.29$, $df = 1$, $P > 0.2$). During collection week 29, the number of *Oc. triseriatus* collected in the yard ovitraps was much higher than the number in the forest traps, but this difference was insignificant because of the large amount of variation

associated with the *Oc. triseriatus* counts for this date (YARD: Sample mean = 1134, SE = 841; FOREST: Sample mean = 313, SE = 73).

In summary, *Ae. albopictus* is well established throughout the study area in southwestern Virginia, preferring to oviposit in open residential areas. The preferences of *Oc. triseriatus* are less clear, with the lowest numbers of this species collected at sites located in dense forest during 1998. Sites in forest borders and open residential areas yielded similar numbers of *Oc. triseriatus* eggs, but maximum numbers for sites in forest edges were higher than those for open areas. During the following year, *Oc. triseriatus* was found to have no preference between the yard and adjacent forests at a human LACE case site. There was no evidence for direct competition between the two species found in this study.

LAC virus infection rates

The species, stages, and numbers of mosquitoes assayed for virus are shown in Table 2.1. Of these mosquitoes, two pools of *Oc. triseriatus* tested positive for La Crosse virus. Both of these pools were collected from the Duncan Gap site which was associated with 3 human cases during 1997. LAC virus-positive specimens were obtained from larval and adult collections taken in June 1998 and July 1999, respectively. This represents the first isolation of LAC virus from field-collected mosquitoes in Virginia.

Table 2.1. Mosquito species collected in Wise County, VA, 1998-2000, and tested for virus.

Mosquito species	1998 collections		1999 Adult collections		2000 Egg collections ^a	Total No. Tested
	Duncan Gap case site ^b	Wise Co. Tire Dump	Duncan Gap case site	Wise Co. Tire Dump	Duncan Gap case site	
<i>Oc. triseriatus</i>	308 ^c	13	229 ^d	7	8,785	9,342
<i>Oc. atropalpus</i>	0	27	0	2	0	29
<i>Oc. trivittatus</i>	8	2	0	0	0	10
<i>Ae. albopictus</i>	28	368	147	857	69	1,469
<i>Ae. vexans</i>	67	0	13	0	0	80

^a Eggs were hatched and reared to adults prior to testing.

^b Includes season-long adult landing collections and 2 larval collections during June 1998.

^c LAC virus was isolated from 1 pool of 12 females collected as larvae in June 1998.

^d LAC virus was isolated from 1 pool of 2 females collected as adults in July 1999.

DISCUSSION

Previous studies in a LAC virus-endemic area in western North Carolina have reported occurrence rates for *Oc. triseriatus* of 80.9% from a peridomestic container survey (Szumlas et al. 1996a) and >98% from an ovitrapping survey (Szumlas et al. 1996b). Ovitrap collections in southwest Virginia during 1998 yielded similarly high relative abundance of *Oc. triseriatus* (90.1%), but the prevalence of *Ae. albopictus* found in our study was higher (9.9%) than those previously reported by Szumlas and others (<1%) (1996a,b). In a more recent study, Jones et al. (1999) also reported similar species composition in an emerging LAC virus focus in Tennessee (80 and 20% *Oc. triseriatus* and *Ae. albopictus*, respectively). *Ae. albopictus* was collected from the Rocky Fork (Figs. 2.6a,b) and Duncan Gap human case sites during 1998 and 1999, indicating the ability of *Ae. albopictus* to become established and overwinter in southwestern Virginia. This is not unexpected, as other workers (Hawley et al. 1989, Swanson et al. 2000) have previously reported *Ae. albopictus* overwintering in colder climates in Indiana and Illinois.

Neither of the two species has been evenly distributed within the three counties studied, indicating that levels of risk for La Crosse virus probably vary in different areas of the counties. Because the numbers of mosquitoes assayed from 1998 and 1999 collections with LAC virus-positive mosquitoes are low, exact minimum field infection rates (MFIRs) cannot be determined. During 2000, when larger numbers of mosquitoes were collected, none of the 8,785 *Oc. triseriatus* tested were shown to be positive by plaque assay, so MFIRs presumably are low (<1/1,000) for this area. This is in agreement with the MFIRs for *Oc. triseriatus* reported previously from LAC virus-endemic areas in western North Carolina, 0.97/1,000 (Kappus et al. 1982) and 0.26/1,000 (Szumlas et al. 1996a); in West Virginia, 0.4 to 7.5/1,000 (Nasci et al. 2000); in Illinois, 1.2/1,000 over a 4-year period (Clark et al. 1983); in Wisconsin, 0.0 to 5.9/1,000 (Lisitz et al. 1977). However, it is lower than other reported MFIRs from overwintered *Oc. triseriatus* in Wisconsin, 5.9 to 12.5/1,000 (Beaty and Thompson 1975); and from a LAC virus-endemic area of Ohio, up to 12.3/1,000, although sample size in this study was small (403 mosquitoes) (Berry et al. 1975).

These data suggest that the typical enzootic cycle of LAC virus is occurring in Wise County, with *Oc. triseriatus* serving as the sole or at least the principal vector of the virus. Virus testing of several mosquito species – mainly *Oc. triseriatus* and *Ae. albopictus* – yielded two isolates of

LAC virus from collections near the Duncan Gap human LACE case site. The first isolate was obtained from a larval collection of *Oc. triseriatus* in 1998, indicating that transovarial transmission of LAC virus is occurring in this site. The second isolate was obtained from *Oc. triseriatus* adults in the same site the following year. This isolate proved that the virus could persist in the local mosquito population through winter, barring the unlikely chance of a reintroduction of virus into the population from an outside source. These data, combined with 3 human LAC virus infections at or near this site during 1997, suggest that the virus was able to persist in a small focus for at least 3 consecutive seasons. Chipmunks and squirrels are common in the deciduous forests in Wise County, so horizontal transmission to and from these potential reservoir hosts probably supplements vertical transmission.

There has been no evidence to show that *Ae. albopictus* has been involved in transmission of LAC virus in Wise County. However, most of the *Ae. albopictus* tested to date have been collected from the Wise County tire dump, not from a human case site. Since the LAC virus-positive *Oc. triseriatus* specimens have been collected from a human case site, further sampling for *Ae. albopictus* will be necessary in these areas to determine the extent of involvement of *Ae. albopictus* in LAC virus transmission. It seems unlikely that *Ae. albopictus* would primarily be responsible for maintenance of LAC virus in southwest Virginia given its recent introduction into the area and its lower transovarial transmission rates compared to *Oc. triseriatus* (Tesh and Gubler 1975), although it is possible that it may serve as a bridge vector, providing a link between viremic reservoir hosts and humans. *Ae. albopictus* feeds on a wide variety of mammalian, avian, and reptilian hosts, including humans and sciurids (Niebylski et al. 1994), and natural transovarial transmission of LAC virus by *Ae. albopictus* recently has been found in endemic areas in Tennessee and North Carolina (Gerhardt et al. 2001), showing that this species is capable of vertical LAC virus transmission in nature and probably also is capable of horizontal transmission to vertebrate hosts given its catholic blood feeding habits. *Ae. albopictus* seems more likely than *Oc. triseriatus* to use breeding areas in the cleared areas around homes, which increases the chance for *Ae. albopictus* to come into contact with humans.

No direct evidence of competition between *Oc. triseriatus* and *Ae. albopictus* was seen in this study. In open areas, both *Oc. triseriatus* and *Ae. albopictus* eggs are commonly deposited, whereas in densely forested areas, *Oc. triseriatus* eggs are considerably more common than those of *Ae. albopictus*. If *Ae. albopictus* proves to be a superior competitor to *Oc. triseriatus* in open

and residential areas as Swanson et al. (2000) have suggested, numbers of *Oc. triseriatus* may gradually decrease in these areas, and considering the relatively recent arrival of *Ae. albopictus* in Wise County, the current coexistence of the two species in open areas may be a result of their relatively short history of competition. Though usage of forest habitat by *Ae. albopictus* for oviposition currently is low, this also may result from a short history of *Ae. albopictus* within the county, as Livdahl and Willey (1991) have predicted stable coexistence of *Oc. triseriatus* and *Ae. albopictus* in treehole habitats based on experimental competition. Also, Mogi (1982) has suggested that forest-inhabiting strains of *Ae. albopictus* in Asia are descendants of anthropophilic strains that have readapted to the forest habitat. Therefore, if *Ae. albopictus* in Wise County originated from an anthropophilic strain introduced in used tires from northern Asia (Hawley 1987), it is possible that U.S. strains of this species will eventually adapt to use forested habitats more effectively, competing more directly with *Oc. triseriatus*.

In conclusion, our study demonstrated that *Oc. triseriatus* is abundant in LAC virus-endemic areas studied in southwestern Virginia, and transovarial transmission of LAC virus was found in *Oc. triseriatus* near at least one LACE human case site. *Ae. albopictus* also is present, and our collections have shown that it is now established and overwintering in southwestern Virginia. Oviposition intensity and relative abundance varied for *Oc. triseriatus* and *Ae. albopictus* based on characteristics of the oviposition habitat.

References Cited

- Balfour, H. H. Jr., C. K. Edelman, F. E. Cook, W. I. Barton, A. W. Buzicky, R. A. Siem, and H. Bauer. 1975. Isolates of California encephalitis (La Crosse) virus from field-collected eggs and larvae of *Aedes triseriatus*: Identification of the overwintering site of California encephalitis. *J. Infect. Dis.* 131(6): 712-716.
- Beaty, B. J. and W. H. Thompson. 1975. Emergence of La Crosse virus from endemic foci: Fluorescent antibody studies of overwintered *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 24(4): 685-691.
- Beaty, B. J., C. H. Calisher, and R. E. Shope. 1995. Arboviruses, pp. 189-212. *In* E. H. Lennette, D. A. Lennette, and E. T. Lennette [eds.], *Diagnostic procedures for viral, rickettsial, and chlamydial infections*, 7th ed. American Public Health Association, Washington, DC.
- Berry, R. L., B. J. LaLonde, H. W. Stegmiller, M. A. Parsons, and G. T. Bear. 1974. Isolation of La Crosse virus (California encephalitis group) from field collected *Aedes triseriatus* (Say) larvae in Ohio (Diptera: Culicidae). *Mosquito News.* 34(4): 454-457.
- Berry, R. L., M. A. Parsons, B. J. LaLonde, H. W. Stegmiller, J. Lebio, M. Jalil, and R. A. Masterson. 1975. Studies on the epidemiology of California encephalitis in an endemic area in Ohio in 1971. *Am. J. Trop. Med. Hyg.* 24(6): 992-998.
- Clark, G. G., H. L. Pretula, W. H. Rohrer, R. N. Harroff, and T. Jakubowski. 1983. Persistence of La Crosse virus (California encephalitis serogroup) in North-Central Illinois. *Am. J. Trop. Med. Hyg.* 32(1): 175-184.
- Cully, J. F. Jr., T. G. Streit, and P. B. Heard. 1992. Transmission of La Crosse virus by four strains of *Aedes albopictus* to and from the eastern chipmunk (*Tamias striatus*). *J. Am. Mosq. Control Assoc.* 8(3): 237-240.
- Gauld, L. W., T. M. Yuill, R. P. Hanson, and S. K. Sinha. 1975. Isolation of La Crosse virus (California encephalitis group) from the chipmunk (*Tamias striatus*), an amplifier host. *Am. J. Trop. Med. Hyg.* 24(6): 999-1005.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. The first isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases* (in press).
- Grimstad, P. R., J. F. Kobayashi, M. Zhang, and G. B. Craig, Jr. 1989. Recently introduced *Aedes albopictus* in the United States: Potential vector of La Crosse virus (Bunyaviridae: California Serogroup). *J. Am. Mosq. Control Assoc.* 5(3): 422-427.

- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: Probable introduction in used tires from northern Asia. *Science*. 236: 1114-1116.
- Hawley, W. A., C. B. Pumpuni, R. H. Brady, and G. B. Craig, Jr. 1989. Overwintering Survival of *Aedes albopictus* (Diptera: Culicidae) Eggs in Indiana. *J. Med. Entomol.* 26(2): 122-129.
- Johnson, T. G. 1992. Forest Statistics for Virginia, 1992. USDA Forest Service, Southeastern Forest Experiment Station Resource Bulletin SE-131.
- Jones, T. F., A. S. Craig, R. S. Nasci, L. E. R. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly recognized focus of La Crosse encephalitis in Tennessee. *Clinical Infectious Diseases*. 28: 93-97.
- Kappus, K. D., C. H. Calisher, R. C. Baron, J. Davenport, D. B. Francly, and R. M. Williams. 1982. La Crosse virus infection and disease in western North Carolina. *Am. J. Trop. Med. Hyg.* 31(3): 556-560.
- Ksiazek, T. G. and T. M. Yuill. 1977. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciurus carolinensis*) and chipmunks (*Tamias striatus*). *Am. J. Trop. Med. Hyg.* 26(4): 815-821.
- Kuno, G., C. J. Mitchell, G. J. Chang, and G. C. Smith. 1996. Detecting bunyaviruses of the Bunyamwera and California serogroups by a PCR technique. *J. Clin. Microbiol.* 34(5): 1184-1188.
- Linley, J. R. 1989a. Scanning electron microscopy of the egg of *Aedes (Protomacleaya) triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 26(5): 474-478.
- Linley, J. R. 1989b. Comparative fine structure of the eggs of *Aedes albopictus*, *Ae. aegypti*, and *Ae. bahamensis* (Diptera: Culicidae). *J. Med. Entomol.* 26(6): 510-521.
- Lisitz, M. A., G. R. DeFoliart, T. M. Yuill, and M. G. Karandinos. 1977. Prevalence rates of La Crosse virus (California encephalitis group) in larvae from overwintered eggs of *Aedes triseriatus*. *Mosquito News*. 37(4): 745-750.
- Livdahl, T. P. and M. S. Willey. 1991. Prospects for an invasion: Competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science*. 253: 189-191.
- McJunkin, J. E., E. C. los Reyes, J. E. Irazuzta, M. J. Caceres, R. R. Khan, L. L. Minnich, K. D. Fu, G. D. Lovett, T. Tsai, and A. Thompson. 2001. La Crosse encephalitis in children. *N. Engl. J. Med.* 344(11): 801-807.
- Miller, B. R., G. R. DeFoliart, and T. M. Yuill. 1977. Vertical transmission of La Crosse virus (California encephalitis group): Transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 14(4): 437-440.

- Miller, B. R., G. R. DeFoliart, and T. M. Yuill. 1979. *Aedes triseriatus* and La Crosse virus: Lack of infection in eggs of the first ovarian cycle following oral infection of females. *Am. J. Trop. Med. Hyg.* 28(5): 897-901.
- Mogi, M. 1982. Variation in oviposition, hatch rate and setal morphology in laboratory strains of *Aedes albopictus*. *Mosquito News.* 42(2): 196-201.
- Moulton, D. W. and W. H. Thompson. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin: Some ecological considerations. *Am. J. Trop. Med. Hyg.* 20(3): 474-482.
- Munstermann, L. E. and L. M. Wasmuth. 1985. *Aedes triseriatus*, pp. 15-24. In P. Singh and R. F. Moore [eds.], *Handbook of Insect Rearing*, Vol. 2. Elsevier, Amsterdam.
- Nasci, R. S. 1988. Biology of *Aedes triseriatus* (Diptera: Culicidae) developing in tires in Louisiana. *J. Med. Entomol.* 25(5): 402-405.
- Nasci, R. S., C. G. Moore, B. J. Biggerstaff, N. A. Panella, H. Q. Liu, N. Karabatsos, B. S. Davis, and E. S. Brannon. 2000. La Crosse encephalitis virus habitat associations in Nicholas County, West Virginia. *J. Med. Entomol.* 37(4): 559-570.
- Niebylski, M. L., H. M. Savage, R. S. Nasci, and G. B. Craig, Jr. 1994. Blood hosts of *Aedes albopictus* in the United States. *J. Am. Mosq. Control Assoc.* 10(3): 447-450.
- Pantuwatana, S., W. H. Thompson, D. M. Watts, T. M. Yuill, and R. P. Hanson. 1974. Isolation of La Crosse virus from field collected *Aedes triseriatus* larvae. *Am. J. Trop. Med. Hyg.* 23(2): 246-250.
- Rust, R. S., W. H. Thompson, C. G. Matthews, B. J. Beaty, and R. W. Chun. 1999. La Crosse and other forms of California encephalitis. *J. Child Neurol.* 14(1): 1-14.
- Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J. Am. Mosq. Control Assoc.* 2(2): 217-219.
- Steinly, B. A., R. J. Novak, and D. W. Webb. 1991. A new method for monitoring mosquito oviposition in artificial and natural containers. *J. Am. Mosq. Control Assoc.* 7(4): 649-650.
- Swanson, J., M. Lancaster, J. Anderson, M. Crandell, L. Haramis, P. Grimstad, and U. Kitron. 2000. Overwintering and establishment of *Aedes albopictus* (Diptera: Culicidae) in an urban La Crosse virus enzootic site in Illinois. *J. Med. Entomol.* 37(3): 454-460.
- Szumlas, D. E., C. S. Apperson, E. E. Powell, P. Hartig, D. B. Francy, and N. Karabatsos. 1996a. Relative abundance and species composition of mosquito populations (Diptera:

- Culicidae) in a La Crosse virus-endemic area in western North Carolina. *J. Med. Entomol.* 33(4): 598-607.
- Szumlas, D. E., C. S. Apperson, and E. E. Powell. 1996b. Seasonal occurrence and abundance of *Aedes triseriatus* and other mosquitoes in a La Crosse virus-endemic area in western North Carolina. *J. Am. Mosq. Control Assoc.* 12(2): 184-193.
- Tesh, R. B. and D. J. Gubler. 1975. Laboratory studies of transovarial transmission of La Crosse and other arboviruses by *Aedes albopictus* and *Culex fatigans*. *Am. J. Trop. Med. Hyg.* 24(5): 876-880.
- Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill, and W. H. Thompson. 1973. Transovarial transmission of La Crosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. *Science.* 182: 1140-1141.
- Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart, and R. P. Hanson. 1974. Overwintering of La Crosse virus in *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 23(4): 694-700.

Chapter 3

Geospatial and statistical modeling of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) distribution in an emerging focus of La Crosse virus

INTRODUCTION

La Crosse encephalitis (LACE), which is caused by La Crosse (LAC) virus, is the most prevalent mosquito-borne disease in the USA during most years. It primarily affects children under 15 years old, causing febrile illness with headache and vomiting. In more severe cases, seizures, disorientation, and aseptic meningitis are evident during the acute phase of illness (McJunkin et al. 1998). Death from LACE is rare (<1% of cases), but recurrent seizures, abnormal electroencephalograms (EEGs), and other sequelae may persist for months after initial infection (Chun 1983).

LAC virus is maintained in an enzootic cycle in which the virus is transmitted vertically from infected female *Ochlerotatus triseriatus* to their progeny (Watts et al. 1973) and horizontally through mammalian amplifiers such as chipmunks and squirrels (Gauld et al. 1975, Moulton and Thompson 1971, Ksiazek and Yuill 1977). Venereal transmission from infected male *Oc. triseriatus* to previously uninfected females also supplements other forms of transmission (Thompson and Beaty 1977).

Oc. triseriatus (Say) is the primary vector of LAC virus (Moulton and Thompson 1971, Sudia et al. 1971), and this mosquito is encountered commonly throughout the deciduous forests of the eastern and midwestern USA. As humans have populated forested areas, contact with *Oc. triseriatus* and LAC virus have increased. *Oc. triseriatus* will breed in water-collecting containers around homes (Hedberg et al. 1985), which further increases the chance of contact between humans and this mosquito. Humans are tangential hosts for LAC virus, being susceptible to infection but incapable of developing a sufficient viremia to infect a susceptible mosquito.

Another mosquito that has become common in many LAC virus-endemic areas is *Aedes albopictus* (Skuse). Following the discovery of the first known breeding population of this mosquito in the continental USA in Harris County, Texas in 1985 (Sprenger and Wuithiranyagool 1986), *Ae. albopictus* has spread throughout most of the eastern United States.

This species is a competent experimental vector of LAC virus, with rates of susceptibility and oral transmission similar to those for *Oc. triseriatus* (Grimstad et al. 1989, Cully et al. 1992). However, transovarial transmission rates for LAC virus in *Ae. albopictus* are lower than those for *Oc. triseriatus* (Tesh and Gubler 1975).

Southwestern Virginia has seen a considerable increase in numbers of LACE cases in recent years. During a 5-year period from 1994 through 1998, 4 counties in the Appalachian Mountains recorded a total of 13 LACE cases (Fig. 3.1), whereas only 1 case was recorded from the entire

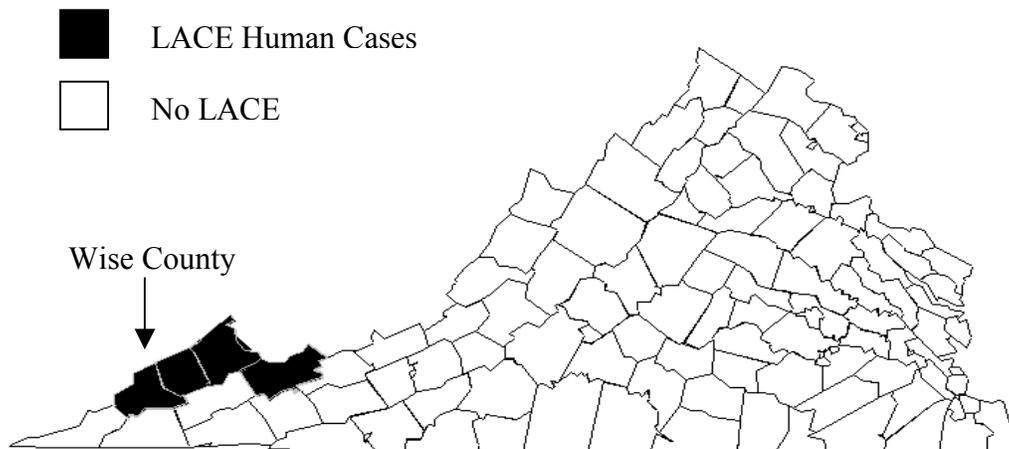


Fig. 3.1. Virginia counties reporting LACE cases (1994-1998).

state during 19 years prior to this period. During 1997, Wise County reported 5 confirmed cases of LACE, constituting a case rate of approximately 13 per 100,000. These recent LACE cases have indicated the need for a better understanding of the LAC virus cycle in this area, including the distribution and habitat preferences of its potential vectors.

LAC virus has been isolated from larval and adult *Oc. triseriatus* collections from a human case site in Wise County, Virginia, indicating that transovarial transmission is occurring in this species (C.M.B. and S.L.P., unpublished data). Also, LAC virus recently has been isolated from *Ae. albopictus* field-collected as eggs in LAC virus-endemic areas in the neighboring states of Tennessee and North Carolina (Gerhardt et al. 2001), demonstrating natural transovarial transmission in this species and supporting the hypothesis that it may serve as an accessory vector in the LAC virus cycle.

Oc. triseriatus and *Ae. albopictus* are found throughout southwestern Virginia, although abundance of these species as measured by oviposition varies with habitat type. *Oc. triseriatus* and *Ae. albopictus* distribution in the region is not uniform, and at a LACE human case site, *Ae. albopictus* was shown to preferentially oviposit in the yard surrounding the home, while *Oc. triseriatus* showed no preference between the habitats, laying eggs in the yard and adjacent forests approximately equally (C.M.B. and S.L.P., unpublished data). In a nearby LAC virus-endemic area in West Virginia, Nasci et al. (2000) found that *Oc. triseriatus* population densities were related to habitat type, with higher densities in mixed northern hardwood habitats primarily containing large maples than in sites with hemlocks mixed with hardwoods or in an abandoned orchard site containing a large number of small maple trees.

Because both *Oc. triseriatus* and *Ae. albopictus* are associated with characteristic habitats, remote sensing techniques can be used to map landscapes and to identify habitats that are associated with breeding of these species. In recent years, geographic information systems and remote sensing have proven useful in numerous studies assessing relationships between environmental variables and disease vector abundance (Rogers and Randolph 1991, Wood et al. 1992, Beck et al. 1994, Roberts et al. 1996, Cross et al. 1996, Baylis et al. 1998). Because both vegetation type and vector abundance are influenced by environmental factors such as rainfall, elevation, and temperature, it seems logical that a relationship would exist between vector population levels and landcover type. Beck et al. (1994) demonstrated that *Anopheles albimanus* abundance near villages in Mexico could be predicted as either high or low based on the landcover composition surrounding the villages. In particular, proportions of two landcover types used as breeding sites by the mosquitoes were indicative of vector abundance. Similarly, Moncayo and others (2000) analyzed the relationship between abundance of eastern equine encephalomyelitis vectors and Landsat Thematic Mapper-derived landscape proportions surrounding human and horse case sites. Wetlands were determined to be the most important landscape class element, accounting for up to 72.5% of observed variation in host-seeking vector mosquito populations. Roberts et al. (1996) also showed that landcover information derived from multispectral satellite data successfully predicted areas of high and low mosquito abundance.

Bayesian modeling procedures can be used to link survey data with satellite imagery to produce distribution maps for a given species. Although Bayesian procedures have been used

mainly to model bird and mammal distributions (Aspinall 1991, Aspinall and Veitch 1993, Hepinstall and Sader 1997, Tucker et al. 1997), this approach also has been used to model insect populations (Wood et al. 1992). Bayesian procedures use survey data or expert knowledge to calculate prior probabilities of finding a species at any point within the landscape, regardless of habitat. The model also requires that conditional probabilities be assigned to habitat variables, given the presence or absence of the species. The end result is a map showing the distribution of the species as a probability of occurrence (Aspinall and Veitch 1993). In a study by Wood et al. (1992), Bayesian procedures were used to predict high and low mosquito-producing rice fields in California. Rice fields predicted to produce high mosquito numbers were identified with 85% accuracy, indicating that Bayesian modeling could be useful for directing vector control efforts or for warning citizens about potential disease risk.

The objective of this study was to predict the distribution of *Oc. triseriatus* and *Ae. albopictus* in Wise County, Virginia using Bayesian modeling procedures based on habitat preferences determined from ovitrapping survey data. Accuracy and other measures of prediction success were used to validate the model and assess its usefulness for predicting mosquito abundance as it relates to LAC virus risk.

MATERIALS AND METHODS

Study Area

This study was conducted in Wise County, Virginia (Fig. 3.1), where a total of 6 cases of LACE occurred during 1997 and 1998. Wise County is in the southern Appalachian Mountains, where mixed hardwood forests predominate, including oak-hickory, maple-beech-birch, and white pine-hemlock forest types (Johnson 1992). Small towns, barren land from coal mining, and occasional pastureland are interspersed among the forest stands. Populated areas are located mainly between 400 m and 800 m elevation, and elevation within the county ranges from approximately 400 m to 1287 m.

Mosquito Survey

Mosquitoes were collected throughout Wise County by ovitrap during a 16-week period from May 29 through September 18, 2000 (Fig. 3.2). Ovitrap consisted of a 450 mL black plastic cup nailed to a tree or other support 1 m or less above ground level. If no support was available, the ovitrap was placed on the ground, usually braced by small rocks to prevent the cup from tipping over. Ovitrap had drain holes approximately halfway up each side, and each cup was

lined with a 5 cm wide strip of seed germination paper as an oviposition substrate (Steinly et al. 1991). Ovitrap collections were made weekly, and traps were distributed throughout the county each week, so that the entire county was represented during each sampling period. During the first 4 weeks of collection, 22 new ovitrap sites (21 during week 1) were set out each week, with a single ovitrap at each site. Each ovitrap site from the first four-week period was sampled

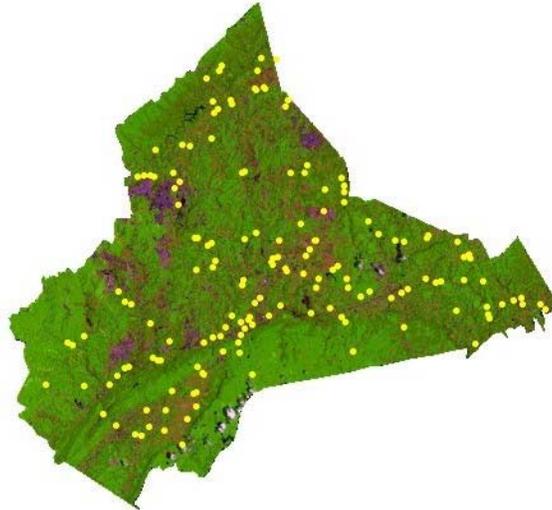


Fig. 3.2. Image of Wise County, Virginia showing ovitrap collection sites.

repeatedly at 4-week intervals through the end of the 16-week period of the study. These traps were placed in various habitat types, with the majority in habitat types where highest numbers of *Oc. triseriatus* and *Ae. albopictus* were expected (either forested areas or urban/residential areas). Remaining ovitraps were placed in habitat types that were expected to have lower numbers of mosquitoes, such as herbaceous rangeland, coniferous forest, shrub and brush rangeland, and barren land. Though the number of ovitraps in a habitat type was based on anticipated numbers of mosquitoes, individual ovitrap sites within each habitat were not chosen based on their suitability as mosquito oviposition sites. Rather, individual sites were chosen that were representative of the habitat type. In addition to these repeated sites, 6 new sites were sampled each week (after the first 4 weeks) from the landcover types where lower numbers of mosquitoes were expected to obtain a more representative estimate of the population in these habitats. A total of 160 ovitrap sites was visited during the collection season. Each ovitrap location was recorded using a hand-held GPS receiver (GPS III Plus, Garmin, Olathe, KS) so that their exact locations could be depicted spatially. In total, 490 1-week ovitrap collections were made, with the following distribution: 200 in forest, 141 in urban/residential, 60 in

herbaceous rangeland, 56 in shrub and brush rangeland, and 33 in barren land. Eggs from each site were identified to species (Linley 1989a,b) and counted weekly, and the numbers were used to determine prior and conditional probabilities for mosquito presence and absence for input into the Bayesian model. A generalized linear model analysis of variance (ANOVA) was used to test for differences among means of square root-transformed $[\sqrt{(y+0.1)}]$ ovitrap counts, and Fisher's least significant difference (LSD) multiple comparison test was used for separation of significantly different means, as determined by the ANOVA.

Image Classification

A landcover map of Wise County was created from Landsat Enhanced Thematic Mapper (ETM+) imagery of the county. All image processing was performed in TNTmips map and image processing software (Microimages, Inc. 2001). The images were classified into landcover types based on landcover classes described by Anderson et al. (1976). These included: Forest, Urban/Residential, Rangeland (Shrub & Brush and Herbaceous), Barren Land, and Water. GPS coordinates were recorded for representative sites in various landcover types and these, along with ovitrap site descriptions, were used in a feature mapping process (Fig. 3.3) to create a



Fig. 3.3. Computer screen showing feature mapping process in TNTmips[®] in which areas of known landcover type are defined for each landcover class. Colorized areas on the map represent areas defined by the user to represent each landcover class. Yellow points on map indicate ovitrap sites and other reference points used to locate representative landcover areas.

ground truth data set to assist in the classification. These training data representing each landcover class and their associated statistics then were used in a maximum likelihood classification of the image to create the landcover map of Wise County. Only six of the Landsat ETM+ spectral bands were used in the classification: blue, green, red, near-infrared, and mid-infrared (2 bands). The thermal band was not used because of its coarser spatial resolution (60m) compared to that of the other bands (28.5m).

Bayesian Classification

There are four main components in the Bayesian model: prior probabilities of high or low species abundance, and conditional probabilities of high or low species abundance. These probabilities are used to calculate a posterior probability of high species abundance for a given condition (landcover type, in this case). Bayes' theorem, as used in this study, is as follows:

$$P(M_{\text{high}} | \text{Habitat}) = \frac{P(M_{\text{high}}) * P(\text{Habitat} | M_{\text{high}})}{P(M_{\text{high}}) * P(\text{Habitat} | M_{\text{high}}) + P(M_{\text{low}}) * P(\text{Habitat} | M_{\text{low}})}, \quad [3.1]$$

where

$P(M_{\text{high}})$ = Prior probability of high mosquito abundance

$P(M_{\text{low}})$ = Prior probability of low mosquito abundance

$P(\text{Habitat}|M_{\text{high}})$ = Conditional probability of finding a specific habitat given high mosquito abundance

$P(\text{Habitat}|M_{\text{low}})$ = Conditional probability of finding a specific habitat given low mosquito abundance, and

$P(M_{\text{high}}|\text{Habitat})$ = Posterior probability of high mosquito abundance

Oviposition activity was used as an indicator of mosquito abundance, and the threshold separating high and low abundance was defined by the seasonal mean for the individual species (126 eggs per trap-week for *Oc. triseriatus*, 23 eggs per trap-week for *Ae. albopictus*). Prior probabilities of high and low mosquito abundance were calculated from the mosquito survey data by dividing the number of sites with egg numbers above and below the seasonal mean, respectively, by the total number of collections during the period of interest. These were used along with conditional probabilities for each landcover type, which were derived also from ovitrap survey data. Conditional probabilities were determined for a given collection period and habitat type by dividing the total number of sites within a certain abundance level (high or low) which were collected from a particular habitat type by the total number of sites within the

abundance level. Prior and conditional probabilities were calculated for each pixel in the landcover map, and rasters containing these pixels were entered into the Bayesian formula to produce a probability surface that showed the posterior probabilities for high mosquito abundance for each pixel in the county based on landcover type. Surfaces were created for eight 2-week periods during the 16-week collection season.

Model Validation

Statistical comparisons based on error matrices (Fielding and Bell 1997) were used to test for agreement between model predictions and actual ovitrapping survey data. A buffer zone of 200 m was created around each ovitrap location (Fig. 3.4), and posterior probabilities for high

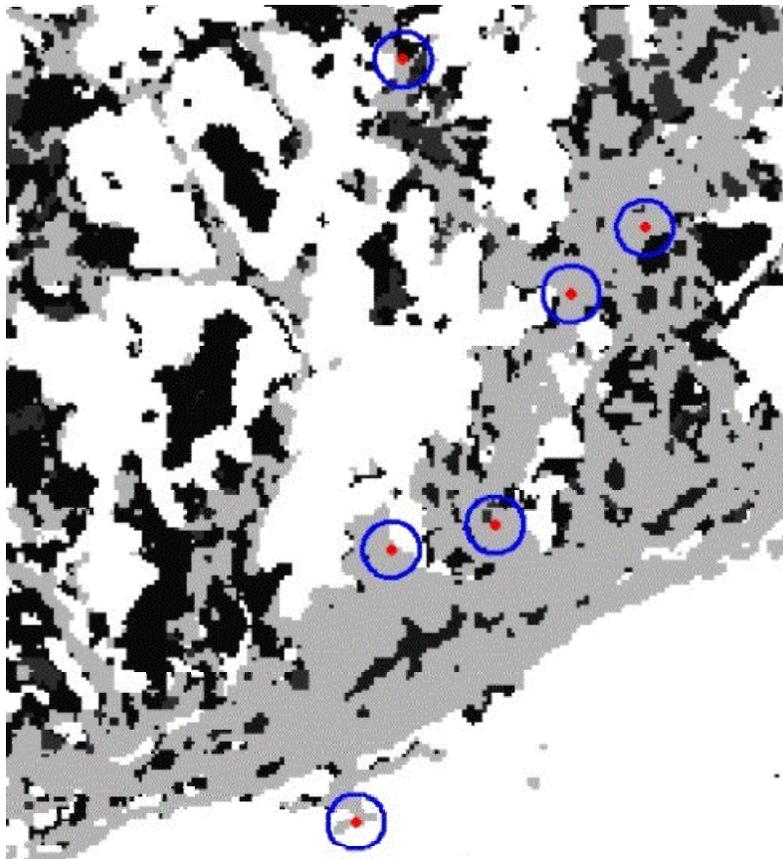


Fig. 3.4. Excerpt from posterior probability map showing 200 m buffer zones surrounding ovitrap sites. Probabilities were averaged within these zones to establish posterior probabilities for comparison with ovitrap data.

mosquito abundance were averaged within this zone for each site. This radius encompasses the flight ranges for most *Oc. triseriatus* and *Ae. albopictus* (Bonnet and Worcester 1946, Mather

and DeFoliart 1984, Hawley 1988, Niebylski and Craig 1994). The average probabilities then were compared with actual mosquito numbers to assess prediction errors and to derive accuracy metrics for the Bayesian model predictions. The following metrics were calculated (Table 3.1):

- Accuracy = proportion of pixels that were correctly classified as having high or low mosquito abundance
- Kappa (K) = proportion of specific agreement
- Prevalence = $p(X_{AHA})$
- False Positive Rate = $p(X_{PHA}|X_{ALA})$
- False Negative Rate = $p(X_{PLA}|X_{AHA})$
- Positive Predictive Power = $p(X_{AHA}|X_{PHA})$
- Negative Predictive Power = $p(X_{ALA}|X_{PLA})$

where X is a trapping site, AHA and ALA are actual, and PHA and PLA are predicted, high and low mosquito abundance, respectively.

Table 3.1. Error matrix and accuracy measures used for assessing predictive capabilities of the Bayesian model (Fielding and Bell 1997).

Error matrix		Measure	Calculation		
		Accuracy	$(a + d)/N^*$		
		Kappa (K)	$\frac{(a + d) - (((a + c)(a + b) + (b + d)(c + d))/N)}{N - (((a + c)(a + b) + (b + d)(c + d))/N)}$		
		Prevalence	$(a + c)/N$		
Predicted	High	a	b	False Positive Rate	$b/(b + d)$
	Low	c	d	False Negative Rate	$c/(a + c)$
			Positive Predictive Power	$a/(a + b)$	
			Negative Predictive Power	$d/(c + d)$	

*N = total number of compared sites.

Average probabilities greater than 50% were assumed to predict high abundance, and actual mosquito numbers were defined as either high or low based on whether they were above or below the mean number of eggs per trap week over the entire collection period for the respective species. Each of the two mosquito species was modeled and analyzed separately.

RESULTS

Ovitrap collections

Oviposition preference differed significantly among the landcover types over all collection dates for *Oc. triseriatus* and *Ae. albopictus* (Table 3.2, Fig. 3.5). Numbers of *Oc. triseriatus*

Table 3.2. Results of GLM ANOVA and Fisher's LSD for square root-transformed [$\sqrt{(y+0.1)}$] ovitrap counts from Wise County, VA. Eggs were collected weekly between May 29 and September 18, 2000.

ANOVA Comparison	Species included in comparison	F	df	P	Fisher's LSD ($\alpha=0.05$)		
					Group	Mean	
Landcover	<i>Oc. triseriatus</i>	32.76	4	< 0.0001	Barren Land	0.594	a*
					Herbaceous Rangeland	1.058	a
					Shrub & Brush Rangeland	1.400	a
					Urban/Residential	3.148	b
					Forest	5.136	c
Landcover	<i>Ae. albopictus</i>	12.95	4	< 0.0001	Forest	0.666	a
					Barren Land	1.267	a,b
					Shrub & Brush Rangeland	1.279	b
					Herbaceous Rangeland	1.446	b
					Urban/Residential	2.048	c
Week	<i>Oc. triseriatus</i>	1.00	15	0.4504	N/A		
Week	<i>Ae. albopictus</i>	20.44	15	< 0.0001	See text		
Landcover x Week	<i>Oc. triseriatus</i>	1.21	60	0.1615	N/A		
Landcover x Week	<i>Ae. albopictus</i>	5.60	60	< 0.0001			
Species	Both	17.75	1	< 0.0001	<i>Ae. albopictus</i>	1.341	a
					<i>Oc. triseriatus</i>	2.267	b
Species x Landcover	Both	35.14	4	< 0.0001	See text		
Species x Week	Both	3.23	15	< 0.0001			

* Means followed by different letters are significantly different based on ANOVA and Fisher's least significant difference multiple comparison test, $P < 0.05$.

eggs were higher in forested areas than in urban/residential areas based on Fisher's LSD at $\alpha=0.05$. The three remaining habitat types (shrub and brush rangeland, herbaceous rangeland, and barren land) did not differ significantly from each other and yielded significantly lower numbers of *Oc. triseriatus* eggs than forested and urban areas. Numbers of *Ae. albopictus* eggs were significantly higher ($\alpha=0.05$) in urban areas than all other habitats and significantly lower in forested areas than all other habitats except barren land. Numbers of *Ae. albopictus* in barren land, herbaceous rangeland, and shrub and brush rangeland were not significantly different from each other at the $\alpha=0.05$ level. *Ae. albopictus* abundance was highest in week 34 (LSD, $\alpha=0.05$), and weeks 30-36 yielded higher numbers of eggs than other collection weeks.

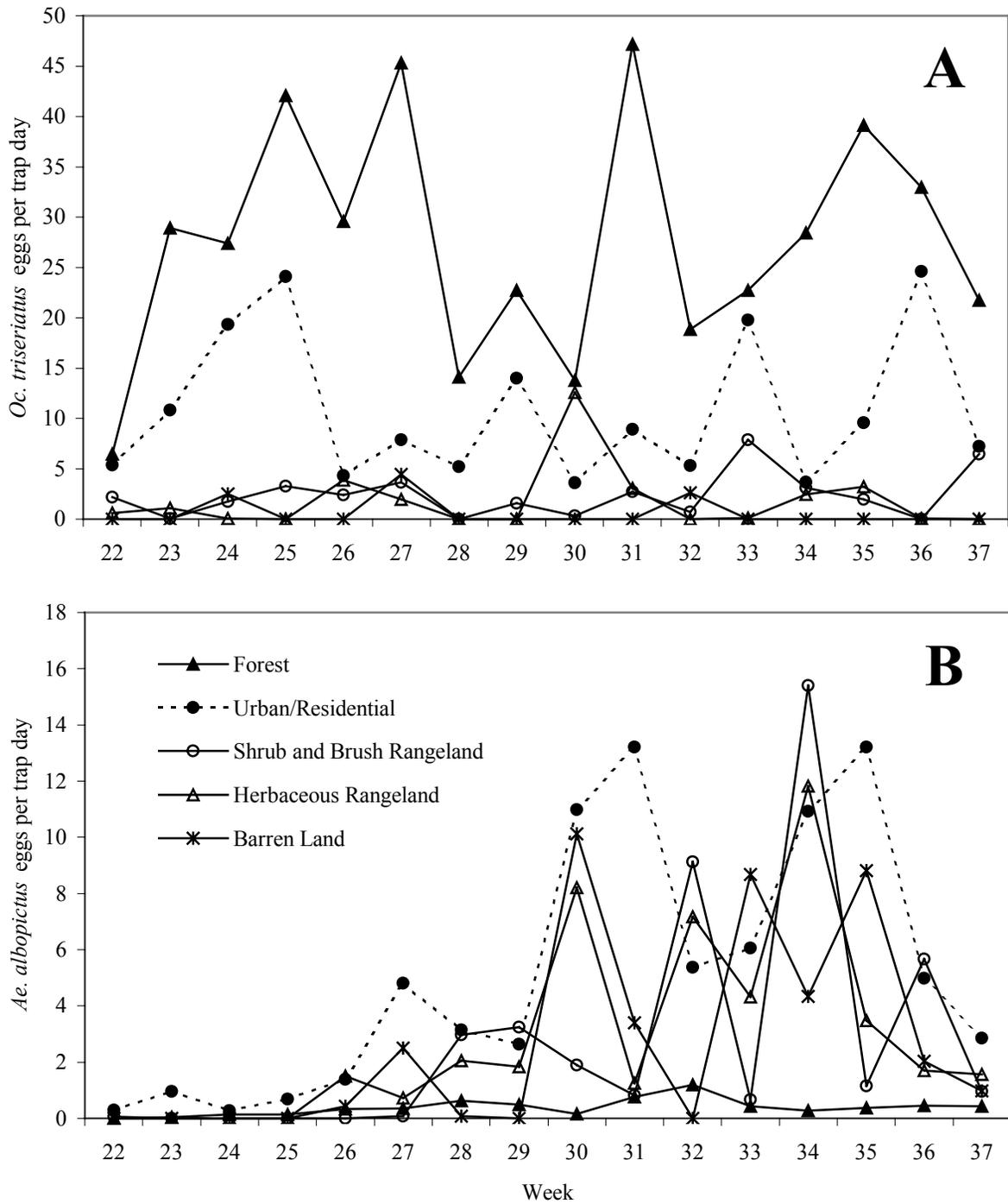


Fig. 3.5. Mean egg counts per trap day for *Oc. triseriatus* (A) and *Ae. albopictus* (B) for various landcover types in Wise County. Eggs were collected weekly between May 29 and September 18, 2000. Numbers displayed in figure are back-transformed from means of $\sqrt{(Y+0.1)}$ transformed data used for statistical comparisons.

Numbers of eggs collected were significantly higher for *Oc. triseriatus* than *Ae. albopictus* for the collection period ($P < 0.0001$) (Table 3.2, Fig. 3.6) and relationships between numbers of eggs

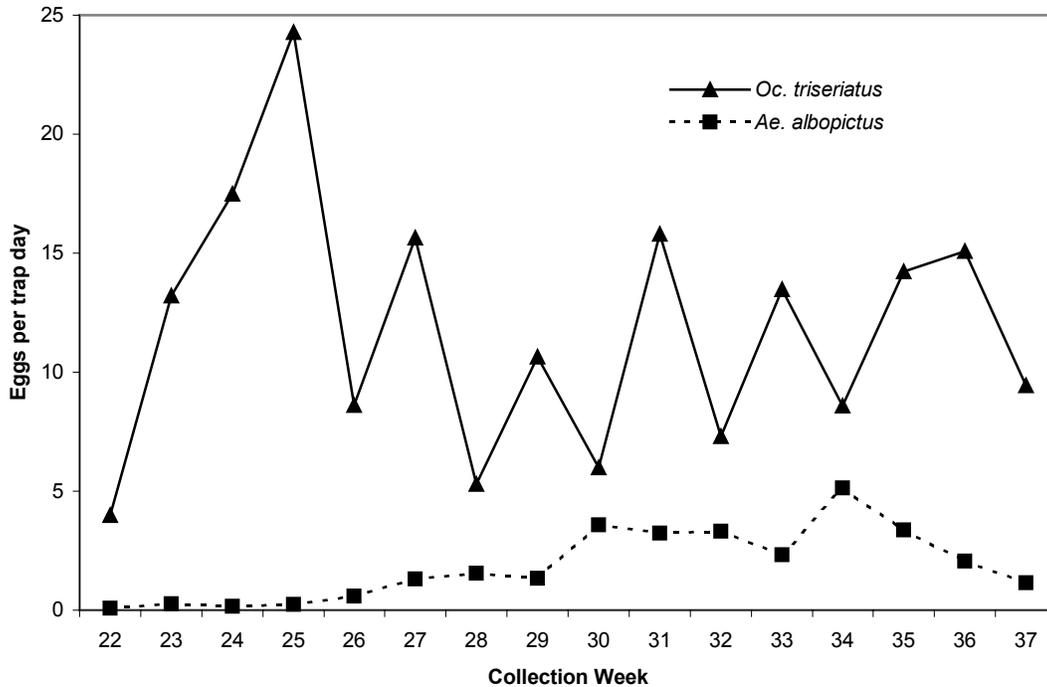


Fig. 3.6. Mean eggs per trap day for all ovitrap sites for *Oc. triseriatus* and *Ae. albopictus*. Eggs were collected between May 29 and September 18, 2000. Values shown in figure are back-transformed from means for square-root transformed data [$\sqrt{(y+0.1)}$] used for statistical analyses.

per species differed over time ($P < 0.0001$) and among landcover classes ($P < 0.0001$) (Table 3.2, Figs. 3.5 and 3.6). A Fisher’s LSD comparison among species-landcover interactions showed that mean numbers of *Oc. triseriatus* in forested areas were higher than for all other species-landcover combinations ($\alpha = 0.05$) (Fig. 3.5).

Landcover classification

The landcover map of Wise County (Fig. 3.7) that was created from the satellite imagery had an overall accuracy of 98% based on a comparison of output classification and user-defined ground truth data (Table 3.3). Accuracy levels for individual landcover classes were also high, indicating that minimal error was introduced in the classification process. The landcover image resulting from the classification consisted of 63.2% forest, 18.0% urban/residential, 10.6% shrub and brush rangeland, 4.9% herbaceous rangeland, 3.1% barren land, and 0.2% water (Table 3.4).

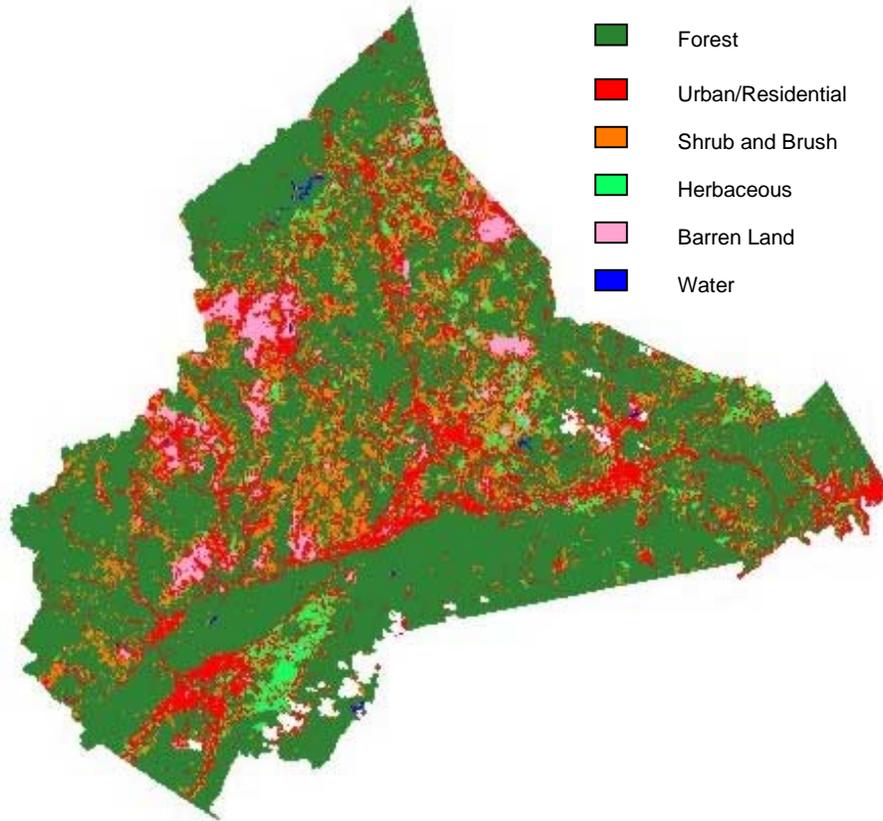


Fig. 3.7. Landcover image of Wise County, Virginia resulting from supervised maximum likelihood classification of visible, near-infrared, and short-wave infrared Landsat ETM+ spectral bands (28.5 m spatial resolution). White areas within county boundary were cloud-covered on image acquisition date and were excluded from analyses.

Table 3.3. Error matrix for supervised landcover classification of Wise County, Virginia.

		Ground Truth Data						Total	Accuracy (%)
		Water	Urban	Shrub & Brush	Herbaceous	Forest	Barren Land		
Classification	Water	279	0	0	0	0	0	279	100.00
	Urban	1	1,533	0	3	48	77	1,662	92.24
	Shrub & Brush	0	4	44	0	0	0	48	91.67
	Herbaceous	0	11	1	359	0	8	379	94.72
	Forest	0	0	0	0	17,112	0	17,112	100.00
	Barren Land	0	275	0	0	0	2,548	2,823	90.26
	Total	280	1,823	45	362	17,160	2,633	22,303	
	Accuracy (%)	99.64	84.09	97.78	99.17	99.72	96.77		
Overall Accuracy = 98.08% Kappa = 95.06%									

Table 3.4. Comparison of relative sampling intensity in landcover classes versus actual prevalence of landcover classes as defined by the supervised landcover map of Wise County.

Landcover Class	Percentage of samples taken	Percentage of total pixels in landcover classification
Forest	41	63
Urban/Residential	29	18
Shrub and Brush Rangeland	11	11
Herbaceous Rangeland	12	5
Barren Land	7	3

Because of the resolution of the Landsat ETM+ imagery used for classification, areas classified as water were limited to large bodies of water such as lakes, and thus were not considered as suitable habitat for the treehole and container-breeding species considered in probability calculations.

Bayesian Model

Posterior probability maps were created for eight 2-week periods during the 16-week collection period for both species, *Oc. triseriatus* and *Ae. albopictus*. *Oc. triseriatus* egg numbers peaked in late June (24.27 eggs/trap day) and again with a smaller peak in early September (15.07 eggs/trap day) (Fig. 3.6). The model reflected these fluctuations, with accuracy levels between 55 and 79% and false negative rates ranging from 0.42 to 1.00 (Fig. 3.8). Predicted and actual numbers of *Oc. triseriatus* were highest in forested areas. Kappa values, which take into account correctly classified pixels in addition to false positives and negatives, varied from very low to moderate values (0.00-0.53) for *Oc. triseriatus*.

Low numbers of *Ae. albopictus* (< 0.3 eggs/trap day) were present during June, and the population increased steadily during July and August, peaking in late August (5.14 eggs/trap day) and declining thereafter (Fig. 3.6). *Ae. albopictus* abundance was generally predicted with better accuracy (70–94%) than *Oc. triseriatus* (Fig. 3.9), and false negative rates ranged from 0.26 to 1.00. Accuracy levels early in the season were very high (94%), and as mosquito populations increased later in the season, accuracy levels declined to 70-75%. Unlike *Oc. triseriatus*, urban areas had the highest actual and predicted numbers of *Ae. albopictus* during most weeks. Kappa values also varied from very low to moderate values (0.00-0.49) for *Ae. albopictus*.

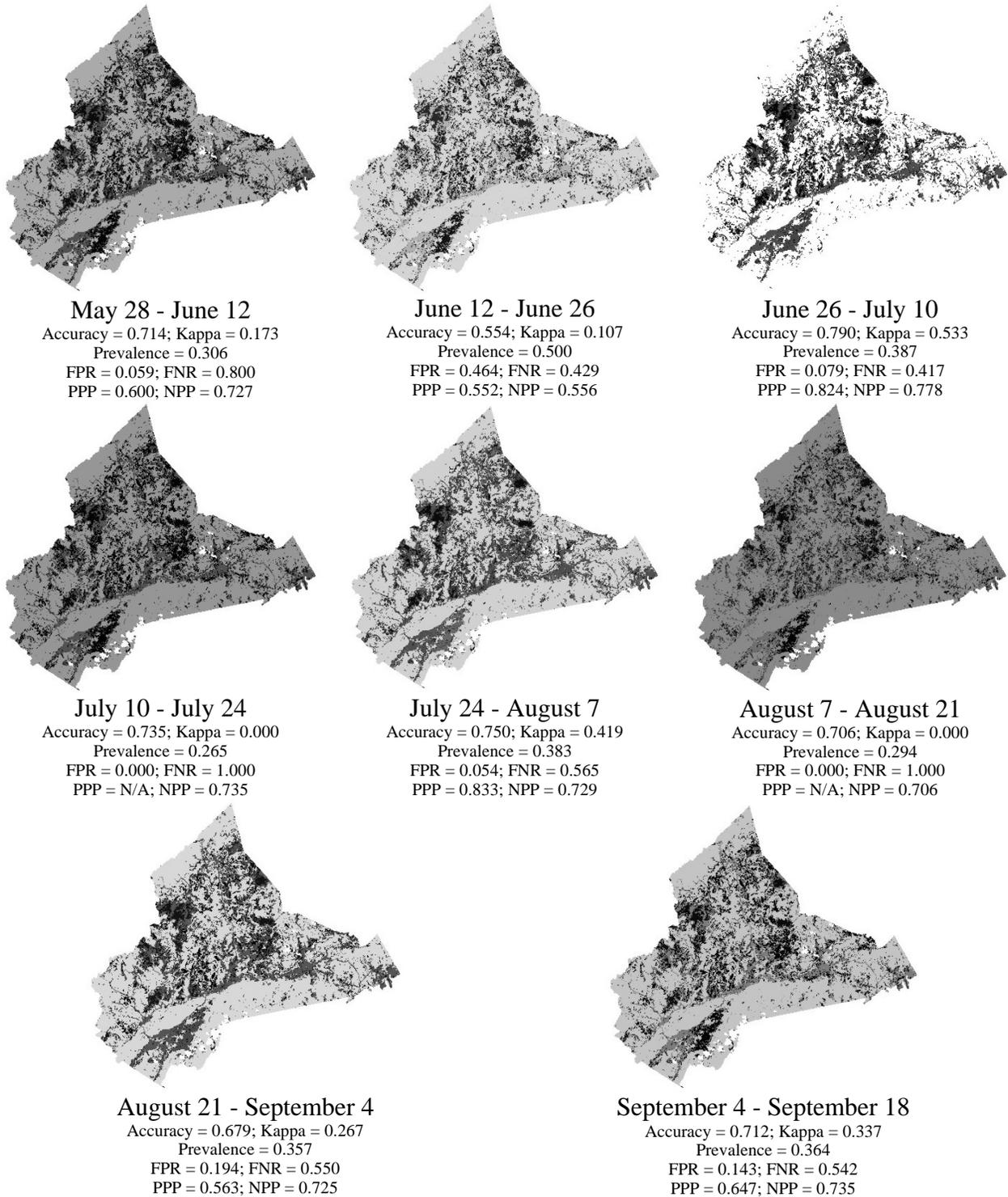


Fig. 3.8. Maps of Wise County, Virginia showing posterior probabilities of high *Oc. triseriatus* abundance. Maps are in grayscale (pure black = 0 % probability of high abundance, pure white = 80 % probability of high abundance). Accuracy and other statistics are indicated. FPR=false positive rate; FNR=false negative rate; PPP=positive predictive power; NPP=negative predictive power. PPP values designated as N/A could not be calculated due to zero values in the denominators.

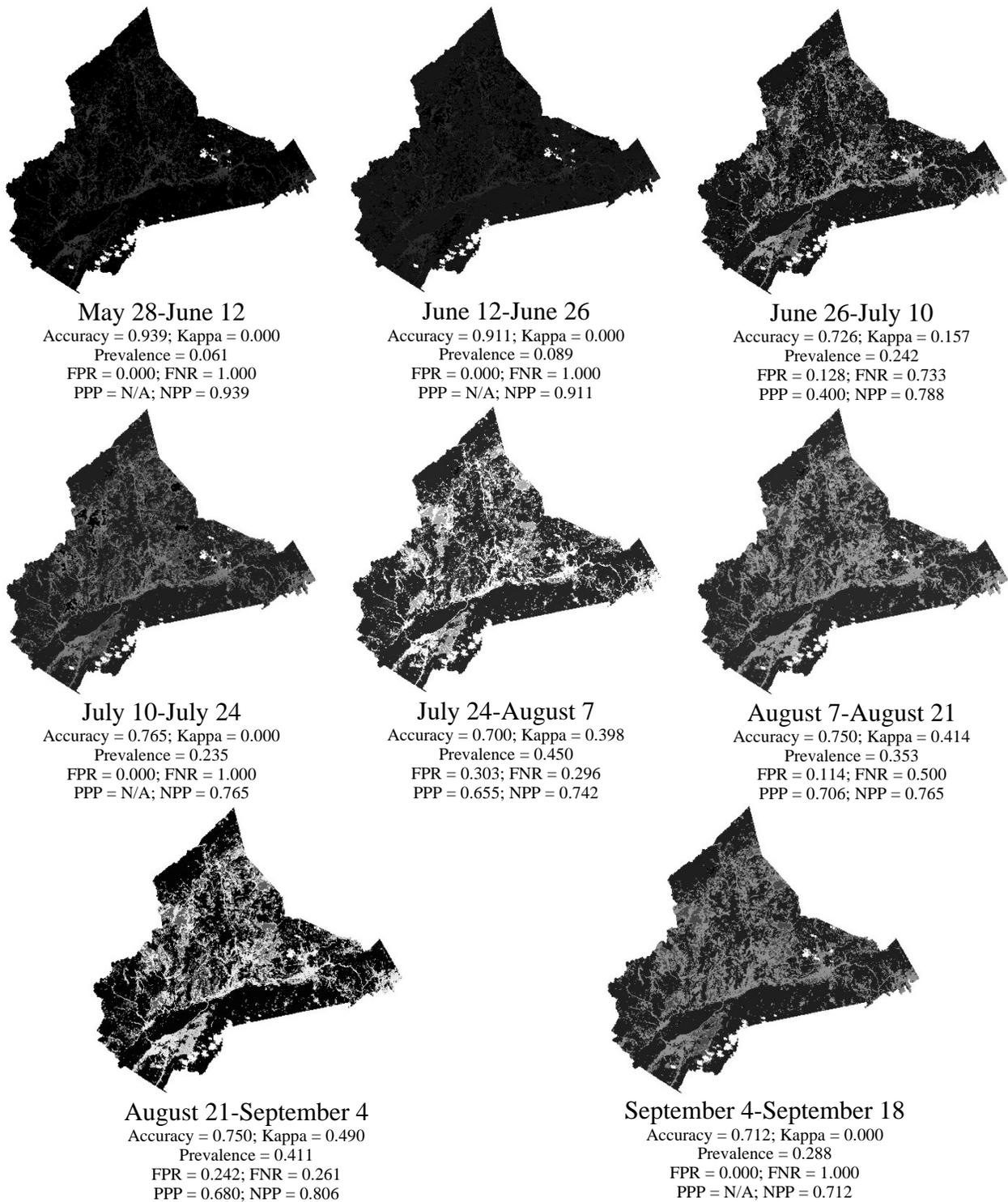


Fig. 3.9. Maps of Wise County, Virginia showing posterior probabilities of high *Ae. albopictus* abundance. Maps are in grayscale (pure black = 0 % probability of high abundance, pure white = 80 % probability of high abundance). Accuracy and other statistics are indicated. FPR=false positive rate; FNR=false negative rate; PPP=positive predictive power; NPP=negative predictive power. PPP values designated as N/A could not be calculated due to zero values in the denominators.

DISCUSSION

The data from our study suggest that the newly sympatric species in southwest Virginia, *Oc. triseriatus* and *Ae. albopictus*, are using different primary habitat types, with overlap in intermediate habitats. Largest numbers of *Oc. triseriatus* were found in forested habitats, while the largest numbers of *Ae. albopictus* eggs were collected in urban and residential areas. A previous season-long evaluation of *Oc. triseriatus* oviposition preferences at a Wise County LACE human case site revealed that *Oc. triseriatus* deposited eggs equally in ovitraps placed in the yard and surrounding forested areas (C.M.B. and S.L.P., unpublished data). However, in this study, *Oc. triseriatus* preferred forested areas over urban and residential areas, which may have resulted from increased sample sizes and a larger sampling area. Because the earlier study was performed at a single site with lower numbers of traps in each habitat type (5 in forest, 5 in yard), the present study provides a better indication of overall oviposition preferences of the species throughout the area. Overall, more *Oc. triseriatus* than *Ae. albopictus* were found in urban and residential areas, but this may be because of newly occurring coexistence in these habitats, and it is possible that *Ae. albopictus* may eventually become more prevalent in these areas. In other areas, such as shrub and brush rangeland, herbaceous rangeland, and barren land, the numbers for both species were lower than in their respective preferred habitats, indicating that both species' use of these habitats is relatively infrequent, at least for oviposition.

Ae. albopictus prefers urban and residential areas in Wise County, but this statement should be qualified by a description of the urban areas that exist there. Areas defined as urban/residential for the purposes of the study were simply places of human habitation, usually a house with a manicured lawn within a residential area or rural housing cluster or a business district within a small town. Large cities are not found in Wise County, and the urban areas in Wise County are similar to suburban areas in major cities with trees and other vegetation interspersed among the houses and other buildings. The preference of *Ae. albopictus* for urban areas and the relatively low abundance found in forests agree well with the proposed origin of this species in the U.S. from used tire shipments from northern Asia (Hawley et al. 1987). Studies of macrohabitat preferences of *Ae. albopictus* in Japan showed that this mosquito is common or regularly occurring in urban, suburban, and rural areas, but is rare in forest (Hawley 1988). However, studies in other parts of Asia found that *Ae. albopictus* was common in forests (Hawley 1988). Mogi (1982) has suggested that forest-dwelling populations of *Ae. albopictus* in

some areas of Asia may result from readaptation of anthropophilic strains of the species to the forest habitat. Thus, *Ae. albopictus* populations in Wise County may eventually adapt to use forested habitats more frequently and compete for resources more directly with native *Oc. triseriatus* in these areas.

The original ovitrap collection data were used to establish prior and conditional probabilities in the Bayesian model and to validate model predictions. The ovitrap data used as input for the model were not extensive enough to allow partitioning for prediction and assessment, and a suitable retrospective mosquito abundance dataset was not available for the study area. Although assessment of predictions based on data used to create a model is generally considered questionable or even invalid, input probabilities for the Bayesian model used in this study are calculated independently of the spatial arrangement of the data. Conversely, validation measures are based on locations of individual sites and their surrounding landcover. Therefore, even though these measures are not as robust as those calculated from a separate dataset, the current assessment provides an accurate depiction of model accuracy. Additional ovitrap collections would provide improved evaluation of model predictions.

Overall accuracy levels for predictions of high mosquito abundance for 2-week periods during the collection season ranged from moderate (0.55) to very high (0.94), and in all cases a majority of pixels in the posterior probability image was correctly classified. However, accuracy levels are dependent on prevalence (proportion of sites that has actual high mosquito abundance), and very high accuracy levels can be achieved even with a poor predictor if nearly all sites have low actual and predicted mosquito abundance. Kappa (K) is a better estimate of prediction success because this statistic takes into account errors of commission and omission and is an indicator of prediction success above that expected by chance (Fielding and Bell 1997). K , which is the proportion of specific agreement, varied from poor ($K < 0.4$) to good ($0.4 < K < 0.75$) for both species, as defined by Landis and Koch (1977). K was highest for *Ae. albopictus* during periods of peak abundance (weeks 9-10, 13-14), and for *Oc. triseriatus*, this is was generally the case (weeks 3-6, 9-10, 13-16), suggesting that the current model's predictive power based on landcover alone was highest during the periods when LAC virus transmission risk was also high. Rates for false negatives were lowest during periods of peak abundance for both species, while rates for false positives were highest during these periods. False positive and false negative rates are measures of the proportion of actual low or high abundance sites, respectively, that

were not predicted as such. For prediction of disease vector abundance, false negative rates must be minimized because false negatives result in neglect of areas with high disease risk by public health and vector control personnel. Decisions based on false positive predictions may result in wasted resources but not in increased risk of human disease.

The moderate levels of accuracy achieved with the current model indicated the need for inclusion of other ecologically relevant factors in the model. Elevation data were obtained for the study area, but their relationship to mosquito oviposition during the study period could not be shown. This agrees with results of a study conducted in woodlands of eastern Kentucky, which found no significant correlation between elevation and number of *Oc. triseriatus* eggs in ovitraps (Ballard et al. 1987). Relationships between rainfall and mosquito oviposition were also assessed for varying lag periods (0-6 weeks) to account for the generation time from egg hatching to oviposition by female mosquitoes, but no clear correlations were found. Rainfall data were obtained from weather stations throughout Wise County and surrounding areas for our study, but a rain gauge placed at each ovitrapping site would allow better evaluation of the relationship between rainfall and oviposition activity. Also, rainfall was relatively frequent during the collection period, so it might be an important variable during years when it is more sporadic and serves to limit mosquito production.

Improvements could be made in sampling design as well. We sampled individual landcover classes unequally, attempting to obtain a cross-section of the landscape in the county representative of the relative prevalence of each landcover type (Table 3.4). This creates some bias toward more prevalent habitats in the conditional probability calculations, since intensely sampled landcover types will tend to have higher conditional probabilities than those that received less sampling attention. However, the bias occurs for probabilities for both high and low mosquito abundance, which negate each other. The best estimates of probabilities would be obtained by sampling in each habitat type at an intensity that is representative of the actual prevalence of the landcover class. In this manner, any inherent effects on probability would not be a result of sampling bias because sampling patterns would represent actual landcover patterns. Because the landcover classification was not completed prior to initiation of sampling in this study, our knowledge of the study area guided our decisions regarding sampling intensity, and our sampling effort was not exactly representative of relative prevalence of the landcover classes but was close (Table 3.4). Equal sampling in all landcover classes was considered before

ovitrapping, but in the Bayesian model, probability estimates would be biased toward over-represented landcover classes with low actual prevalence in the county. Also, equally intensive sampling in less common landcover classes such as barren land would be difficult or impossible based on availability of suitable ovitrapping sites, and would require that traps be more densely arranged within these habitat types. Rotating ovitrap sites were used in this study to increase the number of locations sampled while keeping the number of sites visited per week at a feasible number. Stationary ovitrap sites sampled weekly throughout the season with multiple ovitraps per site to reduce variation due to trap placement selection would provide improved estimates of vector abundance, and when personnel and resources are limited, very intensive sampling during a period of the season when mosquito populations are highest would be appropriate.

Because oviposition trapping was the sole method for assessing mosquito abundance in this study, uses of habitat types by the mosquitoes for other purposes such as host-seeking or resting cannot be directly evaluated. Horsfall (1955) noted that females of *Oc. triseriatus* and *Ae. albopictus* remain close to their larval sites, and since the flight range of these mosquitoes is low (Bonnet and Worcester 1946, Mather and DeFoliart 1984, Hawley 1988, Niebylski and Craig 1994), the other activities of the adult mosquitoes must take place within a short distance of sites used for oviposition, probably in the same or adjacent habitats. Further studies incorporating adult collections in the various habitat types along with ovitrapping would provide insight into differential habitat usage patterns, but adult trapping of these two species is difficult, particularly for *Oc. triseriatus* (Moore et al. 1993).

Predictive models using mosquito abundance as an indicator of disease risk sometimes require timely predictions of periods of high vector abundance before they occur, so that public disease awareness campaigns and vector control measures can be initiated in sufficient time to minimize disease risk. However, an overall map of disease risk is preferable to short-term forecasting of vector abundance in Wise County because: 1) landcover distribution within Wise County is not subject to dramatic interannual variation, 2) the treehole and container-breeding mosquito species in Wise County are not capable of the explosive population growth of species that rely on floodwater and vernal pools for immature development, 3) control of container-breeding mosquitoes important in the LAC virus cycle entirely consists of breeding source reduction through public awareness campaigns, and 4) LAC virus is endemic in this area, so public awareness of disease risk must be constantly maintained. The current Bayesian model

could be used for creating an overall risk map for the county based on mosquito abundance associated with habitat type. Additional studies, such as vertebrate serosurveys and mosquito virus testing, would provide better understanding of LAC virus distribution within the county, which would improve assessment of disease risk associated with predicted mosquito distributions.

In conclusion, the present study demonstrates that remote sensing and statistical modeling can be used to predict the abundance of *Oc. triseriatus* and *Ae. albopictus* based on landcover type and ovitrapping survey data. Additional relevant environmental factors would improve the model's predictive capabilities, and these predictions can improve and focus control strategies or increase awareness of disease risk in communities where mosquito populations are high.

References Cited

- Anderson, J. R., E. E. Hardy, J. T. Roach, and R. E. Witmer. 1976. *A Land Use and Land Cover Classification for Use with Remote Sensor Data*. U. S. Geological Survey Professional Paper 964. Washington, DC: U. S. Government Printing Office, 28 pp.
- Aspinall, R. J. 1991. Use of an inductive modeling procedure based on Bayes Theorem for analysis of pattern in spatial data, pp. 325-339. In D.G. Farmer and M.J. Rycroft [eds.], *Computer Modeling in the Environmental Sciences*, Institute of Mathematics and its Applications Conference Series No. 28. Oxford University Press, Oxford.
- Aspinall, R. and N. Veitch. 1993. Habitat mapping from satellite imagery and wildlife survey data using a Bayesian modeling procedure in a GIS. *Photogrammetric Engineering and Remote Sensing*. 59(4): 537-543.
- Ballard, E. M., J. H. Waller, and F. W. Knapp. 1987. Occurrence and ovitrap site preference of tree hole mosquitoes: *Aedes triseriatus* and *Aedes hendersoni* in eastern Kentucky. *J. Am. Mosq. Control Assoc.* 3(1): 42-44.
- Baylis, M., H. Bouayoune, J. Touti, and H. El Hasnaoui. 1998. Use of climatic data and satellite imagery to model the abundance of *Culicoides imicola*, the vector of African horse sickness virus, in Morocco. *Medical and Veterinary Entomology*. 12(3): 255-266.
- Beck, L. R., M. H. Rodriguez, S. W. Dister, A. D. Rodriguez, E. Rejmankova, A. Ulloa, R. A. Meza, D. R. Roberts, J. F. Paris, M. A. Spanner, R. K. Washino, C. Hacker, and L. J. Legters. 1994. Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. *Am. J. Trop. Med. Hyg.* 51(3): 271-280.
- Bonnet, D. D. and D. J. Worcester. 1946. The dispersal of *Aedes albopictus* in the territory of Hawaii. *Am. J. Trop. Med.* 26(4): 465-476.
- Moore, C. G., R. G. McLean, C. J. Mitchell, R. S. Nasci, T. F. Tsai, C. H. Calisher, A. A. Marfin, P. S. Moore, and D. J. Gubler. 1993. Guidelines for arbovirus surveillance programs in the United States. CDC, Fort Collins, CO.
- Chun, R. W. M. 1983. Clinical aspects of La Crosse encephalitis: Neurological and psychological sequelae, pp. 193-201. In C. H. Calisher and W. H. Thompson [eds.], *California serogroup viruses*. Liss, New York.
- Cross, E. R., W. W. Newcomb, and C. J. Tucker. 1996. Use of weather data and remote sensing to predict the geographic and seasonal distribution of *Phlebotomus papatasi* in southwest Asia. *Am. J. Trop. Med. Hyg.* 54(5): 530-536.
- Cully, J. F., Jr., T. G. Streit, and P. B. Heard. 1992. Transmission of La Crosse virus by four strains of *Aedes albopictus* to and from the eastern chipmunk (*Tamias striatus*). *J. Am. Mosq. Control Assoc.* 8(3): 237-240.

- Fielding, A. H. and J. F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*. 24(1): 38-49.
- Gauld, L. W., T. M. Yuill, R. P. Hanson, and S. K. Sinha. 1975. Isolation of La Crosse virus (California encephalitis group) from the chipmunk (*Tamias striatus*), an amplifier host. *Am. J. Trop. Med. Hyg.* 24(6): 999-1005.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. The first isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases* (in press).
- Grimstad, P. R., J. F. Kobayashi, M. Zhang, and G. B. Craig, Jr. 1989. Recently introduced *Aedes albopictus* in the United States: Potential vector of La Crosse virus (Bunyaviridae: California Serogroup). *J. Am. Mosq. Control Assoc.* 5(3): 422-427.
- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: Probable Introduction in Used Tires from Northern Asia. *Science*. 236: 1114-1116.
- Hawley, W. A. 1988. The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 4(Suppl.1): 1-40.
- Hedberg, C. W., J. W. Washburn, and R. D. Sjogren. 1985. The association of artificial containers and La Crosse encephalitis cases in Minnesota, 1979. *J. Am. Mosq. Control Assoc.* 1(1): 89-90.
- Hepinstall, J. A. and S. A. Sader. 1997. Using Bayesian statistics, thematic mapper satellite imagery, and breeding bird survey data to model bird species probability of occurrence in Maine. *Photogrammetric Engineering and Remote Sensing*. 63(10): 1231-1237.
- Horsfall, W. R. 1955. Mosquitoes, Their bionomics and relation to disease. Hafner Publishing Co., New York. 723 pp.
- Johnson, T. G. 1992. Forest Statistics for Virginia, 1992. USDA Forest Service, Southeastern Forest Experiment Station Resource Bulletin SE-131.
- Ksiazek, T. G. and T. M. Yuill. 1977. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciurus carolinensis*) and chipmunks (*Tamias striatus*). *Am. J. Trop. Med. Hyg.* 26(4): 815-821.
- Landis, J. R. and G. C. Koch. 1977. The measurement of observer agreement for categorical data. *Biometrics*. 33: 159-174.

- Linley, J. R. 1989a. Scanning electron microscopy of the egg of *Aedes (Protomacleaya) triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 26(5): 474-478.
- Linley, J. R. 1989b. Comparative fine structure of the eggs of *Aedes albopictus*, *Ae. aegypti*, and *Ae. bahamensis* (Diptera: Culicidae). *J. Med. Entomol.* 26(6): 510-521.
- Mather, T. N. and G. R. DeFoliart. 1984. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. *J. Med. Entomol.* 21(4): 384-391.
- McJunkin, J. E., R. R. Khan, and T. F. Tsai. 1998. Emerging infectious diseases: California-La Crosse encephalitis. *Infect. Dis. Clin. North Am.* 12(1): 83-93.
- MicroImages, Inc. 2001. TNTmips: the map and image processing system. Version 6.5. MicroImages, Inc., Lincoln, NE.
- Mogi, M. 1982. Variation in oviposition, hatch rate and setal morphology in laboratory strains of *Aedes albopictus*. *Mosquito News.* 42(2): 196-201.
- Moncayo, A. C., J. D. Edman, and J. T. Finn. 2000. Application of geographic information technology in determining risk of eastern equine encephalomyelitis virus transmission. *J. Am. Mosq. Control Assoc.* 16(1): 28-35.
- Moulton, D. W. and W. H. Thompson. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin: Some ecological considerations. *Am. J. Trop. Med. Hyg.* 20(3): 474-482.
- Nasci, R. S., C. G. Moore, B. J. Biggerstaff, N. A. Panella, H. Q. Liu, N. Karabatsos, B. S. Davis, and E. S. Brannon. 2000. La Crosse encephalitis virus habitat associations in Nicholas County, West Virginia. *J. Med. Entomol.* 37(4): 559-570.
- Niebylski, M. L. and G. B. Craig, Jr. 1994. Dispersal and survival of *Aedes albopictus* at a scrap tire yard in Missouri. *J. Am. Mosq. Control Assoc.* 10(3): 339-343.
- Roberts, D. R., J. F. Paris, S. Manguin, R. E. Harbach, R. Woodruff, E. Rejmankova, J. Polanco, B. Wullschleger, and L. J. Legters. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. *Am. J. Trop. Med. Hyg.* 54(3): 304-308.
- Rogers, D. J. and S. E. Randolph. 1991. Mortality rates and population density of tsetse flies correlated with satellite imagery. *Nature.* 351: 739-741.
- Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J. Am. Mosq. Control Assoc.* 2(2): 217-219.
- Steinly, B. A., R. J. Novak, and D. W. Webb. 1991. A new method for monitoring mosquito oviposition in artificial and natural containers. *J. Am. Mosq. Control Assoc.* 7(4): 649-650.

- Sudia, W. D., V. F. Newhouse, C. H. Calisher, and R. W. Chamberlain. 1971. California Group arboviruses: Isolations from mosquitoes in North America. *Mosquito News*. 31(4): 576-600.
- Tesh, R. B. and D. J. Gubler. 1975. Laboratory studies of transovarial transmission of La Crosse and other arboviruses by *Aedes albopictus* and *Culex fatigans*. *Am. J. Trop. Med. Hyg.* 24(5): 876-880.
- Thompson, W. H. and B. J. Beaty. 1977. Venereal transmission of La Crosse (California encephalitis) arbovirus in *Aedes triseriatus* mosquitoes. *Science*. 196: 530-531.
- Tucker, K., S. P. Rushton, R. A. Sanderson, E. B. Martin, and J. Blaiklock. 1997. Modelling bird distributions – a combined GIS and Bayesian rule-based approach. *Landscape Ecology*. 12(2): 77-93.
- Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill, and W. H. Thompson. 1973. Transovarial transmission of La Crosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. *Science*. 182: 1140-1141.
- Wood, B. L., L. R. Beck, R. K. Washino, K. A. Hibbard, and J. S. Salute. 1992. Estimating high mosquito-producing rice fields using spectral and spatial data. *Int. J. Remote Sensing*. 13(15): 2813-2826.

Chapter 4

Summary

La Crosse (LAC) virus is the leading cause of pediatric arboviral encephalitis in the United States in most years. During the acute phase of illness, it causes febrile illness with other possible complications such as seizures, irritability, and disorientation (McJunkin et al. 1998). Possibly the most serious consequences of LAC virus infection are sequelae, which may include behavioral problems and recurrence of seizures for months or years after initial illness (Chun 1983). Virginia has had a recent increase in number of LAC encephalitis (LACE) cases, as 13 cases of LACE encephalitis have been reported from 4 counties in southwestern Virginia since 1994. During the 19-year period prior to 1994, only 1 case of LACE was reported from the entire state. Following this recent increase in the number of cases, it has become apparent that there is a need for better understanding of the LAC virus cycle in southwestern Virginia and the biology of its vectors. The purposes of this study were: 1) to determine the distribution and seasonal abundance patterns for *Ochlerotatus triseriatus*, the primary vector of LAC virus, and *Aedes albopictus*, a potential accessory vector that has been recently introduced in southwestern Virginia, and 2) to use remote sensing and Bayesian modeling procedures to develop maps showing predicted abundance for *Oc. triseriatus* and *Ae. albopictus* based on habitat preferences.

Ovitraping in Wise, Scott, and Lee Counties during 1998 showed that *Oc. triseriatus* and *Ae. albopictus* were present at all collection sites surveyed. Relative abundance of the two species differed among the sites, indicating varying LACE risk among areas in these counties. In Wise County, relative abundance of *Ae. albopictus* was low in densely forested sites, intermediate in forest edges, and highest in open or residential areas. *Oc. triseriatus* outnumbered *Ae. albopictus* in all sites, but relative abundance was lowest in open and residential areas. Overall species composition for *Oc. triseriatus* and *Ae. albopictus* in the three counties was similar to that found from ovitraping surveys in other LAC virus-endemic areas in the southern Appalachian Mountains (Szumlas et al. 1996, Jones et al. 1999).

Numbers of *Oc. triseriatus* in Wise County during 1998 increased to a peak in late June and remained high with some week-to-week variation for approximately nine weeks through late August. After this, numbers gradually declined through the final collection period in early

October. *Ae. albopictus* numbers steadily increased through June and July, reaching a peak in late August, and declining thereafter.

Comparison of oviposition in yard ovitraps versus those in forest at the Duncan Gap human case site in 1999 showed that *Oc. triseriatus* had no preference between the two habitats, while *Ae. albopictus* preferred to oviposit in the yard surrounding the house rather than in adjacent forested areas. This finding agreed with the results from the previous year and with the suggested origin of this species in the U.S. from used tires from northern Asia (Hawley et al. 1987) because Japanese strains have been reported as rare in forest habitats and common or regularly occurring in urban, suburban, and rural areas (Hawley 1988).

Virus testing of several mosquito species – mainly *Oc. triseriatus* and *Ae. albopictus* – yielded two isolates of LAC virus from collections near a human LACE case site. The first isolate was obtained from a larval collection of *Oc. triseriatus* in 1998, indicating that transovarial transmission of LAC virus is occurring in this site. The second isolate was obtained from *Oc. triseriatus* adults in the same site the following year, and barring the unlikely chance of a reintroduction of virus into the population from an outside source, this proved that the virus could persist in the local mosquito population through winter. These data, combined with 3 human LAC virus infections at or near this site during 1997, suggest that the virus was able to persist in a small focus for at least 3 consecutive seasons. However, no virus isolates were obtained from 8,785 mosquitoes tested from this site during 2000, so minimum field infection rates at this site may be low.

Direct evidence of the involvement of *Ae. albopictus* in LAC virus transmission cycles was not shown in this investigation. However, in LAC-virus endemic areas in the neighboring states of Tennessee and North Carolina, natural transovarial infections in *Ae. albopictus* recently have been discovered (Gerhardt et al. 2001), and based on the prevalence of *Ae. albopictus* in our studies and our demonstration of its ability to overwinter and become established in southwestern Virginia, it seems likely that this species may serve as an accessory vector in the LAC virus cycle there.

Results from ovitrapping studies during 1998 and 1999 indicated that *Oc. triseriatus* and *Ae. albopictus* preferred different habitats, at least for oviposition. Thus, a Bayesian model was created based on these habitat preferences as determined by an ovitrapping survey in Wise County during 2000. Ovitrap traps were placed in pre-defined landcover types to determine

oviposition patterns for *Oc. triseriatus* and *Ae. albopictus*. Analyses of variance for the ovitrapping data revealed that the two mosquito species again preferred different habitats for oviposition. Highest numbers of *Oc. triseriatus* were found in forested areas, and highest numbers of *Ae. albopictus* were collected in urban and residential areas. Probability maps were created using a landcover map derived from satellite imagery, along with prior and conditional probabilities derived from ovitrap survey data as input into the Bayesian formula. The posterior probability maps depicted species distribution as a predicted probability of high abundance for all areas of Wise County for 2-week periods during the collection season. Probabilities for high *Oc. triseriatus* abundance were highest in forested areas, while *Ae. albopictus* was most likely to be highly abundant in urban areas. This was in agreement with data from the ovitrapping survey and studies during the previous 2 years that suggested a similar pattern.

Accuracy levels for the model were moderate to very high, but the highest accuracy values were probably somewhat misleading because high accuracy levels can be obtained when the number of actual and predicted high abundance sites is large. Kappa values, which indicate improvement of predictions over random chance, ranged from poor to good. Kappa values were highest during periods of peak mosquito abundance, indicating that the predictive abilities of the model were highest during periods of greatest risk for LAC virus transmission. Rates for false negative predictions were lowest during periods of peak abundance for both species. For prediction of disease vector abundance, false negative rates must be minimized because false negatives result in neglect of areas with high disease risk by public health and vector control personnel. Decisions based on false positive predictions may result in wasted resources but not in increased risk of human disease.

Moderate accuracy measures for the current landcover-based model suggest the need for inclusion of other ecologically relevant factors, such as rainfall and temperature, to improve mosquito abundance predictions. Other possible ways to improve the model include: 1) designing a sampling scheme representative of the actual prevalence of the variable classes to reduce sampling bias, 2) collecting from stationary trap sites with multiple traps per site throughout the season to reduce variation resulting from individual trap site selection, 3) collecting adult mosquitoes to evaluate habitat usage by the mosquitoes for purposes other than oviposition, and 4) including virus and seroprevalence surveys to better assess the relationship between mosquito abundance and disease risk for different areas of the county. Following these

improvements, the model could be used to create an overall risk map for LAC virus in Wise County based on predicted vector mosquito abundance, virus activity, and environmental factors. The resulting risk map would be useful for focusing public disease awareness campaigns to educate citizens in high-risk areas about larval source reduction around homes, use of repellent, and other ways to minimize the risk for La Crosse encephalitis.

References Cited

- Chun, R. W. M. 1983. Clinical aspects of La Crosse encephalitis: Neurological and psychological sequelae, pp. 193-201. *In* C. H. Calisher and W. H. Thompson [eds.], California serogroup viruses. Liss, New York.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. The first isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases* (in press).
- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: Probable Introduction in Used Tires from Northern Asia. *Science*. 236: 1114-1116.
- Hawley, W. A. 1988. The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 4(Suppl.1): 1-40.
- Jones, T. F., A. S. Craig, R. S. Nasci, L. E. R. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly Recognized Focus of La Crosse Encephalitis in Tennessee. *Clinical Infectious Diseases*. 28: 93-97.
- McJunkin, J. E., R. R. Khan, and T. F. Tsai. 1998. Emerging Infectious Diseases: California-La Crosse encephalitis. *Infect. Dis. Clin. North Am.* 12(1): 83-93.
- Szumlas, D. E., C. S. Apperson, and E. E. Powell. 1996. Seasonal occurrence and abundance of *Aedes triseriatus* and other mosquitoes in a La Crosse virus-endemic area in western North Carolina. *J. Am. Mosq. Control Assoc.* 12(2): 184-193.

Vita

Christopher Michael Barker

Christopher M. Barker was born March 27, 1976 in Charlotte, NC. He spent the first five years of his life living near Charlotte on Lake Norman, and during this time, his preschool teacher at Davidson College predicted that he would become an entomologist because he was constantly turning over rocks to examine the insects underneath. On his fifth birthday, he and his family moved to Abingdon, Virginia, and he later attended Patrick Henry High School, where he graduated as class valedictorian in 1994. He lived in Abingdon until he moved to Blacksburg, Virginia to begin undergraduate studies at Virginia Tech in the fall of 1994. He enjoyed his four undergraduate years at Virginia Tech, taking full advantage of the academic and social opportunities that the school had to offer. He particularly enjoyed his experiences in the GERMAN Club, Golden Key National Honor Society, and Mortar Board National Senior Honor Society. During his sophomore year, his interest in fly fishing led him to take a course in aquatic entomology to better understand the insects on which trout feed. This was a wonderful experience, and he continued to take additional courses in entomology. He later undertook a semester of undergraduate research with Dr. Reese Voshell using aquatic macroinvertebrates as indicators of water quality in acid-tolerant and acid-susceptible streams in Shenendoah National Park. The following year, he took a course in medical and veterinary entomology under Dr. Sally Paulson, who would become his major advisor for his M.S. work. He earned a Bachelor of Science degree in Biology in the spring of 1998 and began a graduate program in entomology at Virginia Tech the following fall. His research with Dr. Paulson examined spatial and seasonal distributions of two mosquito species, *Ochlerotatus triseriatus* and *Aedes albopictus*, in southwestern Virginia. He also incorporated remote sensing and GIS work into his research to show relationships between mosquito abundance and habitat type. During 2000, he served as President of the W.B. Alwood Entomological Society, and at the end of that year, he departed for California to begin work at the University of California at Davis's Center for Vector-Borne Disease Research. In California, he will be working on a project examining historical links among mosquito abundance, virus surveillance, and climate data to eventually allow prediction of mosquito abundance and encephalitis virus risk based on short- and long-range climate forecasts. He successfully defended his M.S. thesis at Virginia Tech in July 2001.