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Impact of oviposition trap treatments and environmental factors on seasonal distribution and relative abundance of mosquitoes (Diptera: Culicidae) in eastern Tennessee

Alycia Erin Chapman

To the Graduate Council:

I am submitting herewith a thesis written by Alycia Erin Chapman entitled "Impact of oviposition trap treatments and environmental factors on seasonal distribution and relative abundance of mosquitoes (Diptera: Culicidae) in eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Reid R. Gerhardt, Major Professor

We have read this thesis and recommend its acceptance:

Jerome Grant, Charles Pless

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dr. Reid R. Gerhardt, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Vice Provost and Dean of Graduate Studies

IMPACT OF OVIPOSITION TRAP TREATMENTS AND ENVIRONMENTAL FACTORS ON SEASONAL DISTRIBUTION AND RELATIVE ABUNDANCE OF MOSQUITOES (DIPTERA: CULICIDAE) IN EASTERN TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Alycia E. Chapman

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DEDICATION

This thesis is dedicated to my late grandfather, Franklin Benjamin Chapman, Jr., whose respect and admiration as a scholar and role model has inspired me to never surrender my passion for learning about the natural world.

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I would like to thank the Tennessee Valley Authority for their support of this research. My husband, Greg Schweiger, has provided infinite love, support and guidance, and without whom I never would have made it this far. Many thanks also go to my family for the years of encouragement and believing in all of my lofty goals. I would also like to thank Dr. Arnold Saxton for his hours of assistance and statistical support. Lastly, thanks to Drs. Reid Gerhardt, Jerome Grant and Charles Pless for serving on my committee.

ABSTRACT

Container-inhabiting mosquito oviposition activity and the influences of three oviposition treatments were examined from three Knox County, Tennessee sites May - October 2000 and March - October 2001. Species composition, seasonal distribution and relative abundance of mosquitoes were examined in a ten county region in eastern Tennessee. Due to the increase in La Crosse (LAC) encephalitis cases and the potential for West Nile (WN) virus in eastern Tennessee, there is now a greater need for information regarding the biology and ecology of the exotic species, *Aedes albopictus* (Skuse), and the native species, *Ochlerotatus* (= *Aedes*) *triseriatus* (Say).

Weekly oviposition, larval, pupal and adult collections of *Oc. triseriatus* and *Ae. albopictus* were conducted at three sites in Knox County. Oviposition activity was sampled using five sets of three cup treatments at each site: 1) replenished cups had water replenished and oviposition strips exchanged, 2) replaced cups had the cup, water and oviposition strips exchanged and 3) returned cups had water replenished and the strips were taken to the laboratory, where eggs were counted then the strips were returned to their respective cups. Host-seeking adult collections for *Oc. triseriatus* and *Ae. albopictus* were made using a CO_2- baited Centers for Disease Control (CDC) miniature light trap, with the light removed. More overall oviposition activity was detected in replenished cups (62,351) than replaced cups (40,141), while returned cups were not analyzed due to complications. *Ochlerotatus triseriatus* and *Ae. albopictus* both had more oviposition activity in replenished than replaced cups. This indicates that *Oc. triseriatus* and *Ae. albopictus* both have more oviposition activity in cups with greater

organic content and aged water that may contain larvae or pupae. Surveillance programs that need large numbers of eggs for laboratory rearing and disease testing activities should only add water as necessary to increase levels of oviposition activity by both species.

Biweekly oviposition activity and host-seeking adult collections were conducted at ten counties (Anderson, Blount, Grainger, Hamblen, Jefferson, Knox, Loudon, Roane, Sevier and Union) from May - October 1998, April - October 1999 and 2000 and March - October 2001. Adult collections of all mosquito species were made using a CO_2-baited CDC miniature light trap. Average temperature and average rainfall variables were obtained for Blount County and were analyzed for any effect on *Oc. triseriatus* and *Ae. albopictus* oviposition and *Ae. albopictus* adult activity. The average temperature during, two and four weeks prior to the sampling periods had significant ($p < 0.0001$) impacts on the oviposition activity of *Oc. triseriatus*. *Aedes albopictus* oviposition activity was significantly ($p < 0.0001$) explained by the average temperature during and two weeks prior to the sampling periods. Adult *Ae. albopictus* activity was best described ($p < 0.0001$) by the year, the average rainfall and average temperature four weeks prior to the sampling periods.

Trends for *Oc. triseriatus* peak oviposition, overall oviposition and *Ae. albopictus* adult activity were seen in both studies. *Ochlerotatus triseriatus* peak oviposition activity occurred approximately one month earlier than *Ae. albopictus*, beginning in late May and early June and tapering off in late summer. *Aedes albopictus* peak oviposition activity began in late June and steadily continued into fall. In both studies, more oviposition activity was seen by *Oc. triseriatus* than *Ae. albopictus* except for Hamblen and Sevier

Counties in 1998 and 1998 and 1999 respectively. Although more *Oc. triseriatus* eggs were collected than *Ae. albopictus*, there were a greater number of *Ae. albopictus* adults collected from all sites in the ten counties. This may be due to sampling design or differences in the survivorship of eggs and larvae in field conditions at these sites.

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CHAPTER I

INTRODUCTION

Throughout history, the most medically important group of insects has been the mosquitoes (Diptera: Culicidae). Mosquitoes transmit the disease organisms that cause malaria, dengue fever, yellow fever and encephalitis in humans (Harwood and James 1979, Kappus et al. 1983), as well as heartworms in dogs, cats and humans (Atkins et al. 2000, Boreham and Atwell 1988). Mosquitoes may be broadly classified into three groups based on their larval habitats: 1) permanent pool mosquito larvae are typically found in fresh, clear, shallow water with aquatic vegetation, 2) flood water mosquito larvae are found in areas where there are grassy depressions, low lying and shaded areas that are intermittently flooded, and 3) container-inhabiting mosquito larvae develop in natural and artificial containers with a high organic content (Breeland et al. 1961).

In the eastern United States, the interest in mosquito monitoring and research has grown tremendously due to the emergence of West Nile (WN) virus (Flaviviridae: *Flavivirus* spp.) and increasing La Crosse (LAC) virus (Bunyaviridae: *Bunyavirus* spp.) as human disease agents (Gerhardt et al. 2001). La Crosse encephalitis is the number one reported human encephalitis disease in the United States, and WN virus is now the second most reported human arboviral encephalitis (CDC 2001).

Until 1984, mosquito research in eastern Tennessee was limited to basic research on species composition and seasonal distribution. Prior to 1984, early Tennessee Valley collections of larval and adult mosquitoes yielded 36 species (Shields 1938). Thirteen mosquito species were reported for eastern Tennessee (Arnold 1940). The whole

Tennessee Valley, including Knox County, had 44 mosquito species reported (Breeland et al. 1961) and *Aedes vexans* (Meigen) comprised 45% of all adult species collected in Knox County (Ogg 1975). Hribar (1984) found that *Ochlerotatus* (= *Aedes*) *trivittatus* (Coquillett) is a potential vector of the dog heartworm and reported 32 mosquito species for Knox County (Hribar and Gerhardt 1986). *Aedes albopictus* (Skuse), the Asian tiger mosquito, was found in every county throughout Tennessee (Moore 1998). More recent research in eastern Tennessee has focused on disease surveillance and the importance of mosquitoes as vectors. Jamestown Canyon (JC) virus and LAC virus were isolated from adults reared from field-collected *Ae. albopictus* eggs (Gerhardt et al. 2001, Gottfried et al. 2002). Eastern equine encephalitis virus and Cache Valley virus have both been isolated from naturally infected *Ae. albopictus* in the United States but not in Tennessee (Moore and Mitchell 1997).

i - Container-Inhabiting Mosquitoes

Entomological researchers, health officials, local and national governments in the eastern United States are more closely observing container-inhabiting mosquitoes. The focus on container-inhabiting mosquitoes is due to their habitat preference, placing them in close proximity to residences and areas frequently visited by humans and other animals (Hawley 1988, Comiskey et al. 1999). Container-inhabiting mosquitoes may develop in a variety of natural or artificial containers. Many mosquito species and other aquatic invertebrates develop in phytotelmata, which include plant leaf axils, pitcher plant leaves, bamboo internodes and tree holes (Fish 1983). Phytotelmata may be further divided based on the derivation of the type of liquids present which may originate from plant secretions and rainfall (Kitching 1971, 2000). Artificial containers include tires, rainbarrels, gutters and bird baths, as well as other containers that can hold water.

Both natural and artificial containers have complex interactions effecting the survivorship and success of the developing mosquitoes, including countless other organisms (Kitching 1983, Kitching 2000, Paradise and Dunson 1998). The success of container-inhabiting mosquitoes is influenced by many environmental factors, including physicochemical water characteristics derived from the wood and complex food webs (Jenkins and Carpenter 1946). The temperature, atmospheric deposition (Paradise and Dunson 1998), organic material and the presence or absence of predators (Bradshaw and Holzapfel 1983). Container water and ambient air temperature have a significant impact on development time and habitat stability due to seasonal dehydration in temperate areas (Alto and Juliano 2001a, Sota et al. 1994). *Ochlerotatus triseriatus* (Say), the eastern tree hole mosquito, is more sensitive than *Ae. albopictus* to changes in water temperature (Teng 1993). Adult body size is influenced by the amount of food resources available to larvae (Nasci 1988). The type of water, color of the container and the organic content of the water influence the oviposition that takes place in the container or treehole (Trexler et al. 1998, Yap et al. 1995). Rainfall results in fluctuations of the amount and type of microorganisms present as food for the larval mosquitoes and chemistry of the water also effects the container (Kaufman et al. 1999, Paradise and Dunson 1998).

In eastern Tennessee, the two most important container-inhabiting mosquitoes are *Oc. triseriatus*, due to its implication as the primary vector of LAC virus, and *Ae. albopictus*, which has been collected at approximately 60 sites examined in 13 counties from 1997-1999 (Gerhardt et al. 2001) and receives the most nuisance complaints to officials (Gerhardt pers. comm.). Learning more about the interactions in the tree hole will increase our knowledge of the responses of these two mosquitoes to environmental stressors.

ii - *Ochlerotatus triseriatus*

One of the most intensely researched arthropod vectors is *Oc. triseriatus*, which is the principal vector of LAC virus (Nasci 1988). This species has had WN virus or WN virus RNA detected using molecular tests in the laboratory (Nasci pers. comm.). Due to the vector competency for LAC virus and the possibility of WN virus implications for this mosquito, a lot of research has been conducted on the biology and ecology of larvae and adults. They favor wooded over sunny areas for both feeding and oviposition, and most feeding occurs during the day, typically on rodents (Nasci 1988, Watts et al. 1972). *Ochlerotatus triseriatus* are not strong fliers and disperse between 50-100 m while it is possible that greater distances are common (Mather and DeFoliart 1984). When feeding, it has been observed that they opt for the upper parts over the lower parts of the of the human body and in one study more than 90% of the biting occurred this way while placing the hands above the head (Loor and DeFoliart 1970).

Many biotic and abiotic environmental factors, such as rainfall and temperature (Alto and Juliano 2001b), cannibalistic behavior (Livdahl and Koenekoop 1985) and location of the container affect population dynamics (Nasci 1988). When new mosquito species, such as *Ae. albopictus*, are introduced the competition may displace their distribution (Edgerly et al. 1993). Although *Oc. triseriatus* prefer tree holes and natural containers for oviposition, they also breed in artificial containers, such as discarded tires (Nasci 1988), cups and gutters (Haramis 1984). They coexist well with mosquitoes of different species, surviving competition among their own and other species (Schreiber et al. 1988). However, it has been suggested that some competition and replacement from *Ae. albopictus* in laboratory experiments occurs (Livdahl and Willey 1991) but both *Oc. triseriatus* and *Ae. albopictus* undergo resource partitioning and that low niche overlap may allow for the two species to coexist (Schreiber et al. 1988).

iii - *Aedes albopictus*

Aedes albopictus is a laboratory competent and suspected field vector of LAC virus (Hawley 1988) and was recently discovered to be transovarially infected with LAC virus (Gerhardt et al. 2001). This species has been found, in the laboratory, to contain WN virus or WN virus RNA (Nasci pers. comm.). Although *Ae. albopictus* was occasionally found in the United States prior to 1986, it was then first described by Sprenger and Wuithiranyagool (1986) as being established in Harris County, Texas. Since 1986, *Ae. albopictus* has established itself in large regions of the south central and southeastern United States (Moore and Mitchell 1997). *Aedes albopictus* has been recorded in 26 of the continental United States and every county in Tennessee (Moore 1999). This species is a possible vector of yellow fever and dengue fever (Estrada-Franco and Craig 1995), and a known vector of eastern equine encephalitis (Moore and Mitchell 1997). Adults do not adapt as well to adverse conditions such as cooler temperatures and shorter day length as *Oc. triseriatus* (Hanson and Craig 1995) and have a flight range less than 500 m (Estrada-Franco and Craig 1995). Also, *Ae. albopictus* is a more opportunistic feeder than *Oc. triseriatus* and will feed on many hosts, including rabbits, rats, dogs, humans, deer, birds and turtles plus a variety of other hosts (Nieblyski et al. 1994). With the recent introduction and spread of WN virus, combined with its known vector potential and many unknown factors, there is great concern about the role of this mosquito and its potential as a vector of this disease organism in new areas.

Although *Ae. albopictus* has not displaced *Ae. triseriatus* in Florida (Lounibos et al. 1997), *Ae. albopictus* has displaced *Ae. aegypti* (L.) in some Florida habitats (O'Meara et al. 1995) and in Mobile, Alabama (Hobbs et al. 1991) and this species is currently displacing *Ae. aegypti* in South Carolina (Richardson et al. 1995). Research has suggested that *Ae. albopictus* will spread further northward in the United States due to climate shifts, such as warmer summer temperatures in the northern United States. Thus, it is important to further study the ecology of *Ae. albopictus* and other container-inhabiting species (Alto and Juliano 2001b).

iv - Sampling Container-Inhabiting Mosquitoes

Over time, the monitoring methods for different mosquitoes have changed many times striving for more economical, efficient and accurate measurement tools for the surveillance of mosquitoes. Since surveillance for mosquitoes began, sampling has included larval surveys, egg and adult collecting. Sampling for container-inhabiting mosquitoes has changed to incur more economical and less labor-intensive methods for both oviposition monitoring and adult collecting (Hanson et al. 1988). More efficient sampling methods also make a surveillance program more thorough. Oviposition sampling is the preferred sampling method because it is as sensitive as larval surveys (Furlow and

Young 1970). The host-seeking female trap of choice is the CDC light trap baited with CO_2 (Landry and DeFoliart 1986). No single sampling method is best and most often several methods are used in combination to get the optimum results (Gottfried 1999). One of the first uses of oviposition traps was in Wisconsin where *Oc. triseriatus* were naturally infected with LAC virus. The trap consisted of beer cans with the tops removed and the inside lined with black muslin (Loor and DeFoliart 1969). Red velvet and wooden tongue depressors have also been used as an oviposition substrate in black cups (Craig 1983).

Oviposition activity is effected by the presence of eggs, larvae and pupae from other mosquitoes, as well as the degree of organic material in the container. Mosquitoes lay more eggs in more places where no other mosquito eggs have previously been laid (Allan and Kline 1998, Kitron et al. 1989). Fish oil, hay and grass leaf infusions were found to enhance the water to gravid mosquitoes due to bacteria growing on the decaying matter present in the water (Holck et al. 1988). Hay, grass and oak leaf infusion studies have indicated the significance of water chemistry in oviposition behavior and larval development (Trexler et al. 1998). Although oviposition monitoring is the most common method of sampling, it is more qualitative than quantitative (Berry et al. 1980) since the artificial containers often compete with the natural containers and generate an increase in oviposition in artificial containers when fewer tree holes exist (Landry and DeFoliart 1986, Lang 1990).

Collections of mosquito larvae and pupae, including mosquitoes other than container-inhabiting ones, may be sampled either by dipping water or using an apparatus designed specifically for larval collections (Livdahl and Willey 1991, Undeen and Becnel 1994). Larval collections are not efficient and may cost up to one and one-half times the amount of money as oviposition surveys (Lang 1990). Oviposition sampling is as efficient as larval collections in detecting *Oc. triseriatus* (Furlow and Young 1970).

In the past, human biting counts were frequently utilized for host-seeking adult mosquitoes, but many environmental variables affect the collection (Landry and DeFoliart 1986) as well as ethical concerns surrounding human subjects. Another method is the sampling of resting adult mosquitoes by aspiration, which is also time consuming and labor intensive, especially for long term surveillance (Landry and DeFoliart 1986). Collecting container-inhabiting mosquitoes is also conducted with labor intensive and expensive adult traps, such as Fay traps, gravid traps and CO_2-baited CDC miniature light traps (Landry and DeFoliart 1986). These are often used in addition to oviposition trap sampling.

v - Sampling Other Mosquitoes

Sampling other specific mosquitoes is important for several reasons. Regularly sampling is a good way to establish baseline data for regional mosquito fauna. This is accomplished by using light traps, typically placing them in several locations initially. Baseline data provide information to state, regional and local governments about pest species and may aid in determining the source of breeding. Population trends can be noted when collections are conducted over an extended period of time. This information may also be used to predict the possibility of any arboviral activity or the need for control measures where relative abundance or complaints to regional and local authorities increases. If predictions can be made, authorities and health care workers will know to watch for certain diseases.

vi - Research Objectives

Historically, the focus for the study of mosquitoes in eastern Tennessee has primarily been their species composition and medical and veterinary importance rather than the mosquitoes' relationships with the abiotic and biotic factors of different microhabitats. The main purpose of this research is to continue a previous study on the regional mosquito fauna in eastern Tennessee. The relationship between the oviposition activity of container-inhabiting mosquitoes and three sampling treatments were evaluated to determine the differences between methods in eastern Tennessee. All of this information may be valuable to mosquito abatement programs and other groups that participate in mosquito monitoring and research.

CHAPTER II

OVIPOSITION SAMPLING OF CONTAINER-INHABITING MOSQUITOES IN EASTERN TENNESSEE - WATER TYPE AND ORGANIC DEBRIS' EFFECTS ON OVIPOSITION AND LARVAL PRODUCTION

i - Abstract

Weekly sampling of container-inhabiting mosquitoes was conducted from 2 May - 26 October 2000 and from 14 March - 3 October, 2001, at three similar sites in Knox County that were close in proximity to the University of Tennessee. Oviposition activity of *Ochlerotatus* (= *Aedes*) *triseriatus* (Say), the eastern tree hole mosquito and *Aedes albopictus* (Skuse), the Asian tiger mosquito, were sampled using five sets of three oviposition cup treatments at each site. Replenished cups had water added as necessary and oviposition strips changed weekly. Replaced cups had water and oviposition strips exchanged weekly. Returned cups had water added as necessary and oviposition strips returned to their respective cups after any eggs had been identified and counted. Third and fourth instar larvae and pupae were collected from one of the five cups in 2000 and from two of the five cups in 2001, then reared in the laboratory. Host-seeking adult collections were made using Centers for Disease Control (CDC) miniature light traps baited with CO_2 with the light removed. In 2000 and 2001, 102,527 mosquito eggs were collected from replenished cups (62,351) and replaced cups (40,141). The higher amount of oviposition activity by both species in replenished cups suggests that there is a preference by gravid *Oc. triseriatus* and *Ae. albopictus* to oviposit in cups with a higher organic content and the presence of larvae. *Ochlerotatus triseriatus* (56,847) had larger numbers of eggs collected than *Ae. albopictus* (45,644). Greater numbers of *Ae.*

albopictus adults (800) than *Oc. triseriatus* adults (125) emerged and no apparent difference for cup treatments and numbers of emerged adults was present. The majority of host-seeking adults collected were *Ae. albopictus* (454), while *Oc. triseriatus* comprised only 22.6% (149) of the total collection. This research suggests that disease surveillance programs should only add water to ovitraps to increase the amount of oviposition activity, resulting in greater numbers of eggs that can be reared and analyzed for disease organisms.

ii - Introduction

Sampling is an essential aspect of mosquito control and disease surveillance programs for container-inhabiting mosquitoes (Yap et al. 1995). *Ochlerotatus triseriatus* is readily attracted to artificial oviposition containers but is less attracted to light traps (Furlow and Young 1970) because it is a diurnal mosquito species (Hanson et al. 1988). The use of attractant-enhanced host-seeking traps aid in more accurate monitoring (Landry and DeFoliart 1986). *Aedes albopictus* is also attracted to artificial containers and was found to oviposit more on paper oviposition strips in black cups than bamboo stumps, leaf axils, tires, coconut shells and other artificial containers (Yap et al. 1995), and host-seeking adults are less likely to be attracted to light traps than many other species (Hawley 1988). *Aedes albopictus* is a diurnal mosquito species like *Oc. triseriatus*, displaying bimodal daily biting patterns, one period of biting activity in the morning and one period of biting activity in the evening (Estrada-Franco and Craig 1995).

Oviposition traps are a simple, cheap and effective method of sampling for container-inhabiting mosquitoes (Loor and DeFoliart 1969, Steinly et al. 1991) and are considered the standard for container-inhabiting mosquitoes (Lang 1990). Different collectors may have slight variations in the way they set oviposition traps and service them thereafter. The standard method is to empty the containers and replace all the water when the oviposition strips are changed (Loor and DeFoliart 1969) while others may replenish or augment the water as needed (Gottfried et al. 2001, Hanson et al. 1988).

Ochlerotatus triseriatus preferred to oviposit in cups with organic debris and lined with black muslin sleeves to serve as oviposition substrates instead of no debris or black sleeves in the containers (Loor and DeFoliart 1969). While sampling, they changed water and oviposition sleeves weekly. As a result of these findings, research has been directed to determining the efficacy and attractiveness of the amount and type of organic debris for the collection of mosquitoes. Leaf litter is an important food source for larval tree hole mosquitoes and other container dwellers (Fish and Carpenter 1982). The stemflow provides inorganic ions that nourish microorganisms associated with leaf litter in tree hole ecosystems (Walker et al. 1991). *Ochlerotatus triseriatus* had improved growth when supplemented with plant detritus in field conditions (Lounibos et al. 1993). Oviposition activity in cups with oak leaf infusions increased compared to cups without infusions at certain concentrations (Trexler et al. 1998). *Aedes albopictus* had more oviposition activity in cups with oak leaf infusions at all concentrations (Trexler et al. 1998) and were readily attracted to sod infusion baited ovitraps (Lampman and Novak 1996).

Container-inhabiting mosquitoes rely on physical and chemical cues to find optimum oviposition sites. These factors include oviposition texture, color and optical clarity of the water, temperature, contact chemoreception, olfactory cues (Trexler et al. 1998), preexisting eggs and preexisting larvae in the water of the container (Allan and

Kline 1998). Mosquitoes, as well as other flies, have the ability to detect and respond to previous oviposition activity (Kitron et al. 1989). Gravid *Ae. albopictus* had more oviposition activity when using larval rearing water from a colony and field water taken from a natural container with a known *Ae. albopictus* population (Allan and Kline 1995). Sod infusions (Lampman and Novak 1996) were more attractive than hay infusions (Allan and Kline 1995). Gravid *Ae. albopictus* did not deposit more eggs when either *Ae. albopictus* or *Aedes aegypti* (L.) eggs were present but had an increase in oviposition when larval rearing water of these two species was present in oviposition cups (Allan and Kline 1998). Fewer eggs were laid by *Oc. triseriatus* when an oviposition strip already had *Oc. triseriatus* eggs present and showed a decrease in oviposition (Kitron et al. 1989).

The color of the container influences the amount of oviposition activity by *Ae. albopictus* and they prefer black, red and blue containers (Yap et al. 1995). The effect of semiochemicals from oviposition sites derived from bacterial origins mediate oviposition behavior (Takken 1999), and *Ae. albopictus* is attracted to 3-methylindole, a compound attractive to various *Culex* species (Allan and Kline 1995). Bacteria and heterotrophs in the diet of mosquito larvae increase developmental time in *Anopheles quadrimaculatus* Say when the bacterial community is depressed, while the broad diversity of bacterial food resources associated with their larval habitats may also influence the digestibility (Smith et al. 1998). The primary food source for larval *Oc. triseriatus* is bacteria, but many other organisms, including protozoans, fungi and other microeukaryotes have been found from larval gut analysis (Kaufman et al. 1999). *Ochlerotatus triseriatus* also actively feed on decaying leaves and filter particles from the water column (Merritt et al. 1992). Larvae feed on the microbes on the decaying leaf surfaces and larval growth and survivorship are the greatest when using maple leaves as the decaying material and slower larval development and lower survivorships when using oak leaves (Fish and Carpenter 1982). White oak leaf infusions are attractive to gravid *Ae. albopictus* and less attractive to gravid *Oc. triseriatus* (Trexler et al. 1998) while *Ae. albopictus* prefer aged oak leaf and sod infusions (Lampman and Novak 1996). Chemical analysis of tree hole water showed that *Oc. triseriatus* were associated with water that had high potassium, low sodium, chloride and water hardness levels while *Orthopodomyia signifera* (Coquillett) and *An. barberi* Coquillett were associated with lower water measurements (Vrtiska and Pappas 1984).

The two mosquito species studied in this research are *Oc. triseriatus* and *Ae. albopictus*. They are important due to the rise in emerging infectious diseases such as La Crosse encephalitis and the threat of West Nile virus in eastern Tennessee. More information is needed on the biology of these species and their response to different sampling methods. One approach is to examine the sampling methods that a typical mosquito worker may use to collect eggs and how that might affect suspected patterns in relative abundance. This research was designed to determine the difference in ovipostition and numbers of adults reared of *Ae. albopictus* and *Oc. triseriatus*: 1) when the ovitrap water, oviposition strip and ovitrap were changed each week, 2) when ovitrap water was replenished as needed and oviposition strip was replaced each week and 3) when the ovitrap water was replenished each time and the oviposition strip was returned and reused each week.

iii - Materials and Methods

Collection Sites: Mosquito egg and adult collections were made for both *Oc. triseriatus* and *Ae. albopictus* in Knox County, Tennessee at three different sites. Collections were made from the Hackberry Site (HACK) (2 May -26 October 2000) and (14 March - 3 October 2001), Knoxville Experiment Station Plant Science Farm (PSF) (6 June - 26 October 2000) and (14 March - 3 October 2001) and Island Home Airport (IH) (8 May - 26 October 2000) and (14 March - 3 October 2001). Sites were chosen based on proximity to the Department of Entomology and Plant Pathology, the known presence of both species of mosquitoes and similarity of sites to each other. Trash, including tires, cups, beer cans and woody debris were present at all three sites.

Hackberry Site: This collection site was located on Fletcher Luck Lane directly between the greenhouses and the bicycle trail on the University of Tennessee Agriculture campus (N35° 56', W83° 56') in Knoxville, Tennessee. The primary overstory species present at this site were hackberry (*Celtis occidentalis* L.), red mulberry (*Morus rubrus* L.), white pine (*Pinus* spp. L.) and black cherry (*Prunus serotina* Ehrh.). The understory species included honeysuckle (Family Caprifoliaceae), privet (*Ligustrum* spp.), box elder (*Acer negundo* L.) and red mulberry. Various containers, building supplies and plant debris were discarded off the side of the road and behind various small buildings located at this site.

Plant Science Farm Site: This collection site was an oak-hickory forest adjacent to the Tennessee River located approximately 6 km from the University of Tennessee Agriculture campus at the Knoxville Experiment Station Plant Science Farm (N35° 53', W83° 57') in Knoxville, Tennessee. The primary overstory species were white oak (*Quercus alba* L.), chestnut oak (*Quercus prinus* L.), northern red oak (*Quercus rubra* L.), southern red oak (*Quercus falcata* Michx.), winged elm (*Ulmas alata* Michx.) and bitternut hickory (*Carya cordiformis* (Wangenh.) K. Koch). The understory species were Carolina buckthorn (*Ramnus carolina* Walt.), eastern redbud (*Cercis canadensis* L.), white ash (*Fraxinus americana* L.), eastern redcedar (*Juniperus virginiana* L.) and greenbriar (*Smilax* spp.). This site had little trash present, and the general area is well maintained by University of Tennessee employees.

Island Home Site: This collection site is near a residential area located adjacent to the Tennessee River (N35° 57', W83° 52') approximately 6.8 km from the University of Tennesse Agriculture campus across from the runway to Island Home Airport in Knoxville, Tennessee. The location, driving directions, overstory and understory species are given in Gottfried (1999). This site is the most urban and trash littered of the three sites. This site is within a residential neighborhood and also borders a school. It is evident that trash is often discarded consisting of glass bottles, organic material and a variety of household waste.

Oviposition activity: Initially, five sets of three types of oviposition cups were placed at each site and were serviced weekly. The three types of cups were: 1) replenished cups numbered and painted in yellow which indicated a cup that had the oviposition strip collected and replaced weekly with a new strip and water replenished, 2) replaced cups numbered and painted in white had the cups, oviposition strips and water replaced and 3) returned cups were not labeled and the strips were dried in the lab, the eggs were counted and the strips were returned to their respective cups within 24 hrs and water was replenished.

Each trap consisted of a 16 oz (473 ml) black plastic cup attached approximately 0.5 m from the base of the north side of trees or a stationary object (Loor and DeFoliart 1969, Steinley et al. 1991). Oviposition activity was monitored using 76 lb seed germination paper (Anchor Paper Company, Saint Paul, Minnesota) cut into 5 cm x 25.5 cm strips. Each strip was held by a single paper clip on the side of the cup with approximately 400 ml of water inside the cup. The water added was distilled and aged at least 24 hours. Each cup had holes punched 2 cm from the rim on opposite sides of one another to hang the cup and to provide drainage holes.

When the water was added as necessary in the replenished and the returned cups, the water level was either below the hole punch mark on the side of the cup or the cup had been disturbed, such as laying on the ground or tipped over. Replenished cups and returned cups were replaced only when they had holes chewed in the bottom or were otherwise unusable. Strips from all three cup types were dried (Pratt and Kidwell 1969) and any eggs were identified, counted and recorded.

Larval and pupal collections: Weekly field collections of third and fourth instar larvae and pupae were taken from the replenished and the returned cups at all three sites during 2000 and 2001. Collections of larvae were based on site identification and an estimation of their instar. In 2000, one cup from each of the three sites and two cups from each site were sampled in 2001. Cups were randomly chosen and placed in order before each collection season to prevent any set of cups from being sampled two weeks in a row.

Ovitraps were processed in the field to replace the existing water in the replenished and returned cups and to return any early instar mosquito larvae not large enough to rear in the laboratory. The ovitrap contents were emptied into a 2.25 l, clear, rectangular Ziploc® container. The third and fourth instar larvae, pupae and water were collected from the ovitrap contents using a 20 cc syringe with the tip cut off and a 20 ml disposable plastic pipette. After the larvae, pupae and water were collected, they were placed in labeled, 145 ml plastic cups with secure fitting lids and taken to the laboratory. Approximately 50 ml of ovitrap water was collected to provide a food resource to the larvae as no other food resource was added after returning to the laboratory.

Once in the laboratory, the contents in the cup were transferred to a small mosquito emergence chamber consisting of two 145 ml clear plastic cups, joined by two tight fitting lids stapled together with a 2.54 cm circular hole punched in the middle to serve as a resting ledge (Figure 2.1). The cup on the top of the chamber had small holes melted into the sides to allow the passage of air into the chamber. These were labeled and placed in an incubator at 27 ± 2 °C for seven days. Seven days was seen as an adequate amount of time since it takes an average of ten days for development from first instar to adult at 26 °C (Estrada-Franco and Craig 1995). Any *Oc. triseriatus* and *Ae. albopictus* adults that emerged were identified to species, sexed and counted.

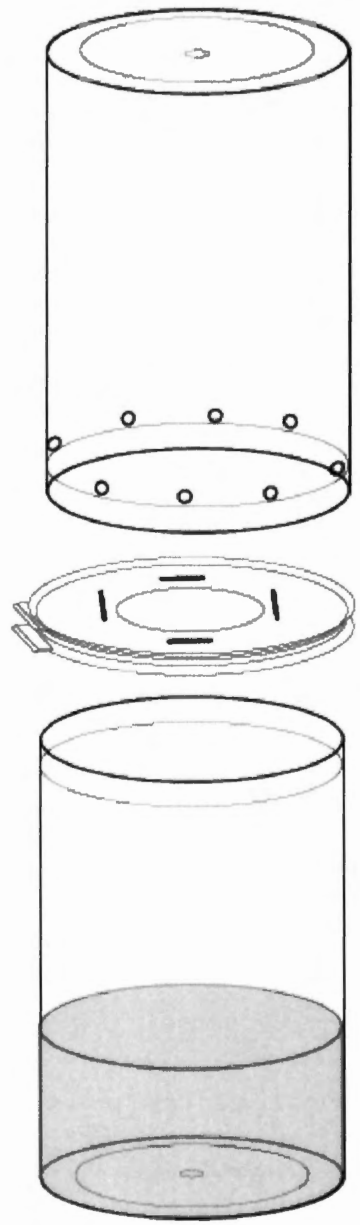


Figure 2.1. Mosquito emergence chamber used for adult rearing of field collected mosquito larvae and pupae in the laboratory.

Host seeking adult collections: On a weekly basis, a day before collections of oviposition strips and larval collections were made, CDC miniature light traps baited with CO_2 and the lights removed were set at each site. Dry ice was contained in a 1.9 l water cooler suspended under the CDC miniature light trap. The nozzle on the cooler was completely opened to allow the gaseous CO_2 to escape in the trap area. Collections were made for approximately 24 hrs and once the collection cup was removed, they were placed in a cooler with dry ice to kill the mosquitoes. They were then taken to the laboratory and identified to species (Darsie and Ward 1981, Darsie 1986), sexed and counted. Only *Oc. triseriatus* and *Ae. albopictus* adults were recorded in these experiments.

Statistical analyses: Several formulae were used for the statistical analysis based on the numbers of eggs for *Oc. triseriatus* and *Ae. albopictus*. Calculations used in analysis were:

Prevalence = T_{egg} / T; Mean intensity = egg / T_{egg} ; Mean density = prevalence x mean intensity where the variable T = the total number of cups per sampling period minus any missing cups, T_{egg} = the total number of cups with eggs per sampling period and egg = the total numbers of eggs in traps per sampling period (Kitron et al. 1989).

The overall effects of the site, treatment (replenished versus replaced cups), and site and treatment interactions on the mean intensity, mean density and average number of eggs of both species and replenished and replaced cups were analyzed for the three sites using PROC GLM of SAS® (SAS 2001) with a least squares means statement. Data for returned cups were not included in the analysis. Normality of the data was analyzed using PROC UNIVARIATE of SAS® and all dependent variables were not normally distributed and required transformation using log (x + 1). Chi-square analysis was performed examining the prevalence for both species of mosquitoes using PROC FREQ of SAS®. Means for untransformed variables were established using PROC GLM of SAS® without the statistical tests statement. All data were analyzed using SAS® system version 8.2 (SAS 2001) for elementary statistics with an alpha level of $\alpha = 0.05$.

iv- Results

The returned strips in this study were not analyzed due to several complications that arose. Many of the oviposition strips disintegrated and could no longer be reused after several weeks in the cups. Eggs on the strips became difficult to count after a few weeks of accumulation of oviposition activity. The large amount of accumulated eggs also made calculating the average weekly oviposition activity impossible when some eggs from previous weeks could have fallen off the strips or eclosed and were impossible to identify.

At all three sites, both *Oc. triseriatus* and *Ae. albopictus* were abundant. During 2000 and 2001, 102,527 *Oc. triseriatus* and *Ae. albopictus* eggs were collected from the three sites. In 2000, 57,712 eggs from both species were collected. Island Home site had the largest number of eggs (24,356) of the three sites in 2000, and 33,693 (58.4%) *Ochlerotatus triseriatus* and 24,019 (41.6%) *Ae. albopictus* (Table 2.1) collected from all three sites. In 2001, 44,815 eggs of both species were collected (Table 2.2) and 34,595 (77.2%) of the eggs were collected at the PSF and IH sites. During both collection years,

Table 2.1. Number of *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) eggs collected for each treatment of oviposition cup used at the Hackberry, Plant Science Farm and Island Home sites in Knox County, TN, from 2 May - 26 October 2000.

Site	TRI	ALB	Replenished	Replaced	Total
Hackberry	3,677	8,921	8,260	4,338	12,598
Plant Science Farm	13,407	7,351	11,889	8,869	20,758
Island Home	16,609	7,747	14,518	9,838	24,356
Total	33,693	24,019	34,667	23,045	57,712

Table 2.2. Number of *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) eggs collected for each treatment of oviposition cup used at the Hackberry, Plant Science Farm and Island Home sites in Knox County, TN, from 14 March- 3 October 2001.

Site	TRI	ALB	Replenished	Replaced	Total
Hackberry	4,489	5,731	6,833	3,387	10,220
Plant Science Farm	8,735	9,788	11,360	7,160	18,523
Island Home	9,930	6,106	9,491	6,545	16,072
Total	23,154	21,625	27,684	17,096	44,815

the lowest number of eggs was found at the HACK site each year. Also, replenished cups had the greatest number of eggs collected in 2000 and 2001 with 34,667 (60.1%) and 27,684 (61.8%), respectively (Table 2.3, 2.4). *Ochlerotatus triseriatus* and *Ae. albopictus* had 925 adults reared from the three sites in 2000 and 2001 (Table 2.5). Collections of adults yielded 657 *Oc. triseriatus* and *Ae. albopictus* adults collected during the study, 344 (52 %) from the IH site (Table 2.6).

Ochlerotatus triseriatus: In 2000 and 2001, 33,693 and 23,154 eggs, respectively, were collected (Table 2.1, 2.2). During 2000, 19,984 eggs were collected from replenished cups and 13,709 eggs were collected from replaced cups (Table 2.3). In 2001, 14,758 eggs were collected in replenished cups and 8,458 eggs were collected in replaced cups (Table 2.4). Overall, site and treatment were significant effects for mean intensity, mean density and average number of *Oc. triseriatus* at all three sites. Using the least squares means for mean intensity, mean density and average for *Oc. triseriatus* and the effects of treatment used, all three dependent variables were significant. The mean intensity was significant ($p = 0.03$) with a mean for replenished cups ($\bar{x} = 53.02$) greater than the mean for replaced cups ($\bar{x} = 33.23$). The mean density was significant ($p = 0.01$) with a mean for replenished cups ($\bar{x} = 46.45$) greater than the mean for replaced cups ($\bar{x} = 29.10$). The average number of eggs was significant ($p = 0.03$) with a mean for replenished cups ($\bar{x} = 45.20$) greater than the mean for replaced cups ($\bar{x} = 29.69$).

Among the three sites, the PSF ($\chi^2 = 0.01$, $p = 0.92$) and IH ($\chi^2 = 0.45$, $p = 0.5$) sites had no significance for treatments and prevalence using Chi-square analysis. Prevalence had significant differences for treatments at HACK using Chi-square analysis

Table 2.3. Number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs by cup type for the Hackberry, Plant Science Farm and Island Home sites from 2 May - 26 October 2000.

	Oc. triseriatus			*Ae. albopictus*		
Site	Replenished	Replaced	Total	Replenished	Replaced	Total
Hackberry	2,481	1,196	3,677	5,779	3,142	8,921
Plant Science Farm	7,606	5,801	13,407	4,283	3,068	7,351
Island Home	9,897	6,712	16,609	4,621	3,126	7,747
Total	19,984	13,709	33,693	14,683	9,336	24,019

Table 2.4. Number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs by cup type for the Hackberry, Plant Science Farm and Island Home sites from 14 March - 3 October 2001.

	Oc. triseriatus			*Ae. albopictus*		
Site	Replenished	Replaced	Total	Replenished	Replaced	Total
Hackberry	3,061	1,428	4,489	3,772	1,959	5,731
Plant Science Farm	5,669	3,066	8,735	5,691	4,097	9,788
Island Home	6,028	3,964	9,992	3,463	2,617	6,080
Total	14,758	8,458	23,216	12,926	8,673	21,599

Table 2.5. Number of reared *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) adults from larval and pupal collections at the Hackberry, Plant Science Farm and Island Home sites from 2 May - 26 October 2000 and 14 March - 3 October 2001.

Site	Year	TRI ♂	TRI ♀	ALB ♂	ALB ♀	Total
Hackberry	2000	0	0	24	17	41
	2001	8	19	64	193	284
Plant Science Farm	2000	2	7	1	12	22
	2001	6	18	66	139	229
Island Home	2000	0	17	30	35	82
	2001	13	35	83	136	267
Total		29	96	268	532	925

Table 2.6. Number of *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) adults collected at the Hackberry, Plant Science Farm and Island Home sites from 2 May - 26 October 2000 and 14 March - 3 October 2001.

	Hackberry			Plant Science Farm			Island Home		
Year	TRI	ALB	Total	TRI	ALB	Total	TRI	ALB	Total
2000	23	81	104	15	54	69	54	104	158
2001	19	65	84	12	44	56	26	106	186
Total	42	146	188	27	98	125	80	210	344

($\chi^2 = 4.08$, $p = 0.04$) (Figure 2.2, 2.3).

Aedes albopictus: In 2000 and 2001, 24,019 and 21,625 eggs, respectively, were collected (Table 2.1, 2.2). In 2000, 14,683 eggs were collected from replenished cups and 9,336 eggs were collected from replaced cups (Table 2.3). In 2001, 12,926 eggs were collected from replenished cups and 8,673 eggs were collected from replaced cups (Table 2.4). Overall, site and treatment were significant effects for mean intensity and mean density while only method was significant for the average number of *Ae. albopictus* at all three sites. Using the least squares means for mean intensity, mean density and average for *Ae. albopictus* and the effects of treatment used, all three dependent variables were significant. The mean intensity was significant ($p = 0.02$) with a mean for replenished cups ($\bar{x} = 37.82$) greater than the mean for replaced cups ($\bar{x} = 24.29$). The mean density was significant ($p = 0.01$) with a mean for replenished cups ($\bar{x} = 36.40$) greater than the mean for replaced cups ($\bar{x} = 23.19$). The average number of eggs was significant ($p = 0.05$) with a mean for replenished cups ($\bar{x} = 36.01$) greater than the mean for replaced cups ($\bar{x} = 24.07$).

Among the three sites, the HACK ($\chi^2 = 0.73$, $p = 0.39$) and the PSF ($\chi^2 = 0.91$, $p = 0.34$) sites had a significant difference for prevalence among treatments using Chi-square analysis. Island Home site had no significant difference among the treatments used for prevalence ($\chi^2 = 0.46$, $p = 0.5$) (Figure 2.4, 2.5).

Larval and pupal collections: During 2000 and 2001, 925 adult *Oc. triseriatus* and *Ae. albopictus* mosquitoes were reared from larval and pupal collections (Table 2.5). There were 145 adults reared in 2000, 26 (17.9%) of these were *Oc. triseriatus* adult

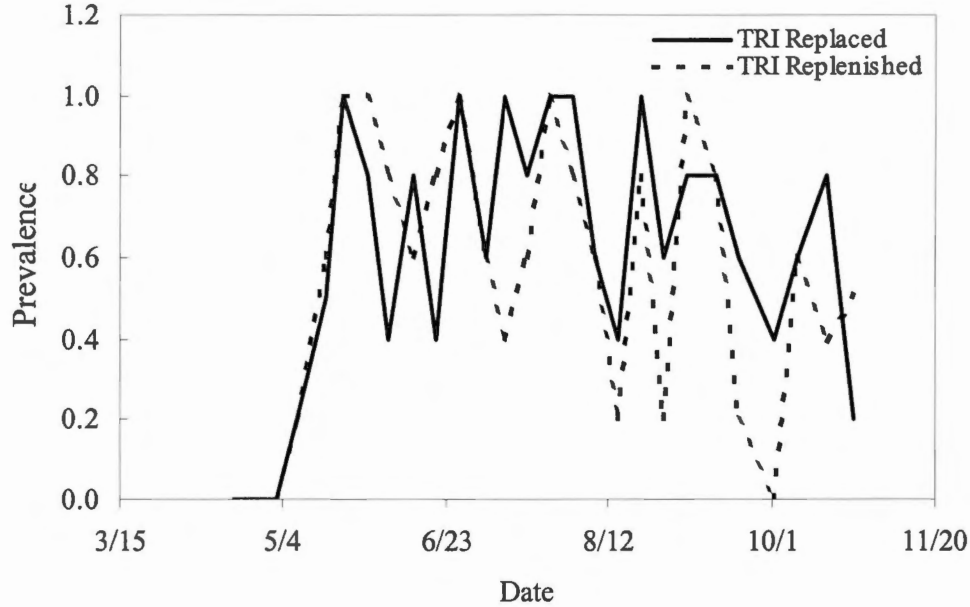


Figure 2.2. The prevalence (total number of cups with eggs/ total number of cups) of *Ochlerotatus triseriatus* (TRI) at the Hackberry site, Knox County, Tennessee, from 2 May - 26 October 2000.

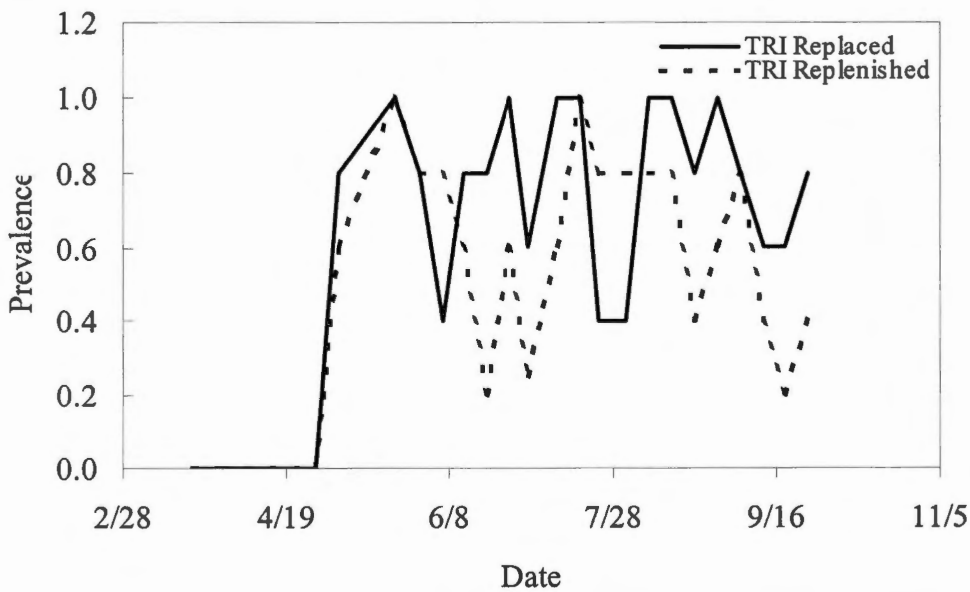


Figure 2.3. The prevalence (total number of cups with eggs/ total number of cups) of *Ochlerotatus triseriatus* (TRI) at the Hackberry site, Knox County, Tennessee, from 14 March - 3 October 2001.

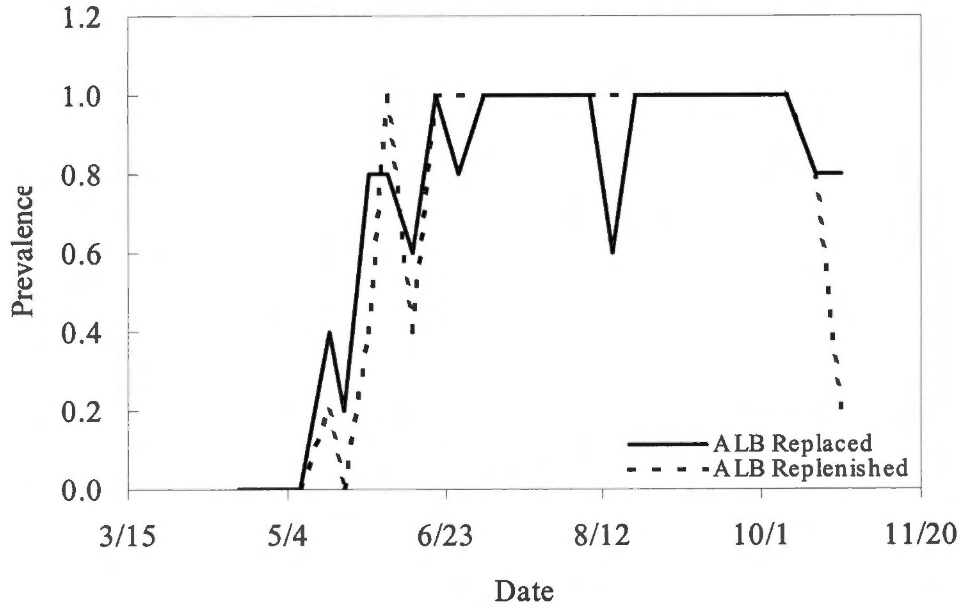


Figure 2.4. The prevalence (total number of cups with eggs/ total number of cups) of *Aedes albopictus* (ALB) at the Island Home site, Knox County, Tennessee, from 8 May - 26 October 2000.

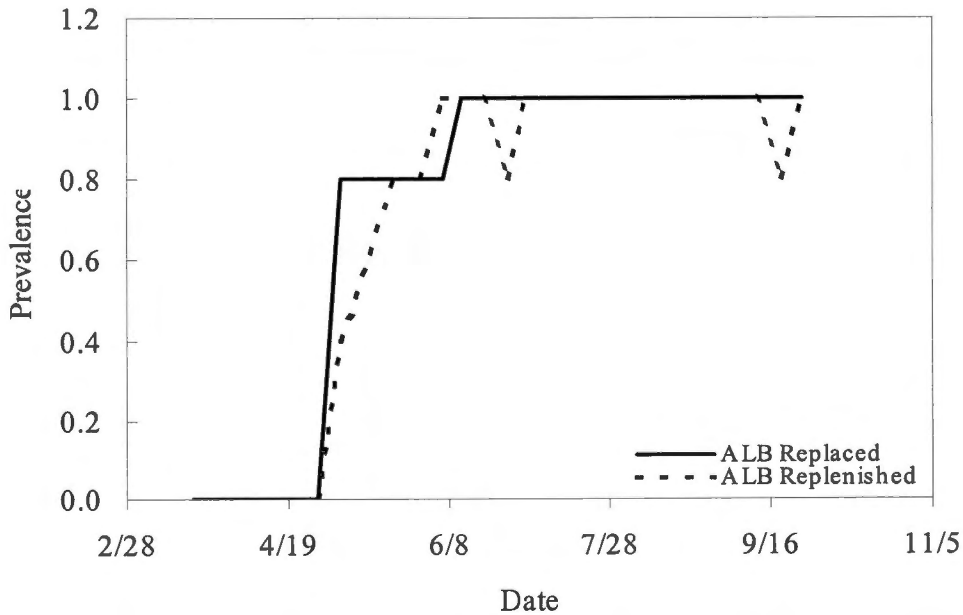


Figure 2.5. The prevalence (total number of cups with eggs/ total number of cups) of *Aedes albopictus* (ALB) at the Island Home site, Knox County, Tennessee, from 14 March - 3 October 2001.

males (2) and females (24). *Aedes albopictus* constituted 82.1% (119) while males (55) and females (64) had similar total numbers. Replenished cups had 50 emerged adults and returned cups had 92 emerged adults in 2000.

In 2001, 780 adult *Oc. triseriatus* and *Ae. albopictus* mosquitoes were reared from the three sites (Table 2.5). *Ochlerotatus triseriatus* accounted for 12.7% (99) of the adults reared and 72 (72.7%) of these were female. *Aedes albopictus* had 213 (31.3%) male and 468 (68.7%) females totaling 681 (87.3%) of the 2001 adults reared from larval and pupal collections. The replenished cups had 417 adults and returned cups had 363 adults reared during 2001.

Host seeking adult collections: Ochlerotatus triseriatus and *Ae. albopictus* had 657 adult mosquitoes collected in 2000 and 2001, 149 (22.7%) of these were *Oc. triseriatus* and 508 (77.3%) were *Ae. albopictus* (Table 2.6). The IH site had the most adults collected during the two years of collections and the PSF site had the least amount of adult activity in collections during the two periods. During 2000, a combined collection of 331 adults of *Oc. triseriatus* and *Ae. albopictus* had 92 (27.8%) *Oc. triseriatus* and 239 (72.2%) *Ae. albopictus*. In 2001, there was a combined collection of 326 *Oc. triseriatus* and *Ae. albopictus* adults consisting of 57 (17.5%) *Oc. triseriatus* and 269 (82.5%) *Ae. albopictus*.

v - Discussion

Oviposition activity was greater for both *Oc. triseriatus* and *Ae. albopictus* in replenished cups than replaced cups. The greater levels of oviposition activity may be due to higher levels of organic material, which is inhabited by many types of microorganisms that may themselves release olfactory cues enhancing the attractiveness of certain containers (Trexler et al. 1998). The bacteria and fungi present also provide a valuable food source since bacterial populations are lower when mosquito larvae are present, indicating that the larvae are actively feeding on these microorganisms (Fish and Carpenter 1982, Walker et al. 1991).

Ochlerotatus triseriatus had more overall oviposition activity than *Ae. albopictus* at all three sites, for replenished and replaced cups years of the study. All three sites had more oviposition activity for both species in replenished cups than replaced cups (Table 2.3, 2.4). Overall, there was more oviposition activity measured by prevalence in replenished than replaced cups but this was significant only at the HACK site when examined by Chi-square analysis ($\chi^2 = 4.08$, $p = 0.04$). Since this significance occurred only at the one of the three sites, there was no significant difference between treatments used regarding prevalence.

The mean intensity, mean density and average number of eggs were all found to be significantly higher in replenished cups rather than replaced cups. The means for each variable were higher for replenished than replaced cups suggesting that *Oc. triseriatus* oviposits more eggs in replenished than replaced cups. *Ochlerotatus triseriatus* more readily oviposits in containers that have water that once contained larvae (Bentley et al. 1976). However, Kitron et al. (1989) found that *Oc. triseriatus* had more oviposition activity in cups with surfaces that had no eggs on them. Although it been suggested that *Ae. aegypti* prefers to avoid oviposition activity in cups with eggs or larvae present (Chadee et al. 1990), for *Oc. triseriatus*, these cups may have features that exceed the risks of competition and crowding.

Aedes albopictus: Aedes albopictus eggs are more likley to be found in any cup than *Oc. triseriatus* eggs on any date for both types of cups (Figure 2.4, 2.5) suggesting that they are less discriminatory or more efficient than *Oc. triseriatus* regarding oviposition behavior. *Aedes albopictus* used a larger number of cups for oviposition than *Oc. triseriatus* (Trexler et al. 1998) while these data suggest that both *Oc. triseritaus* and *Ae. albopictus* oviposit in many more replenished cups than replaced cups. Both statistical procedures used found a significant difference among treatments used and oviposition activity of *Ae. albopictus*. Only IH site showed no significance for prevalence ($\chi^2 = 0.46$, $p = 0.49$) using Chi-square analysis for treatments (Figure 2.4, 2.5). Since this significance occured at two of the three sites, there was a significant difference among treatments used for prevalence.

Overall, as with *Oc. triseriatus*, mean intensity, mean density and average numbers of eggs were significantly different among treatments. Replenished cups had higher means than the replaced cups, for all three variables, suggesting that *Ae. albopictus* prefers cups with a higher organic content than one mainly containing cleaner water. The results agree with Trexler et al. (1998) that *Oc. triseriatus* and *Ae. albopictus* had more oviposition activity in cups that contain higher organic content using oak leaf infusions. They also found that *Ae. albopictus* laid more eggs in oak leaf infusion water that contained conspecific larvae and pupae than water alone. The older larvae and pupae present in the replenished cups in this study may have increased oviposition activity of *Oc. triseriatus* and *Ae. albopictus*.

Larval and pupal collections: In 2000, 145 adult *Oc. triseriatus* and *Ae. albopictus* emerged form larval and pupal collections (Table 2.5). Fewer adults were reared in 2000 than 2001 because there were a greater number of larval and pupal collections. In 2000, many of the field collections reared poorly because many of the larvae and pupae died. A problem with using the adult emergence chamber (Figure 2.1) was that many adults would emerge within 24 hrs of taking them into the lab, when they died before the week was up the carcasses would disintegrate in the water.

Ochlerotatus triseriatus had fewer adults emerge than *Ae. albopictus*. *Ochlerotatus triseriatus* has a higher larval mortality and longer development time than *Ae. albopictus* in the laboratory. Both in conspecific and heterospecific cultures, *Oc. triseriatus* had 26 - 40% larval mortality when reared in culture with *Ae. albopictus* and *Ae. aegypti* larvae and it took an average of eight days for 50% pupation (Ho et al. 1989).

Host seeking adult collections: Approximately the same numbers of adults were collected each year (Table 2.6). At all sites, more *Ae. albopictus* than *Oc. triseriatus* adults were collected. Plant Science Farm had the fewest numbers of adults collected overall and the least numbers of *Oc. triseriatus* and *Ae. albopictus* during both collection years. The greatest number of adults were collected at IH site both years which is expected as this is the most urban and littered site. The large amount of containers and trash present may provide additional sources of larval habitats. Relative to the numbers of eggs collected of both species, few adults of both species were collected. Low levels of host-seeking adult collections were expected, since both species are not readily attracted to the traps, even when a CO_2 source is added (Leiser 1981, Hawley 1988).

The amount of oviposition activity by *Oc. triseriatus* was greater than *Ae. albopictus* at all three sites during the two study years. However, when examining the numbers of reared adults (Table 2.5) and the numbers of host-seeking adult collections (Table 2.6), there are always greater levels of *Ae. albopictus* than *Oc. triseriatus*. This discrepancy may be explained by a high mortality of *Oc. triseriatus* larvae and a longer developmental time, but *Ae. albopictus* larvae may also have a high mortality rate of up to 80% but a low egg mortality in some field studies (Hawley 1988). In other mosquito rearing activities in our laboratory, where *Oc. triseriatus* eggs outnumber *Ae. albopictus*, overall, more *Ae. albopictus* adults are reared than *Oc. triseriatus* (Gerhardt pers. comm.).

The greater amount of oviposition activity in cups that were replenished may be due to factors relating to the quality of the water associated with these cups. Although *Oc. triseriatus* and *Ae. albopictus* are both density dependent species (Hawley 1988, Mahmood et al. 1997), *Oc. triseriatus* and *Ae. albopictus* both prefer to oviposit more in ovitraps that have a higher organic content and those either containing larvae and pupae or ovitraps that once contained larvae and pupae (Allan and Kline 1998, Trexler et al. 1998, Yap et al. 1995). *Aedes albopictus* has shown a better resistance to starvation and develops faster on decaying leaf litter than liver powder and yeast (Barrera 1996). It may be possible that the gravid *Oc. triseriatus* and *Ae. albopictus* are seeking oviposition sites that are ideal and have a greater amount of food resources regardless of the risks due to crowding and competition.

The indications, resulting from these data, for researchers regarding the techniques employed in sampling and monitoring mosquitoes are complex. Based on the significance of these data for cup treatments and *Oc. triseriatus* and *Ae. albopictus* oviposition activity the type of sampling and monitoring would dictate which treatment to use in sampling. If the goal of the research is to collect the most mosquito eggs for disease surveillance, then leaving the water in the ovitraps to attract more gravid females would be most effective. Replenishing water rather than replacing water in ovitraps may also increase the amount of adult activity present since there would be a continuing source for larval production and increased oviposition activity. Routine surveillance of *Oc. triseriatus* and *Ae. albopictus* without disease surveillance activity would indicate a need to replace ovitraps with clean water regularly. If both methods are employed in the same research, there may be significant variation in the amount of oviposition activity detected. Either programs for disease surveillance or routine surveillance for baseline data could use the two types of treatments as long as the same method was used consistently. The returned cup treatment that was excluded from data analysis would not be valuable for oviposition sampling due to the many complications.

CHAPTER III

SEASONAL DISTRIBUTION AND RELATIVE ABUNDANCE OF MOSQUITOES (DIPTERA: CULICIDAE) IN TEN EASTERN TENNESSEE COUNTIES

i - Abstract

The seasonal distribution and relative abundance of container-inhabiting mosquitoes were examined biweekly at ten eastern Tennessee counties from 1999 - 2001. This study was a continuance of the same study conducted in 1998 and 1999 by Gottfried (1999). Five oviposition traps for *Ochlerotatus* (= *Aedes*) *triseriatus* (Say), the eastern tree hole mosquito and *Aedes albopictus*, the Asian tiger mosquito, eggs were placed in each county and host-seeking adult mosquitoes were collected using a single Centers for Disease Control (CDC) miniature light trap baited with dry ice. Daily average temperature and average rainfall variables from Blount County were examined for any effects on oviposition activity of *Oc. triseriatus* and *Ae. albopictus* and *Ae. albopictus* adult activity. A combined total of 321,270 eggs of *Oc. triseriatus* (256,862) and *Ae. albopictus* (64,408) were collected from 1998 - 2001. These data suggest that *Oc. triseriatus* has a greater amount of oviposition activity than *Ae. albopictus*, and it appears that *Oc. triseriatus* peak oviposition activity occurs approximately one month earlier than *Ae. albopictus*. These differences may allow for these two species to coexist with limited competition. The majority (64%) of adults collected in host-seeking traps were *Oc. triseriatus* and *Ae. albopictus*. The variability in *Oc. triseriatus* and *Ae. albopictus* oviposition data were best described by month and average temperature variables two to four weeks before and during the sampling period. *Aedes albopictus* adult activity

variability was best described by year, average rainfall and average temperature variables four weeks before and during the sampling period. In Blount County, it is apparent that temperature has a greater impact than rainfall on container-inhabiting mosquitoes.

ii - Introduction

In eastern Tennessee, several studies examining mosquito surveillance and abundance have been conducted over the past 60 years. An early study was by Arnold (1940) who surveyed and reported 13 mosquito species in eastern Tennessee. A later study listed 44 species of mosquitoes in the whole Tennessee Valley (Breeland et al. 1961). Twenty four adult mosquito species were reported from species composition research in Knox County (Ogg 1975). Mosquito composition was evaluated again in Knox County and 32 species were reported (Hribar and Gerhardt 1986). The vector potential of *Oc. trivittatus* (Coquillett) for dog heartworm in Knox county was determined by Hribar (1984). Gottfried (1999) provided a study of the seasonal abundance and relative distribution of mosquito species in eastern Tennessee. The environmental relationships in case sites of the La Crosse encephalitis (LAC) virus in eastern Tennessee were examined and found no correlation between the amount of trash or tree line but found correlations with the total number of tree holes, total mosquito load and the occurrence of disease (Smith 2001). Recent research findings also include an isolation of Jamestown Canyon (JC) virus from *Ae. albopictus* (Gottfried et al. 2002) which has been found in 26 states and every county in Tennessee (Moore 1998). La Crosse encephalitis virus was also isolated from *Ae. albopictus* (Gerhardt et al. 2001). Both findings are significant because they showed that transovarial transmission occurred in naturally infected eggs. This was the first time JC virus was reported from Tennessee and the first time LAC virus had been reported in *Ae. albopictus*.

To further understand the biology of the mosquitoes as potential and known vectors, it is important to fully understand the biology, ecology, seasonal and temporal distribution of each species. Significant influences that affect mosquitoes are resource limitation and starvation (Haramis 1985), larval density and competition (Mahmood et al. 1997), rainfall, dessication and temperature patterns (Alto and Juliano 2001a). Determining the effects of weather patterns on mosquitoes may help aid in the prediction of mosquito abundance, population fluctuations and potential disease risks throughout the season. Vandyk and Rowley (1995) found that in Iowa individual mosquito species responded differently to abnormally high rainfall in 1993. *Culex pipiens complex* L., *Anopheles punctipennis* (Say) and *Ae. vexans* (Meigen) populations increased and the other species including *Oc. trivittatus* (Coquillett) and *Cx. tarsalis* Coquillett, remained unchanged or decreased in collections. In contrast, a study in Kern County, California, found the amount of rainfall between 1990-1998 only explained 13% of the variability of total numbers of *Cx. tarsalis* female mosquitoes captured, while 67% of the variability in mosquito abundance could be explained by river runoff (Wegbreit and Reisen 2000). Szumlas et al. (1996) found that the seasonal occurrence of rain rather than the total amount of rainfall had a significant impact on *Oc. triseriatus* (Say), the eastern tree hole mosquito, oviposition activity in North Carolina. While several studies showed a correlation between weather patterns and mosquito populations, one study generated a regression model that failed to predict the abundance of *Oc. taeniorhynchus* (Weidemann) but showed a positive correlation between rainfall, temperature, tides and adult light trap counts (Ailes 1998).

To aid in determining patterns of seasonal distribution and relative abundance in eastern Tennessee, surveillance in ten eastern Tennessee counties began in 1998 and continued through mid-summer 2001. This study was twofold in purpose: 1) to determine the seasonal distribution and relative abundance of *Oc. triseriatus*, *Ae. albopictus* other mosquito species and 2) to examine the effects of weather patterns on mosquito populations in eastern Tennessee.

iii - Materials and Methods

Collection sites: Ten eastern Tennessee counties (Anderson, Blount, Grainger, Hamblen, Jefferson, Knox, Loudon, Roane, Sevier and Union) were chosen in 1998 to begin a multiple year surveillance program of the mosquito species (Gottfried 1999). Sites in each of the counties were chosen to be relatively close to Tennessee Valley Authority regulated reservoirs. Locations and directions to each site are listed in Gottfried (1999). Previous collections in 1998 and 1999 (Gottfried 1999) were continued in the same ten sites from 13 March - 24 October 2000 and from 2 March - 22 October 2001.

Oviposition activity: Five ovipostion traps similar to those described by Steinley et al. (1991) were placed at each site (Loor and DeFoliart 1969). The oviposition substrate was a 76 lb seed germination paper (Anchor Paper Company, Saint Paul, MN) cut into 5 cm x 25.5 cm strips and placed inside the cup and held securely by a paper clip (Steinly et al. 1991). Strips were replaced at the same time adult collections were conducted. New oviposition strips were placed in the black cups on a biweekly basis.

Once collected, the oviposition strips were taken to the laboratory and dried. The eggs were then identified to species (Pratt and Kidwell 1969) and recorded.

Host seeking adult mosquito collections: All adult mosquitoes were collected on a biweekly basis using one CO_2 baited CDC miniature light trap at each site. Every week, collections were taken at five of the ten counties. Dry ice was placed in a 1.9 l water cooler with an open spout to release the CO_2 gas. Traps were placed at the sites for approximately 24 hours. As the adult trap collection cups were retrieved they were immediately placed in a cooler with dry ice to kill the mosquitoes, taken to the laboratory and stored in a refrigerator to be identified to species (Darsie and Ward 1981, Darsie 1986).

Blount County weather data: The collection in Blount County was within 5 km of the local airport (McGhee Tyson Airport) and all weather data for Blount County were obtained from accessing the National Oceanic and Atmospheric Administration's (NOAA) National Climate Data Center's web site (http.//www.ncdc.noaa.gov/). Data consisted of average daily temperature and average daily rainfall. The biweekly average rainfall patterns are measured in inches and the biweekly average temperatures are measured in degrees °F for the three years examined. These variables were analyzed and graphed in these formats. Data from NOAA were used for examining any relationships between weather patterns and *Oc. triseriatus* and *Ae. albopictus* egg collections and *Ae. albopictus* adult mosquito population fluctuations.

All statistical tests were performed using SAS® (SAS 2001) and oviposition data for both species and adult data for *Ae. albopictus* were tested for normality using PROC

UNIVARIATE of SAS® and PROC REG of SAS® was used to test several independent variables (Table 3.1) for multiple regression. These variables were also tested for analysis of variance PROC GLM of SAS®. Using the Shapiro-Wilk test for normality from the SAS® results, average *Oc. triseriatus* egg and *Ae. albopictus* adult data were normal and did not need transformed. The average egg data for *Ae. albopictus* were not normally distributed and required transformation using log (x + 0.5). All data were analyzed using the SAS® system version 8.2 for elementary statistical analysis (SAS 2001) and used an alpha level $\alpha = 0.05$.

Three model statements were chosen to represent the data from the analysis that best described the effects of different weather variables for *Oc. triseriatus* and *Ae. albopictus* oviposition activity and *Ae. albopictus* adult activity. Each model statement was chosen based on the strength of the r-square values and by the simplest amount of reasonable explanations represented in the independent variables as they related to our knowledge of the biologies for these mosquitoes.

iv - Results

Eggs of *Oc. triseriatus* were more abundant than *Ae. albopictus* eggs in oviposition traps in all counties except Hamblen and Sevier Counties. The ten counties can be placed into three groups according to the ratios of *Oc. triseriatus* to *Ae. albopictus* eggs in the oviposition traps. The first group of seven counties was those where the *Oc. triseiratus* to *Ae. albopictus* ratios were high over the four sample years, with *Oc. triseriatus* having much higher oviposition activity than *Ae. albopictus* (Table 3.2). A second group includes the three counties in which the *Oc. triseriatus* to *Ae. albopictus*

Table 3.1. Variables used for analyzing weather patterns, *Ochlerotatus triseriatus* and *Aedes albopictus* oviposition and *Ae. albopictus* host-seeking adult activity for Blount County from 1998 - 2001.

x_n	Name	Description	Unit
x_1	MONTH	Roman numeral designation	1 - 12
x_2	YEAR	Roman numeral designation	1 - 3
x_3	AVETEMP	Average temperature	° F (Fahrenheit)
x_4	RAINPRE2	Average rainfall two previous sampling periods	Inches
x_5	TEMPRE	Average temperature one previous sampling period	° F (Fahrenheit)
x_6	TEMPRE2	Average temperature two previous sampling periods	° F (Fahrenheit)
x_7	TEMP_QUAD	Average temperature squared	° F $(\text{Fahrenheit})^2$
x_8	TEMPRE_QUAD	Average temperature one previous sampling period squared	° F $(\text{Fahrenheit})^2$
x_9	TEMPRE2_QUAD	Average temperature two previous sampling periods squared	° F $(\text{Fahrenheit})^2$

Table 3.2. The level of *Ochlerotatus triseriatus* to *Aedes albopictus* oviposition activity and their ratios by county for the four years each site was sampled.

		Ratio of *Oc. triseriatus* : *Ae. albopictus*			
County	Activity	1998[1]	1999[2]	2000	2001
Anderson	High	20.1 : 1.0	67.8 : 1.0	39.2 : 1.0	36.4 : 1.0
Loudon	High	3.0 : 1.0	5.5 : 1.0	19.4 : 1.0	6.9 : 1.0
Union	High	11.4 : 1.0	11.7 : 1.0	11.3 : 1.0	7.6 : 1.0
Blount	Medium	1.9 : 1.0	3.8 : 1.0	4.4 : 1.0	2.6 : 1.0
Jefferson	Medium	2.6 : 1.0	3.1 : 1.0	7.5 : 1.0	3.0 : 1.0
Roane	Medium	2.3 : 1.0	2.6 : 1.0	8.3 : 1.0	2.9 : 1.0
Grainger	Low	2.5 : 1.0	1.7 : 1.0	2.3 : 1.0	1.7 : 1.0
Hamblen	Low	0.5 : 1.0	1.2 : 1.0	1.1 : 1.0	1.3 : 1.0
Knox	Low	1.8 : 1.0	3.6 : 1.0	2.9 : 1.0	2.2 : 1.0
Sevier	Low	0.4 : 1.0	0.6 : 1.0	1.6 : 1.0	1.8 : 1.0

1 = Gottfried 1999
2 = Gottfried 1999, In part

ratios had medium levels of activity (Table 3.2). The third group consists of four counties, which had lower ratios overall during the four years of sampling (Table 3.2). The overall pattern for *Oc. triseriatus* was to have peak oviposition in late May and early June then taper off in late summer. *Aedes albopictus* peak oviposition activity occurred approximately one month after *Oc. triseriatus* and continued into early fall. These trends occurred for the four years collectively sampled for this study.

County oviposition patterns: Overall, *Oc. triseriatus* oviposition activity peaked in late May and early June and typically four to five weeks prior to the peak oviposition activity of *Ae. albopictus*. This trend occurred at nine of the ten sites over the four year seasonal sampling period with few exceptions as seen in Appendix 1. In Grainger County, for all four years, peak oviposition activity for both *Oc. triseriatus* and *Ae. albopictus* occurred at approximately the same time each year. Complete oviposition and adult collection data for individual sites during 1998 and part of 1999 are provided in Gottfried (1999).

The seasonal oviposition totals for all ten sites in 1999 include 61,835 *Oc. triseriatus* eggs and 15,983 *Ae. albopictus* eggs (3.9 : 1.0) (Table 3.3). Anderson and Union counties had the most *Oc. triseriatus* oviposition activity with 13,705 and 12,813 eggs respectively. The lowest number of *Oc. triseriatus* eggs (1,527) were collected from Sevier County while *Ae. albopictus* had significantly less oviposition activity at all ten sites. The fewest number of *Ae. albopictus* eggs (202) were collected from Anderson County. The greatest number of *Ae. albopictus* eggs (2,772) were collected from Sevier County.

Table 3.3. The number of *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) eggs and adults collected, the number of oviposition trap periods (5x the number of biweekly periods minus any missing traps) and the mean density (eggs/ trap/ ovitrap period) of each species by county for 12 April - 25 October 1999 (In part Gottfried 1999).

	Eggs		Ovitrap	Mean Density		Egg Ratio
County	TRI	ALB	Periods	TRI	ALB	TRI : ALB
Anderson	13,705	202	64	214.14	3.16	67.8 : 1.0
Union	12,813	1,099	60	213.55	18.32	11.7 : 1.0
Loudon	6,115	1,112	60	101.92	18.53	5.5 : 1.0
Blount	4,781	1,274	58	82.43	21.97	3.8 : 1.0
Knox	5,124	1,424	64	80.06	22.25	3.6 : 1.0
Jefferson	6,854	2,187	59	116.17	37.07	3.1 : 1.0
Roane	5,425	2,107	65	83.46	32.42	2.6 : 1.0
Grainger	3,346	2,000	59	56.71	33.90	1.7 : 1.0
Hamblen	2,145	1,806	56	38.30	32.25	1.2 : 1.0
Sevier	1,527	2,772	59	25.88	46.98	0.6 : 1.0
Total	61,835	15,983	604	102.38	26.46	3.9 : 1.0

In 2000, 71,955 *Oc. triseriatus* and 13,378 *Ae. albopictus* eggs (5.4 : 1.0) were collected (Table 3.4). Four of ten sites, Anderson, Jefferson, Loudon and Union Counties, had similar *Oc. triseriatus* oviposition activity ranging from 10,411 to 12,296 eggs. The largest number of *Oc. triseriatus* eggs (12,296) were collected from Union County. The least amount of *Oc. triseriatus* eggs (1,661) were collected from Hamblen County. The site with the most *Ae. albopictus* eggs (2,955) collected was from Sevier County while Anderson County had the fewest number of *Ae. albopictus* eggs (268) collected.

In 2001, 68,094 *Oc. triseriatus* and 17,704 *Ae.albopictus* (3.6 : 1.0) were collected (Table 3.5). Oviposition activity of *Oc. triseriatus* was greatest in Anderson and Union Counties, with 13,246 and 14,495 eggs collected, respectively. The least amount of *Oc. triseriatus* oviposition activity was detected in Grainger and Hamblen Counties, where 2,780 and 2,095 eggs were collected, respectively. The greatest number of *Ae. albopictus* eggs (2,783) were collected from Sevier County. The fewest number of *Ae. albopictus* eggs (364) were collected from Anderson County.

Anderson County: This collection site had the most variability in ratios of *Oc. triseriatus* to *Ae. albopictus* eggs over four years (Table 3.2). The ratios of *Oc. triseriatus* to *Ae. albopictus* oviposition activity were high all four years and were variable (Table 3.2). Each year this site had consistently high levels of *Oc. triseriatus* oviposition activity compared to all other sites. In contrast, *Ae. albopictus* had low levels of oviposition activity over the four years (Figure 3.1) resulting in higher ratios.

Hamblen and Sevier Counties: These collections sites were the only two where there was more oviposition activity by *Ae. albopictus* than *Oc. triseriatus* resulting in low

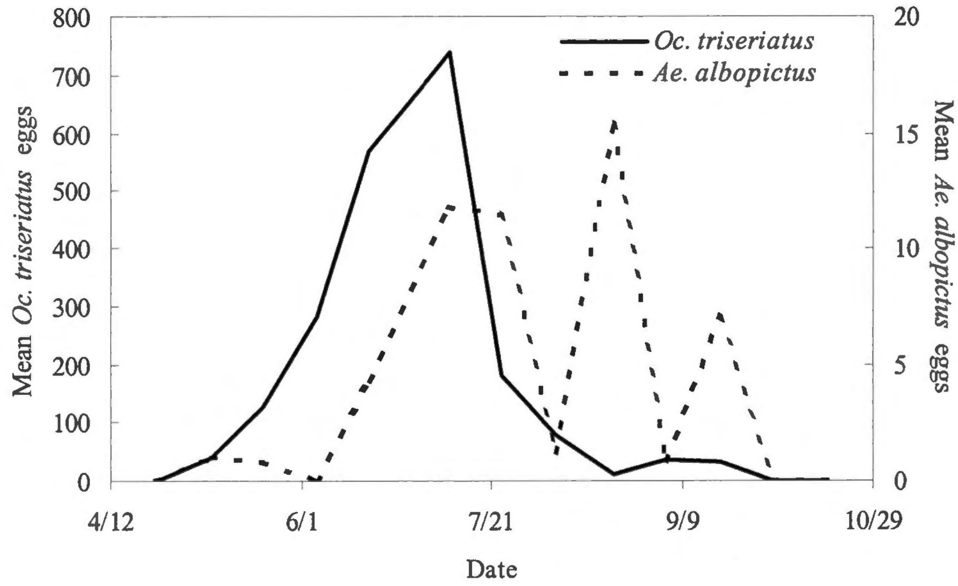
Table 3.4. The number of *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) eggs and adults collected, the number of oviposition trap periods (5x the number of biweekly periods minus any missing traps) and the mean density (eggs/ trap/ ovitrap period) of each species by county for 10 April - 24 October 2000.

	Eggs		Ovitrap	Mean Density		Egg Ratio
County	TRI	ALB	Periods	TRI	ALB	TRI : ALB
Anderson	10495	268	63	39.16	4.25	39.1 : 1.0
Loudon	11241	578	64	175.64	9.03	19.4 : 1.0
Union	12296	1084	66	186.30	16.42	11.3 : 1.0
Roane	5668	687	60	94.47	11.45	8.3 : 1.0
Jefferson	10411	1395	65	160.17	21.46	7.5 : 1.0
Blount	7130	1621	64	111.41	25.33	4.4 : 1.0
Knox	4158	1429	64	64.97	22.33	2.9 : 1.0
Grainger	4177	1846	69	60.54	26.75	2.3 : 1.0
Sevier	4718	2955	70	67.40	42.21	1.6 : 1.0
Hamblen	1661	1515	69	24.07	21.96	1.1 : 1.0
Total	71,955	13,378	654	110.02	20.46	5.4 : 1.0

Table 3.5. The number of *Ochlerotatus triseriatus* (TRI) and *Aedes albopictus* (ALB) eggs and adults collected, the number of oviposition trap periods (5x the number of bi-weekly periods minus any missing traps) and the mean density (eggs/ trap/ ovitrap period) of each species by county for 21 March - 22 October 2001.

	Eggs			Mean Density		Egg Ratio
County	TRI	ALB	Ovitrap Periods	TRI	ALB	TRI : ALB
Anderson	13,246	364	64	206.97	5.69	36.4 : 1.0
Union	14,495	1,901	75	193.27	25.35	7.6 : 1.0
Loudon	8,318	1,204	65	127.97	18.52	6.9 : 1.0
Jefferson	8,168	2,756	69	118.38	39.94	3.0 : 1.0
Roane	6,029	2,116	67	89.99	31.58	2.9 : 1.0
Blount	5,211	1,997	70	74.44	28.53	2.6 : 1.0
Knox	2,893	1,335	69	41.93	19.35	2.2 : 1.0
Sevier	4,859	2,783	75	64.79	37.11	1.8 : 1.0
Grainger	2,780	1,601	69	40.29	23.20	1.7 : 1.0
Hamblen	2,095	1,647	75	27.93	21.96	1.3 : 1.0
Total	68,094	17,704	698	97.56	25.36	3.9 : 1.0

A.



B.

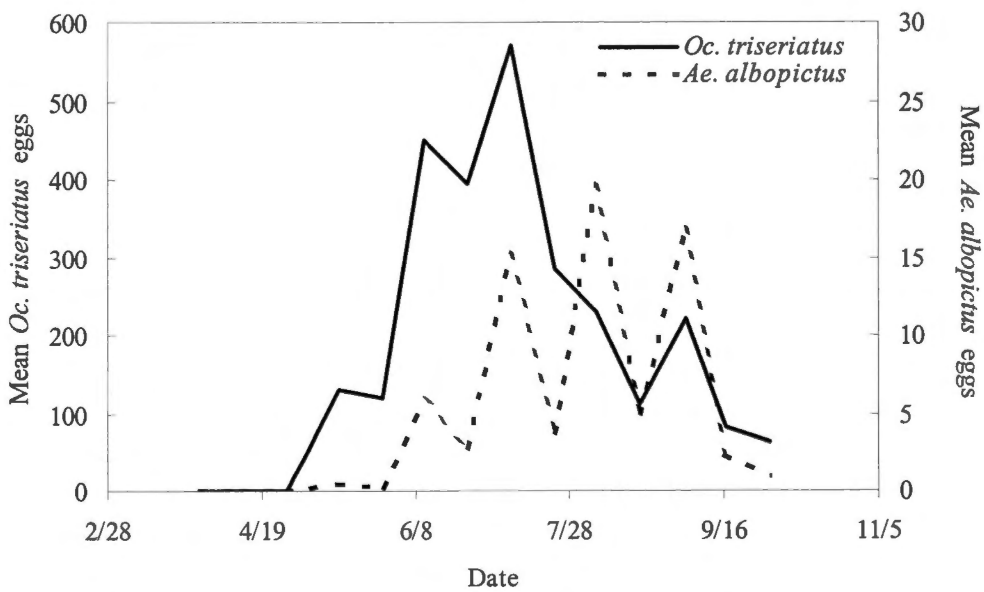


Figure 3.1. Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from The University of Tennessee Arboretum, Anderson County, from 10 April - 17 October 2000 (A) and 30 March - 14 October 2001(B).

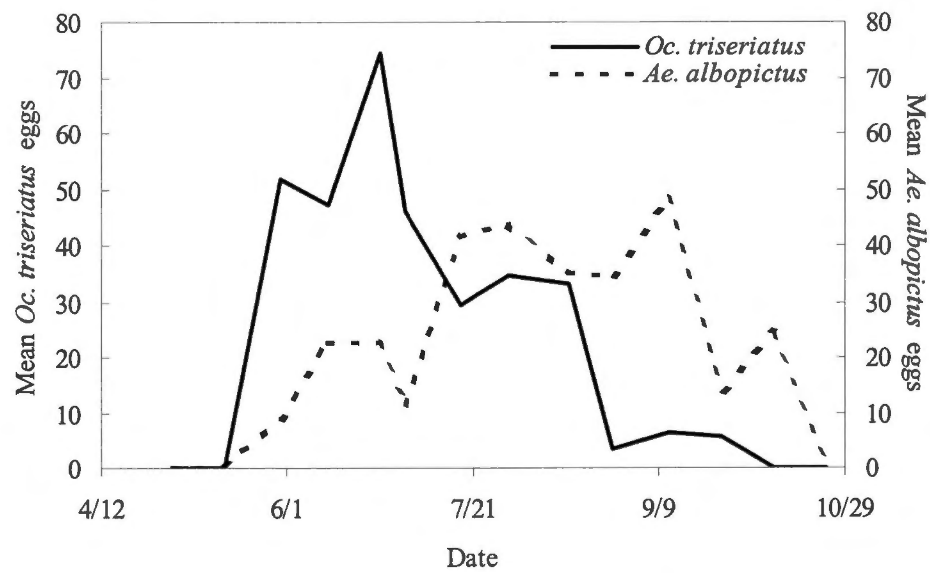
ratios (Table 3.2, Figure 3.2). The ratios of *Oc. triseriatus* to *Ae. albopictus* increased from year to year for both sites. These sites are also the most urban and have a lot of human litter surrounding the sites.

Union County: This site is uniquely the most consistent for *Oc. triseriatus* and *Ae. albopictus* oviposition activity, for both all four years (Figure 3.3). Each year the ratios and total egg collections of *Oc. triseriatus* and *Ae. albopictus* are similar (Table 3.2). This site is rural and located directly next to a lake, similar to two of the other sites' environs, including Loudon and Union Counties, which are also rural and located next to the lakeshore.

Host seeking adult mosquito collections: Collectively, 8,239 adult mosquitoes were collected between 1998 and 2001, with *Oc. triseriatus* and *Ae. albopictus* constituting 64% of adults collected. Complete adult data for individual sites during 1998 and part of 1999 are provided in Gottfried (1999). All individual adult collections for the ten sites during the four years of study are shown in Appendix 2. These totals include host seeking female and male mosquitoes. During the four years, 4,292 (52%) *Ae. albopictus* were collected from all ten sites and 1,014 (12%) *Ochlerotatus triseriatus* were collected (4.2 : 1.0). Nine genera and 25 species of adult mosquitoes were collected from all sites during the four years. These totals do not include the species of mosquitoes that were in poor condition and could not be identified in the laboratory.

During 1999, *Ae. albopictus* was the most commonly collected adult mosquito, comprising 49% (1,233) of all adults. *Ochlerotatus triseriatus* had 14% (343) of the total collection. The largest collections of *Ae. albopictus* (783) and *Oc. triseriatus* (150) were

A.



B.

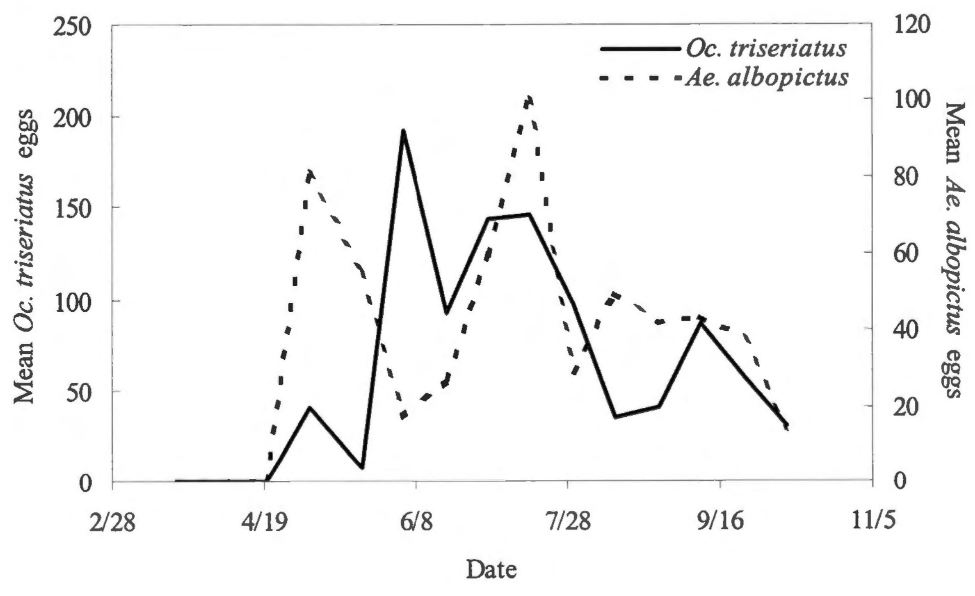


Figure 3.2. Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Morristown, Hamblen County, from 17 April - 24 October 2000 (A) and from Sevierville, Sevier County, from 21 March - 22 October 2001 (B).

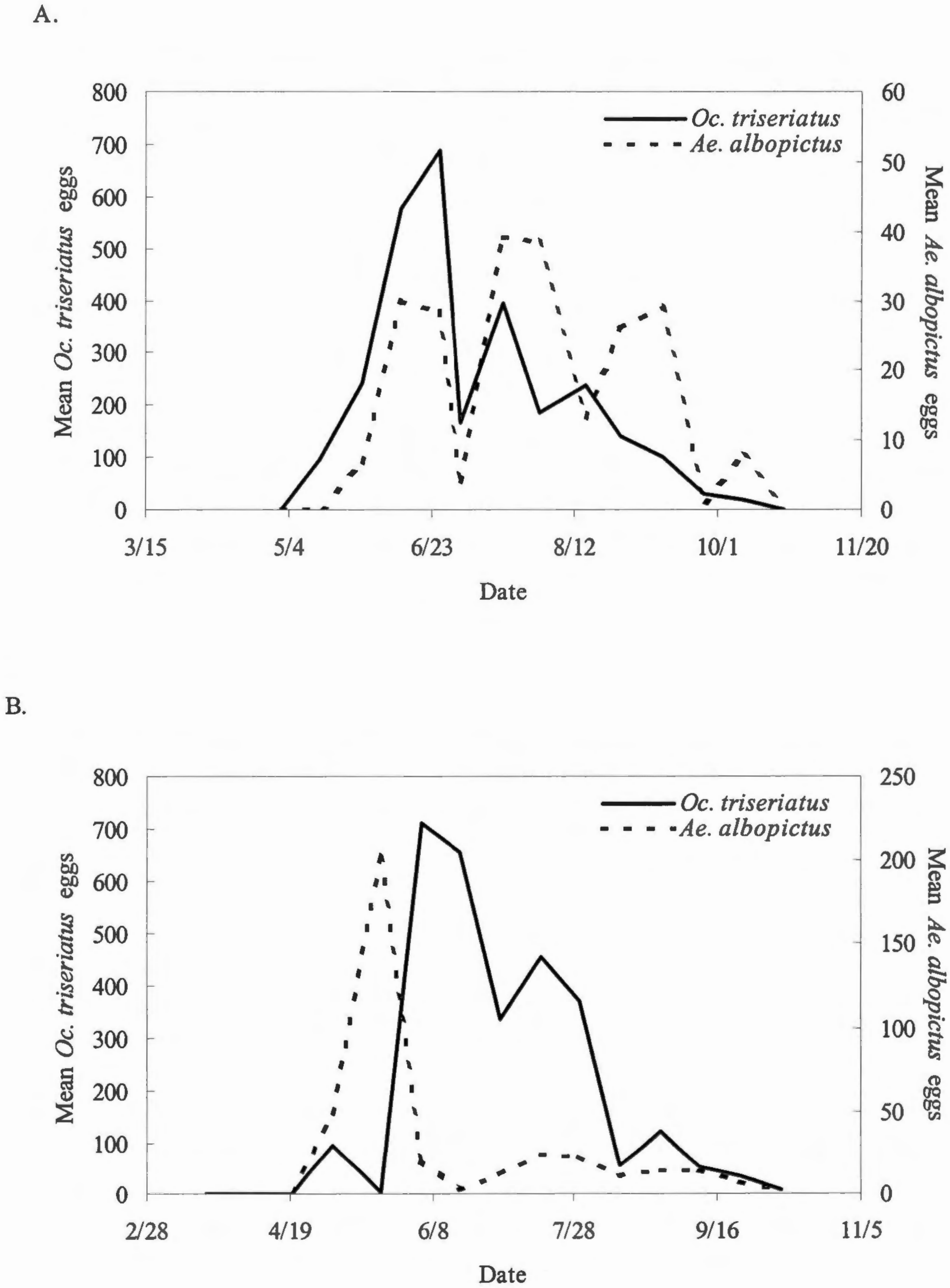


Figure 3.3. Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Maynardville, Union County, from 17 April - 24 October 2000 (A) and 21 March - 22 October 2001 (B).

in Hamblen County. The fewest number of *Oc. triseriatus* adults were collected from Roane County (6) and Sevier County (1). Only 6% (157) were *Anopheles* species and 35% (55) of these were collected in Blount County.

In 2000, 1,523 adult mosquitoes were collected and *Ae. albopictus* constituted 49.7 % (757) of the total collection. The largest number of *Oc. triseriatus* (47) were collected from Jefferson County. The most *Ae. albopictus* adults (384) collected were taken from Hamblen County. The fewest numbers of *Oc. triseriatus* (3) were collected from Blount County and Anderson County had the fewest *Ae. albopictus* (6) adults collected. In 2000, few specimens of Anopheline mosquitoes (94) were collected from all sites.

In 2001, 1,780 adults were collected from the ten sites. The majority (57.1%) of adults collected were *Ae. albopictus* (1,027) while *Oc. triseriatus* accounted for 13.8% (249) of the total. The most *Oc. triseriatus* (78) and *Ae. albopictus* (422) were collected from Hamblen County. The fewest number of *Oc. triseriatus* (7) were from Knox County. The fewest number of *Ae. albopictus* (2) were collected from Anderson County. Sevier County had 39 (31.2%) of the 125 Anopheline specimens collected.

Blount County weather patterns: Seasonal fluctuations in rainfall and temperature occured from 1998 - 2000 in Blount County (Figure 3.4, 3.5, 3.6). *Ochlerotatus triseriatus* data was best described by month and different temperature variables: $y = -6529.77 - 39.63\ x_1 + 121.13\ x_5 + 78.92\ x_6 - 0.83\ x_8^2 - 0.61\ x_9^2$, where y = average *Oc. triseriatus* eggs, x_1 = month, x_5 = previous average temperature, x_6 = two previous average temperatures, x_8^2 = previous average temperature squared and x_9^2 = two previous

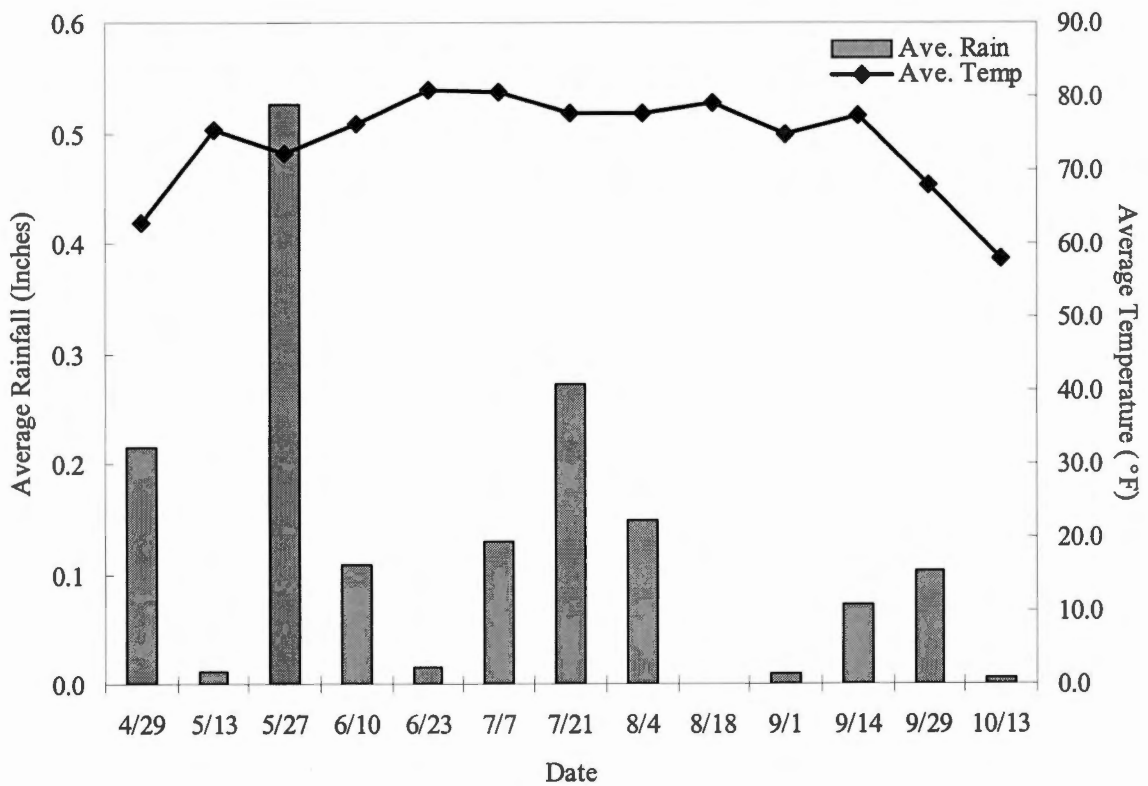


Figure 3.4. Biweekly average temperature and biweekly average rainfall amounts collected from the Knoxville McGhee Tyson Airport, Blount County, Tennessee, from 29 April - 27 October 1998.

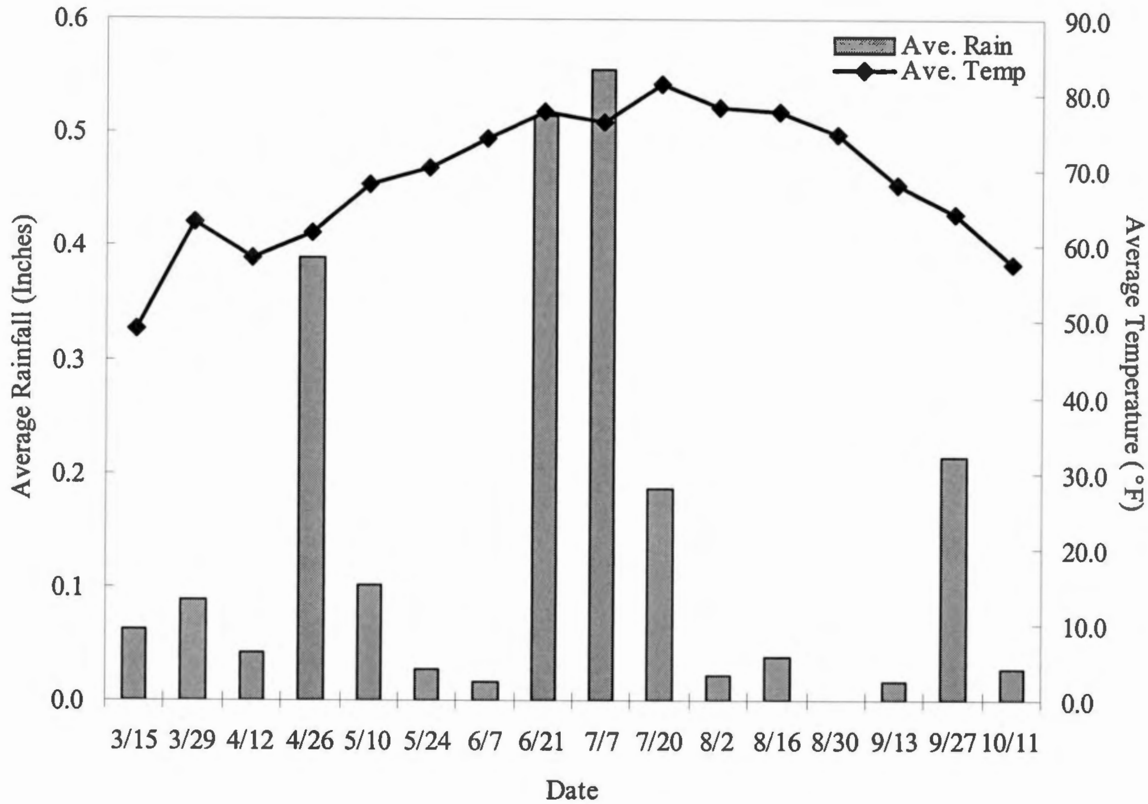


Figure 3.5. Biweekly average temperature and biweekly average rainfall amounts collected from the Knoxville McGhee Tyson Airport, Blount County, Tennessee, from 15 March - 25 October 1999.

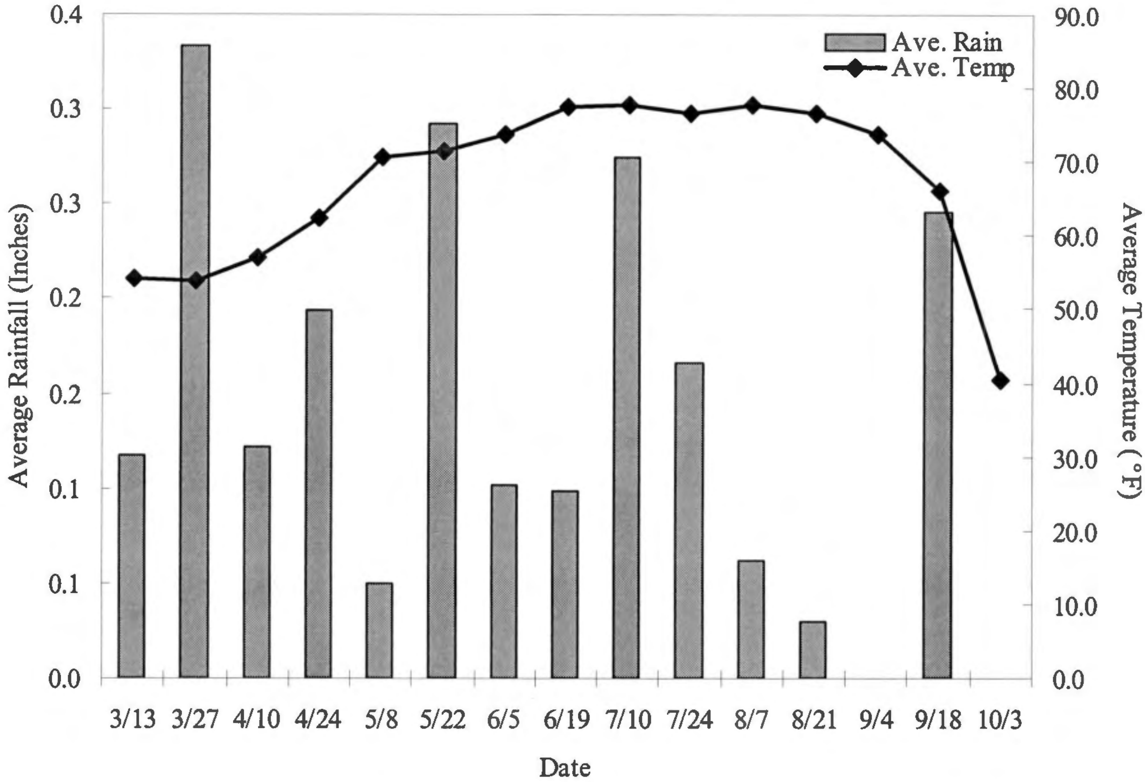


Figure 3.6. Biweekly average temperature and biweekly average rainfall amounts collected from the Knoxville McGhee Tyson Airport, Blount County, Tennessee, from 13 March - 17 October 2000.

average temperatures squared. The r-square (0.70) of this statement is significant ($p < 0.0001$) with the combination of these variables.

Oviposition data of *Ae. albopictus* required transformation and were best described by temperature variables: $y = -51.85 + 0.053\ x_3 + 1.38\ x_5 - 0.01\ x_7^2$, where y = log (AVEA + 0.5), x_3 = average temperature, x_5 = previous average temperature and x_7^2 = average temperature squared. The r-square (0.61) of this statement is significant ($p < 0.0001$) for this combination of variables.

Aedes albopictus adult data were best described by year, rainfall and temperature variables: $y = -10.04 + 1.17\ x_2 + 0.11\ x_6 + 4.41\ x_4$, where y = average *Ae. albopictus* adults, x_2 = year, x_4 = two previous average rainfalls and x_6 = two previous average temperatures. The r-square (0.52) of this statement is significant ($p < 0.0001$) for these combined variables. To generate variables with the previous temperature and rainfall data, the beginning of the second and third years were given missing data points to prevent the use of previous year's data in the analysis.

v - Discussion

At all ten sites, *Ae. albopictus* eggs or adults were present each year of this study (Appendix 1, 2). In 1998, no *Oc. triseriatus* adults were collected from Anderson and Blount Counties (Gottfried 1999) but were collected from all sites 1999 - 2001. Since these two species occupy the same habitat and utilize the same food resources, the question of whether competitive displacement would occur has some answers. In Florida and Alabama, research suggests that *Ae. albopictus* is displacing *Ae. aegypti* (L.) populations (Hobbs et al. 1991, O'Meara et al. 1995). In Texas, after *Ae. albopictus* became established, the population of *Ae. albopictus* was found to be three and twenty times the populations of *Ae. aegypti* and *Oc. triseriatus*, respectively (Livdahl and Willey 1991). If *Ae. albopictus* successfully out-competes *Oc. triseriatus* in any areas of the country, including eastern Tennessee, a significant impact on the amount and type of disease activity and transmission in these areas is likely to occur (Oberg et al. 1996). Depending on the site, oviposition activity indicates that both *Oc. triseriatus* and *Ae. albopictus* activity fluctuates. This is possibly due to abiotic and biotic factors and the effects of sampling design.

At the ten sites sampled, a variation of fluctuations in oviposition activity was common. Overall, Oc. triseiratus oviposition activity peaks in June and Ae. albopictus peak oviposition occurs approximately one month later. The only site where oviposition activity of *Ae. albopictus* increased each year was in Sevier County, while at this same site *Oc. triseriatus* oviposition activity also increased. Four sites (Anderson, Grainger, Loudon and Roane Counties) had decreasing levels of oviposition activity during the four year study. Three sites (Hamblen, Knox and Union Counties) had similar amounts of *Ae. albopictus* oviposition activity. No obvious common traits are displayed by these two groups of sites.

Adult collections of *Oc. triseriatus* are typically lower than other adult female mosquitoes. It has been more difficult to monitor this species until oviposition traps were introduced because it is a diurnal species (Hanson et al. 1988). *Aedes albopictus* are diurnal as well but were found more abundantly in adult traps. In contrast to adult collections, more eggs of *Oc. triseratus* than eggs *Ae. albopictus* were collected in oviposition traps during all four years of this study. Ovitraps are not the most effective sampling methods for monitoring mosquito populations because they are influenced by competing oviposition sites and rainfall (Landry and DeFoliart 1986). The use of both of these sampling methods, or combinations of these and other methods, may be the best way to get a clearer picture of the mosquito populations at individual sites in eastern Tennessee.

The climate data indicate that temperature is more important than rainfall for oviposition activity of *Oc. triseriatus* and *Ae. albopictus*. The month was also a significant factor which would seem to play a role in temperature fluctuations depending on the season. Container-inhabiting mosquito eggs are more susceptible to dessication than other types of mosquito eggs (Loor and DeFoliart 1970, Wilton 1968), but *Oc. triseriatus* has relatively drought-resistant eggs (Bradshaw and Holzapfel 1984). The effect of air temperature and its impact on water temperatures in small containers influences the development times of container-inhabiting mosquitoes (Haramis 1984, Hawley 1988). In western North Carolina, the seasonal occurrence of rainfall, and not the total amount of rainfall, had a greater effect on *Oc. triseriatus* oviposition activity (Szumlas et al. 1996). Desiccation, predation, temperature and photoperiod impact seasonal distribution of tree hole mosquitoes. *Ochlerotatus triseriatus* is susceptible to predation but can benefit from drought periods when predators may be reduced (Bradshaw and Holzapfel 1984). Benefits to container-inhabiting mosquitoes from periods without rainfall and higher temperatures include decreased developmental time, thus making them less available for predation.

Adult *Oc. triseriatus* data analysis was not performed due to the low and variable amounts of adult collections. *Aedes albopictus* adult data were impacted by year, temperature and rainfall. Increased temperature result in decreased larval development time and higher adult emergence in the laboratory while not effecting the size or survival of adult females (Alto and Juliano 2001a).

CHAPTER IV

CONCLUSIONS

i - Treatment Effects on Oviposition Sampling

The oviposition activity by *Ochlerotatus* (= *Aedes*) *triseriatus* and *Aedes albopictus* was significantly higher in cups where water was replenished than cups that had water replaced. This could be due to the higher organic content and the presence of larvae and pupae or the previous presence of larvae and pupae in the replenished cups (Allan and Kline 1998, Trexler et al. 1998). *Ochlerotatus triseriatus* had higher levels of oviposition activity than *Ae. albopictus* both years and at all sites. The higher level of oviposition activity for *Oc. triseriatus* (56,847) is not reflected in the number of reared adults (125) and host-seeking adult (149) *Oc. triseriatus* collected. *Aedes albopictus* had low levels of oviposition activity (45,644) but greater numbers of reared (800) and host-seeking (454) adults than *Oc. triseriatus* were collected. The discrepancy between ovitrap, reared and host-seeking adult collections may be due to environmental factors, differing developmental times and survivorship or differences in sampling methods.

More adults of *Ae. albopictus* than *Oc. triseriatus* emerged from field collected larvae and pupae in the laboratory, but no difference between the cup treatment was evident. In similar rearing activities in our laboratory, more *Ae. albopictus* adults emerge than *Oc. triseriatus* when there are more *Oc. triseriatus* eggs. In the future, identification and enumeration of the larvae and pupae would allow for calculation of survivorship for these two species.

The returned cups were not analyzed because of the many difficulties surrounding the use of this treatment in the study. Although the data were not analyzed, it is significant that this treatment was found not to be a useful measure of oviposition activity. Disease surveillance programs that need the greatest number of *Oc. triseriatus* and *Ae. albopictus* eggs should replenish the ovitrap water. Routine surveillance programs should replace the ovitrap water, which would reduce adult emergence from the artificial oviposition sites and would not provide additional oviposition sites. Either technique should be used consistently to reduce the amount of variation among traps since adding water as necessary, rather than replacing the water, will increase the appeal of the oviposition trap.

ii - Seasonal Distribution and Relative Abundance

Generally, *Oc. triseriatus* peak oviposition activity begins in May, approximately one month prior to the peak oviposition activity of *Ae. albopictus* in eastern Tennessee. The *Oc. triseriatus* oviposition activty wanes in late summer and *Ae. albopictus* oviposition activity decreases and continues into early fall. The difference in peak oviposition activity for the two species may aid in these two species coexistence and limit resource competition. There was also greater *Oc. triseriatus* oviposition activity than *Ae.albopictus* except for Hamblen and Sevier Counties. These sites are different from the others because they have greater amounts of trash present. The various containers present provide many sources for oviposition sites in addition to the ovitraps placed at these sites.

The presence of both species at all sites is a significant factor in recognizing the vector potential of these species in eastern Tennessee. The majority of adults collected were *Ae. albopictus*, *Ae. vexans* and some *Oc. triseriatus* were collected during the four year study. Since few flood water mosquitoes were collected during the four years, mosquito complaints reported to officials are likely the result of container-inhabiting mosquitoes.

iii - Blount County Weather Analysis

Temperature variables are significant factors impacting *Oc. triseriatus* and *Ae. albopictus* oviposition activity in Blount County. The temperatures may be the average temperature during and up to one month before the sampling period. *Aedes albopictus* adults were influenced by average temperature and average rainfall four weeks prior to the sampling period. The temperature effect on oviposition activity may be a result of the temperature influence on the adults. Rainfall is likely to have a significant impact on container-inhabiting mosquitoes in natural containers, but in this research it may not have because oviposition cups were supplied water each week.

In future research, it may be important to examine the effects of monthly and seasonal climatic factors and their impact on oviposition and adult activity of *Oc. triseriatus* and *Ae. albopictus*.

REFERENCES CITED

Ailes, M. C. 1998. Failure to predict abundance of saltmarsh mosquitoes *Aedes sollicitans* and *Ae. taeniorhynchus* (Diptera: Culicidae) by using variables of tide and weather. J. Med. Entomol. 35: 200-204.

Allan, S. A. and D. L. Kline. 1995. Evaluations of organic infusions and synthetic compounds mediating oviposition. J. Chem. Ecology 21: 1847-1859.

Allan, S. A. and D. L. Kline. 1998. Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Aedes albopictus.* J. Med. Entomol. 35: 943-947.

Alto, B. W. and S. A. Juliano. 2001a. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. J. Med. Entomol. 38: 548-556.

Alto, B. W. and S. A. Juliano. 2001b. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): Implications for range expansion. J. Med. Entomol. 38: 646-656.

Arnold, F. T. Jr. 1940. The mosquitoes of east Tennessee. M.S. Thesis, The University of Tennessee, Knoxville.

Atkins, C. E., T. C. DeFrancesco, J. R. Coats, J. A. Sidley and B. W. Keene. 2000. Heartworm infection in cats: 50 cases (1985-1997). J. Am. Vet. Med. Assoc. 217: 355-358.

Barrera, R. 1996. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. Ecol. Entomol. 21: 117-127.

Bentley, M. D., I. N. McDaniel, H. Lee, B. Stiehl and M. Yatagai. 1976. Studies of *Aedes triseriatus* oviposition attractants produced by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). J. Med. Entomol. 13: 112-115.

Berry, W. J., D. Fish and G. B. Craig. 1980. The use of an ovitrap grid for measuring adult movement and population density of the treehole mosquito, *Aedes triseriatus*. Proc. Ind. Acad. Sci. 89: 208-209.

Boreham, P. F. and R. B. Atwell. 1988. Dirofilariasis. CRC Press, Boca Raton, Florida.

Bradshaw, W. E. and C. M. Holzapfel. 1983. Predator-mediated, non-equilibrium coexistence of tree-hole mosquitoes in southeastern North America. Oecologia 57: 239-256.

Breeland, S. G., W. E. Snow and E. Pickard. 1961. Mosquitoes of the Tennessee Valley. J. Tenn. Acad. Sci. 36: 249-319.

Centers for Disease Control. 2001. Epidemic/epizootic West Nile virus in the United States: Revised guidelines for surveillance, prevention and control. (http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-apr-2001.pdf)

Chadee, D. D., P. S. Corbet and J. J. D. Greenwood. 1990. Egg-laying yellow fever mosquitoes avoid sites containing eggs laid by themselves or by conspecifics. Entomol. Exp. Appl. 57: 295-298.

Comiskey, N. M., R. C. Lowrie, Jr. and D. M. Wesson. 1999. Role of habitat components on the dynamics of *Aedes albopictus* (Diptera: Culicidae) from New Orleans. J. Med. Entomol. 36: 313-320.

Craig, G. B., Jr. 1983. Biology of *Aedes triseriatus*: Some factors affecting control, pp. 329-341. *In* Calisher, C. H. and W.H. Thomson [eds.], California serogroup viruses. A.R. Liss, Inc., New York.

Darsie, R. F, Jr. 1986. The identification of *Aedes albopictus* in the Nearctic region. J. Am. Mosq. Control Assoc. 2: 336-340.

Darsie, R. F., Jr. and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Mosq. Syst. Supplement 1: 1-313.

Edgerly, J. S., M. S. Willey and T. P. Livdahl. 1993. The community ecology of *Aedes* egg hatching: Implications for a mosquito invasion. Ecol. Entomol. 18: 123-128.

Estrada-Franco, J. G. and G. B. Craig, Jr. 1995. Biology, disease relationships, and control of *Aedes albopictus*. Tech. Paper 42. Pan American Health Organization, Washington, D.C.

Fish, D. 1983. Phytotelmata: Flora and fauna, pp. 1-28. *In* Frank, J. H. and L. P. Lounibos [eds.], Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publ., Inc., Medford, New Jersey.

Fish, D. and S. R. Carpenter. 1982. Leaf litter and larval mosquito dynamics in tree-hole ecosystems. Ecology 63: 283-288.

Furlow, B. M. and W. W. Young. 1970. Larval surveys compared to ovitrap surveys for detecting *Aedes aegypti* and *Aedes triseriatus*. Mosq. News 30: 468-470.

Gerhardt, R. R., K. L.Gottfried, C. S. Apperson, B. S. Davis, 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 Infect. Dis. 7: 807-811.

Gottfried, K. L. 1999. Seasonal distribution and abundance of the mosquito species (Diptera: Culicidae) in eastern Tennessee. The University of Tennessee, Knoxville.

Gottfried, K. L., R. R. Gerhardt, R. S. Nasci, N. Karabatsos, B. S. Davis, N. A. Panella and D. J. Paulsen. 2002. Temporal abundance, parity, survival rates, and arbovirus isolation of field collected container-inhabiting mosquitoes in eastern Tennessee. J. Am. Mosq. Control Assoc. *In Press.*

Hanson, S. M. and G. B. Craig, Jr. 1995. *Aedes albopictus* (Diptera: Culicidae) eggs: Field survivorship during northern Indian winters. J. Med. Entomol. 32: 599-604.

Hanson, S. M., M. Song and G. B. Craig, Jr. 1988. Urban distribution of *Aedes triseriatus* in northern Indiana. J. Am. Mosq. Control Assoc. 4: 15-19.

Haramis, L. D. 1984. *Aedes triseriatus*: A comparison of density in tree holes versus discarded tires. Mosq. News 44: 485-489.

Haramis, L. D. 1985. Larval nutrition, adult body size, and the biology of *Aedes triseriatus*, pp. 431- 438. *In* Lounibos, L. P., J. R. Rey and J. H. Frank [eds.], Ecology of mosquitoes: Proceedings of a workshop. Florida Medical Entomology Laboratory, Vero Beach, Florida.

Harwood, R. F. and M. T. James. 1979. Entomology in human and animal health. pp. 169-233. 7th edition. MacMillan Publ. Co., Inc., New York.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4 (Suppl.1): 1-40.

Ho, B. C., A. Ewert and L. Chew. 1989. Interspecific competition among *Aedes aegypti*, *Ae. albopictus* and *Ae. triseriatus* (Diptera: Culicidae) larval development in mixed cultures. J. Med. Entomol. 26: 615-623.

Hobbs, J. H., E. A. Hughes and B. H. Eichold II. 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile, Alabama. J. Am. Mosq. Control Assoc. 7: 488-489.

Holck, A. R., C. L. Meek and J. C. Holck. 1988. Attractant enhanced ovitraps for the surveillance of container breeding mosquitoes. J. Am. Mosq. Control Assoc. 4: 97-98.

Hribar, L. J. 1984. A potential vector of dog heartworm in Knox County, Tennessee. M.S. Thesis. The University of Tennessee, Knoxville.

Hribar, L. J. and R. R. Gerhardt. 1986. A checklist of the mosquitoes (Diptera: Culicidae) occurring in Knox County, Tennessee. J. Tenn. Acad. Sci. 61: 6-7.

Jenkins, D. W. and S. J. Carpenter. 1946. Ecology of the tree hole breeding mosquitoes of nearctic North America. Ecol. Mono. 16: 32-47.

Jones, T. F., A. S. Craig, R. S. Nasci, L. E. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery and W. Schaffner. 1999. Newly recognized focus of La Crosse encephalitis in Tennessee. Clin. Infec. 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.31-42. *In* Calisher, C.H. and W. H. Thompson [eds.], California serogroup viruses. A. R. Liss, Inc., New York.

Kaufman, M. G., E. D. Walker, T. W. Smith, R. W. Merritt and M. Klug. 1999. Effects of larval mosquitoes (*Aedes triseriatus*) and stemflow on microbial community dynamics in container habitats. App. Env. Micro. 65: 2661-2673.

Kitching, R. L. 1971. An ecological study of the water-filled tree holes and their position in the woodland ecosystem. J. Anim. Ecol. 40: 281-302.

Kitching, R. L. 1983. Community structure in water-filled tree holes in Europe and Australia- Comparisons and explanations, pp. 205-222. *In* Frank, J. H. And L. P. Lounibos [eds.], Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Inc., Medford, New Jersey.

Kitching, R. L. 2000. Food webs and container habitats: The natural history and ecology of phytotelmata. Cambridge University Press, Cambridge, UK.

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: 462-467.

Lampman, R. L. and R. J. Novak. 1996. Attraction of *Aedes albopictus* adults to sod infusion. J. Am. Mosq. Control Assoc. 12: 119-124.

Landry, S. V. and G. R. DeFoliart. 1986. Attraction of *Aedes triseriatus* to carbon dioxide. J. Am. Mosq. Control Assoc. 2: 355-357.

Lang, J. T.1990. Ovipositional response of *Aedes triseriatus* females to horizontally and vertically open ovitraps in southern Illinois. J. Am. Mosq. Control Assoc. 6: 530-531.

Leiser, L. 1981. Distribution of *Aedes triseriatus* (Say) in an urban area comparison of two survey methods. Proc. Ind. Acad. Sci. 90: 248-253.

Livdahl, T. P. and R. K. Koenekoop. 1985. The nature of egg hatching in *Aedes triseriatus*: Ecological implications and evolutionary consequences, pp.439-458. *In* Lounibos, L. P., J. R. Rey and J. H. Frank [eds.], Ecology of mosquitoes: Proceedings of a workshop. Florida Medical Entomology Laboratory, Vero Beach, Fla.

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. 1969. Oviposition trap for detecting the presence of *Aedes triseriatus* (Say). Mosq. News 29: 487-488.

Loor, K. A. and G. R. DeFoliart. 1970. Field observations on the biology of *Aedes triseriatus*. Mosq. News 30: 60-64.

Lounibos, L. P., N. Nishimura and R. L. Escher. 1993. Fitness of a tree hole mosquito: Influences of food type and predation. Oikos 66: 114-118.

Lounibos, L. P., R. L. Escher, N. N. Shimura and S. A. Juliano. 1997. Long-term dynamics of a predator used for biological control and decoupling from mosquito prey in a subtropical tree hole ecosystem. Oecologia 111: 189-200.

Mahmood, F., W. J. Crans and N. S. Savur. 1997. Larval competition in *Aedes triseriatus* (Diptera: Culicidae): Effects of density on size, growth, sex ratio and survival. J. Vector Ecol. 22: 90-94.

Mather, T. N. and G. R. DeFoliart. 1984. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. J. Med. Entomol. 21: 384-391.

Merritt, R. W, R. H. Dadd and E. D. Walker. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annu. Rev. Entomol. 37: 349-376.

Moore, C. G. 1999. *Aedes albopictus* in the United States: Current status and prospects for further spread. J. Am. Mosq. Control Assoc.15: 221-227.

Moore, C. G. and C. J. Mitchell. 1997. *Aedes albopictus* in the United States: Ten year presence and public health implications. Emerg. Infect. Diseases 3: 329-334.

Moore, J. P. 1998. *Aedes albopictus* (Diptera: Culicidae) occurrence throughout Tennessee, with biological notes. Entomol. News 109: 363-365.

Nasci, R. S. 1988. Biology of *Aedes triseriatus* (Diptera: Culicidae) developing in tires in Louisiana. J. Med Entomol. 25: 402- 405.

Nieblyski, 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: 447- 450.

Oberg, A. L., L. J. Young and L. G. Higley. 1996. A comparison of two measures of competition. J. Ag. Bio. Environ. Statistics 1: 393-403.

Ogg, N. 1975. Species composition, relative abundance, and seasonal distribution of mosquitoes (Diptera: Culicidae) of Knox County. M. S. Thesis. The University of Tennessee, Knoxville.

O'Meara, G. F., L. F. Evans Jr., A. D. Gettman and J. P. Cuda. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera:Culicidae) in Florida. J. Med. Entomol. 32: 554-562.

Paradise, C. J. and W. A. Dunson. 1998. Relationship of atmospheric deposition to the water chemistry and biota of treehole habitats. Environ. Toxicol. Chem. 17: 362-368.

Pratt, H. D. and A. S. Kidwell. 1969. Eggs of mosquitoes found in *Aedes aegypti* oviposition traps. Mosq. News 29: 545-548.

Richardson, J. H., W. E. Barton and D. C. Williams. 1995. Survey of container-inhabiting mosquitoes in Clemson, South Carolina, with emphasis on *Aedes albopictus*. J. Am. Mosq. Control Assoc.11: 396-400.

SAS System for Windows, Release 8.2. 2001. SAS Institute Inc., Cary, North Carolina.

Schreiber, E. T., C. L. Meek and M. M. Yates. 1988. Vertical distribution and species coexistence of tree hole mosquitoes in Louisiana. J. Am. Mosq. Control Assoc. 4: 9-14.

Shields, S. E. 1938. Tennessee valley mosquito collection. J. Econ. Entomol. 31: 426-430.

Smith, T. W., E. D. Walker and M. G. Kaufman. 1998. Bacterial density and survey of cultivable heterotrophs in the surface water of a freshwater marsh habitat of *Anopheles quadrimaculatus* larvae (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 14: 72-77.

Smith, A. B. 2001. A study of La Crosse encephalitis in eastern Tennessee. M. S. Thesis. The University of Tennessee, Knoxville.

Sota, T., M. Mogi and E. Hayamizu. 1994. Habitat stability and the larval community in treeholes and other communities on a temperate island. Res. Popul. Ecol. 36: 93-104.

Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 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: 649-650.

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: 184-193.

Takken, W. 1999. Chemical signals affecting mosquito behaviour. Invert. Reprod. Develop. 36: 67-71.

Teng, H. 1993. Environmental determinants of intra and interspecific competition in the container-breeding mosquitoes *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae). Ph.D. Dissertation. North Carolina State University, Raleigh, North Carolina.

Trexler, J. D., C. S. Apperson and C. Schal. 1998. Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. J. Med. Entomol. 35: 967-976.

Undeen, A. H. and J. J. Becnel. 1994. A device for monitoring populations of larval mosquitoes in container habitats. J. Am. Mosq. Control Assoc. 10: 101-103.

Vandyk, J. K. and W. A. Rowley. 1995. Response of Iowa mosquito populations to unusual precipitation patterns as measured by New Jersey light trap collections. J. Am. Mosq. Control Assoc. 11: 200-205.

Vrtiska, L. A. and L. G. Pappas. 1984. Chemical analysis of mosquito larval habitats in southeastern Nebraska. Mosq. News 44: 506-509.

Walker, E. D., D. L. Lawson, R. W. Merritt, W. T. Morgan and M. J. Klug. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. Ecology 72: 1529-1546.

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: 125-127.

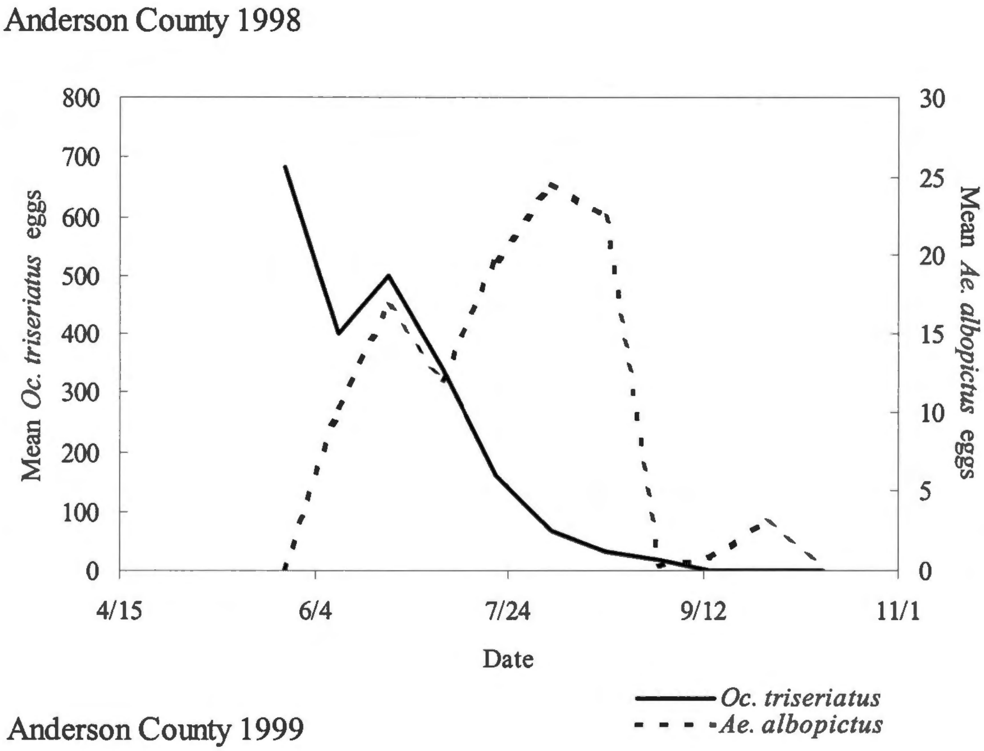
Wegbreit, J. and W. K. Reisen. 2000. Relationships among weather, mosquito abundance, and encephalitis virus activity in California: Kern County 1990-1998. J. Am. Mosq. Control Assoc. 16: 22-27.

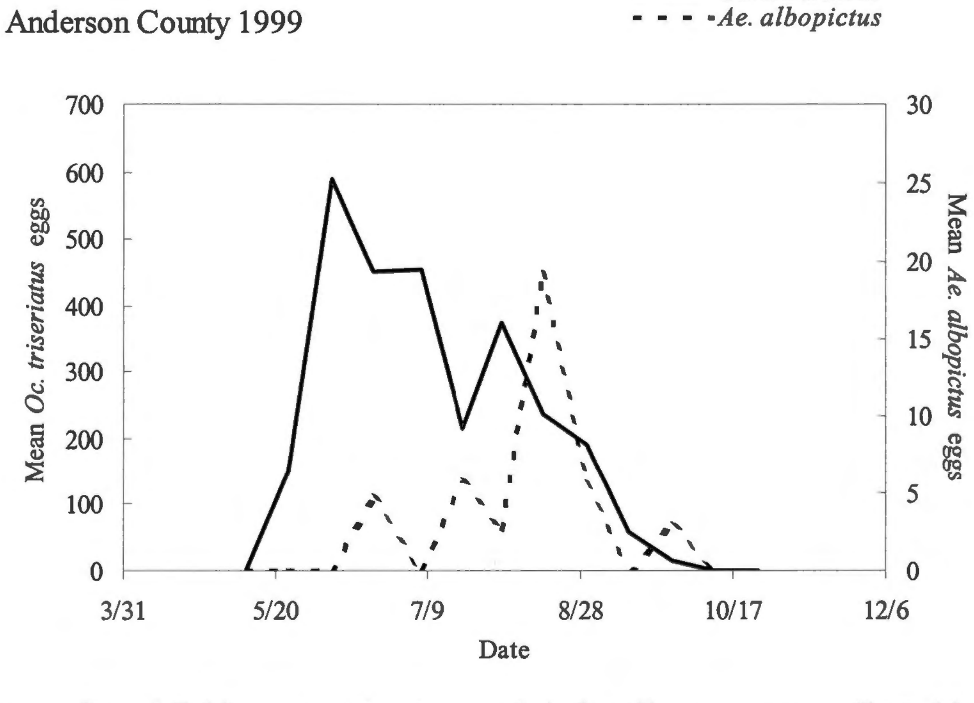
Wilton, D. P. 1968. Oviposition site selection by the tree-hole mosquito, *Aedes triseriatus* (Say). J. Med. Entomol. 5: 189-194.

Yap, H.H., C.Y. Lee, N.L. Chong, A.E.S. Foo and M.P. Lim. 1995. Oviposition site preference of *Aedes albopictus* in the laboratory. J. Am. Mosq. Control Assoc. 11: 128-132.

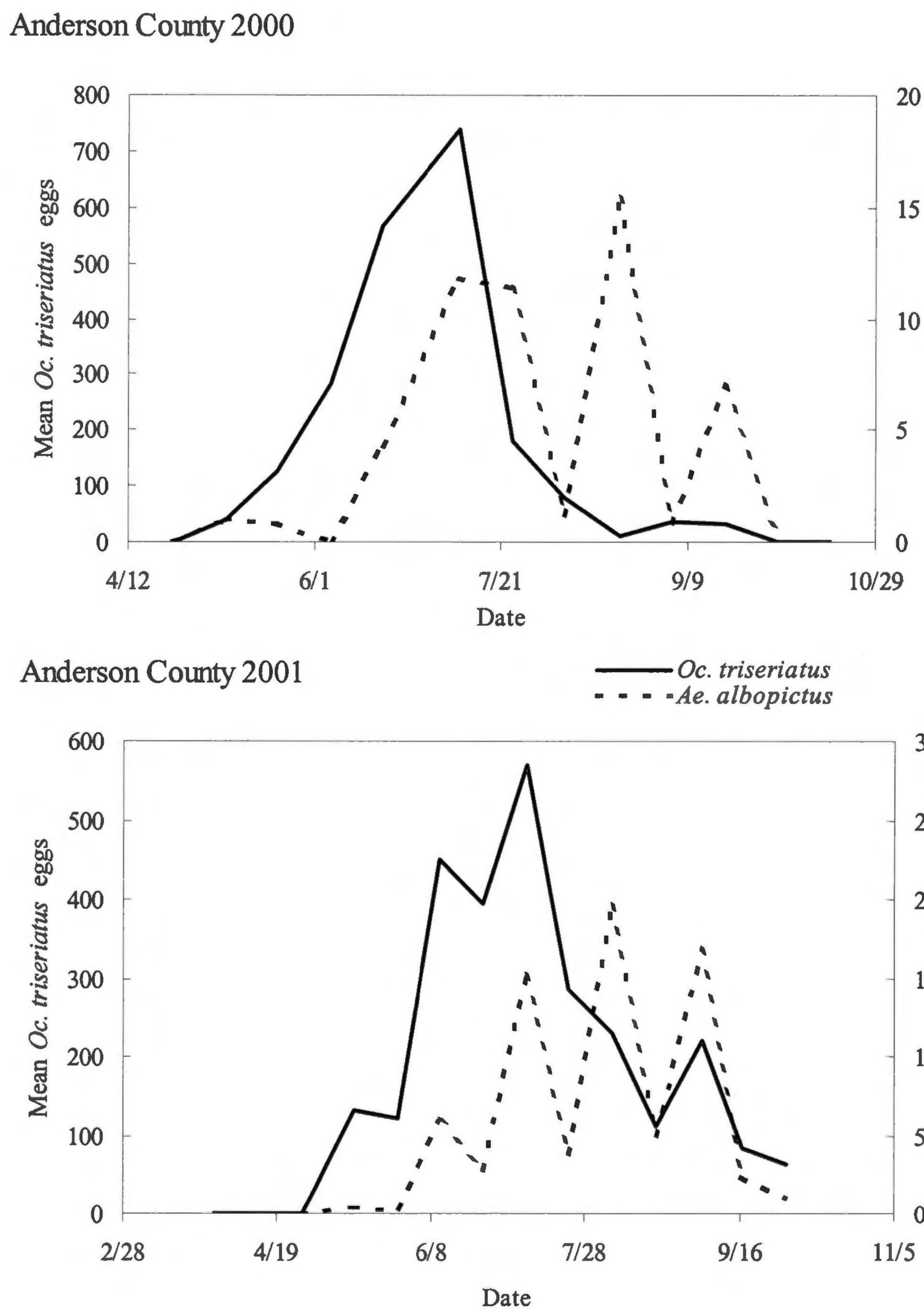
APPENDICES

APPENDIX 1. Four Year *Ochlerotatus triseriatus* and *Aedes albopictus* Oviposition Data for Tennessee Valley Authority Collections



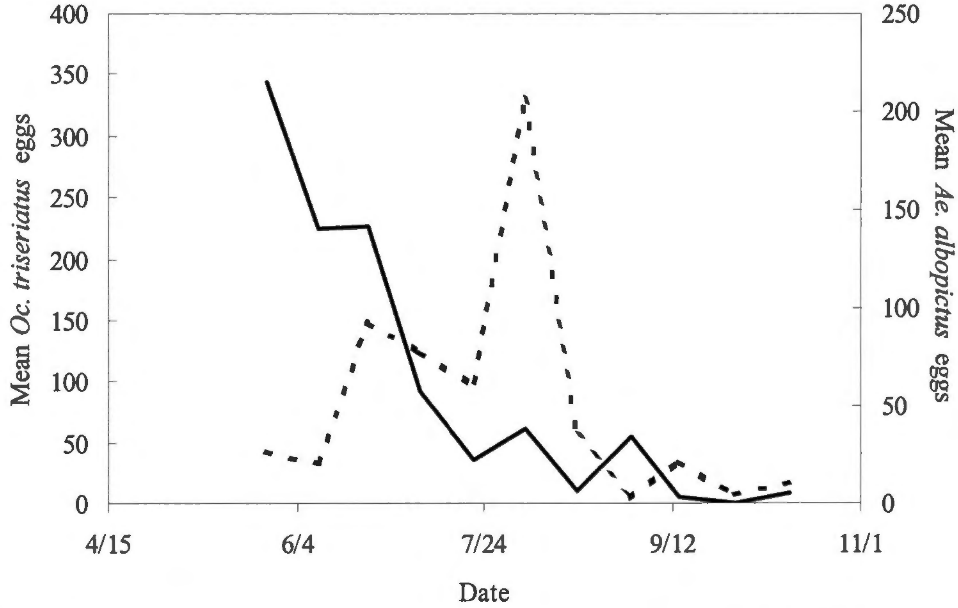


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from The University of Tennessee Arboretum, Anderson County, from 9 June - 26 October 1998 and 26 April - 25 October 1999.

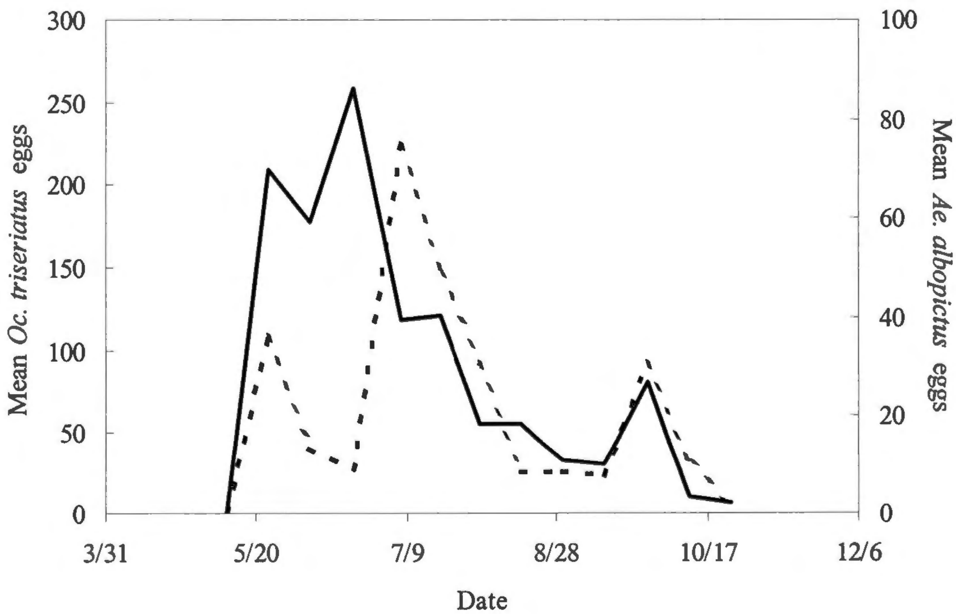


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from The University of Tennessee Arboretum, Anderson County, from 13 March - 17 October 2000 and 30 March - 14 October 2001.

Blount County 1998

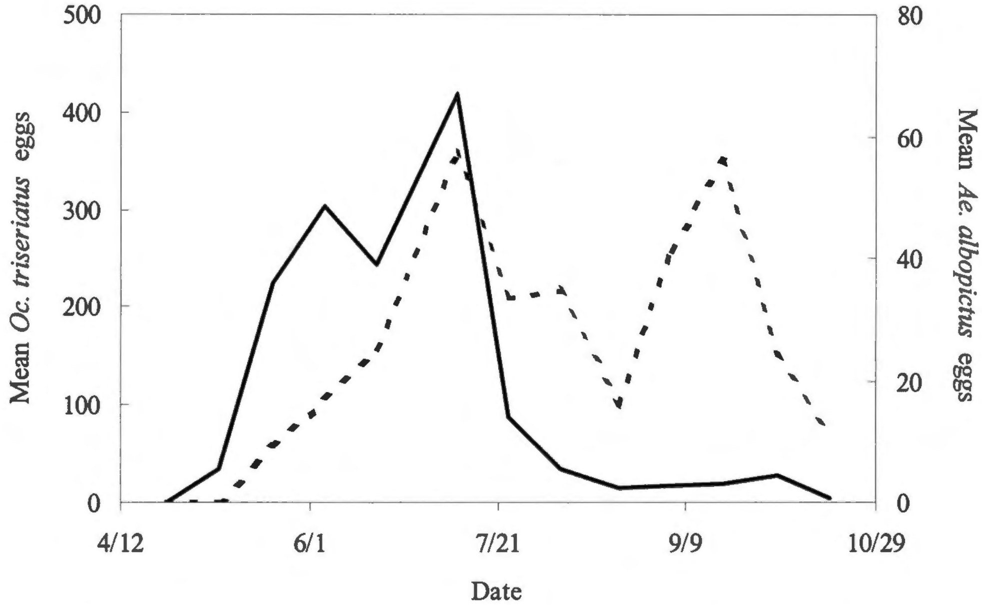


Blount County 1999

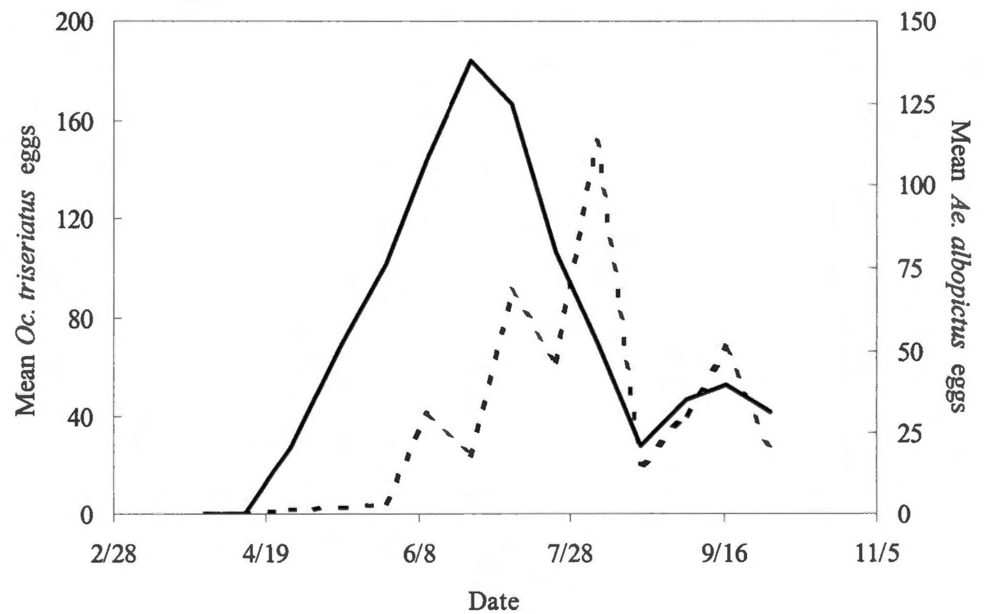


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Lousiville, Blount County, from 9 June - 26 October 1998 and 26 April - 25 October 1999.

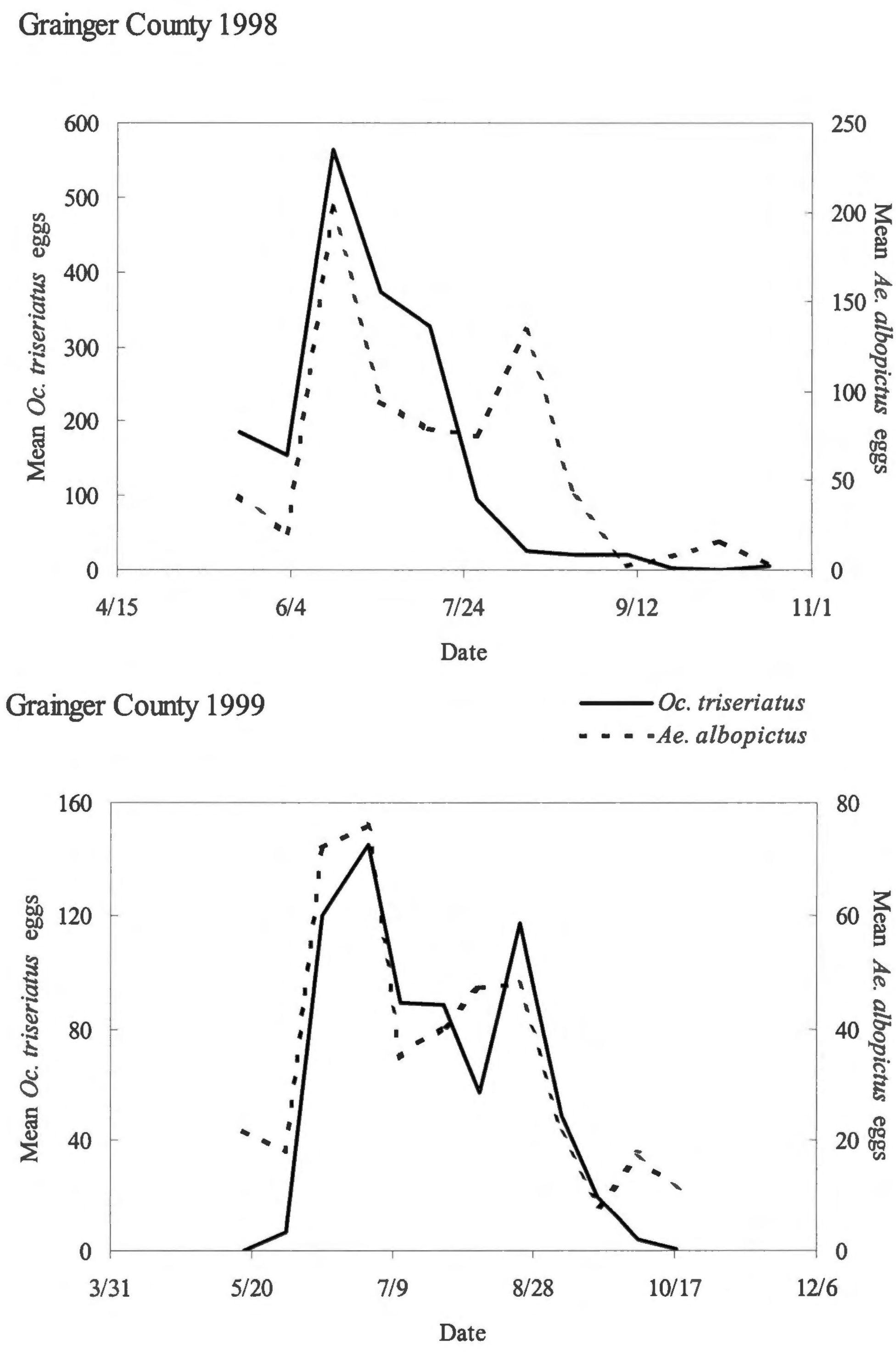
Blount County 2000



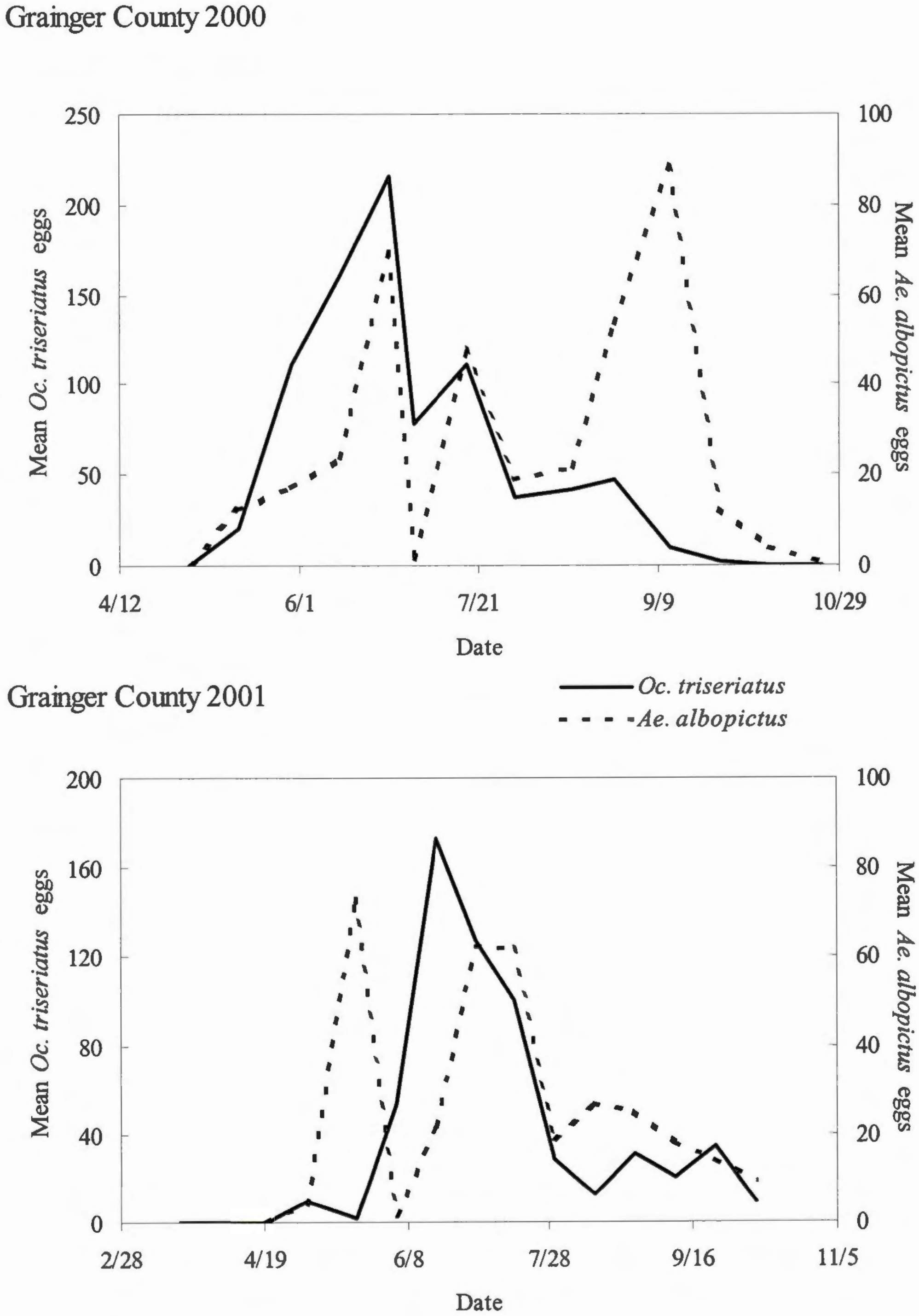
Blount County 2001



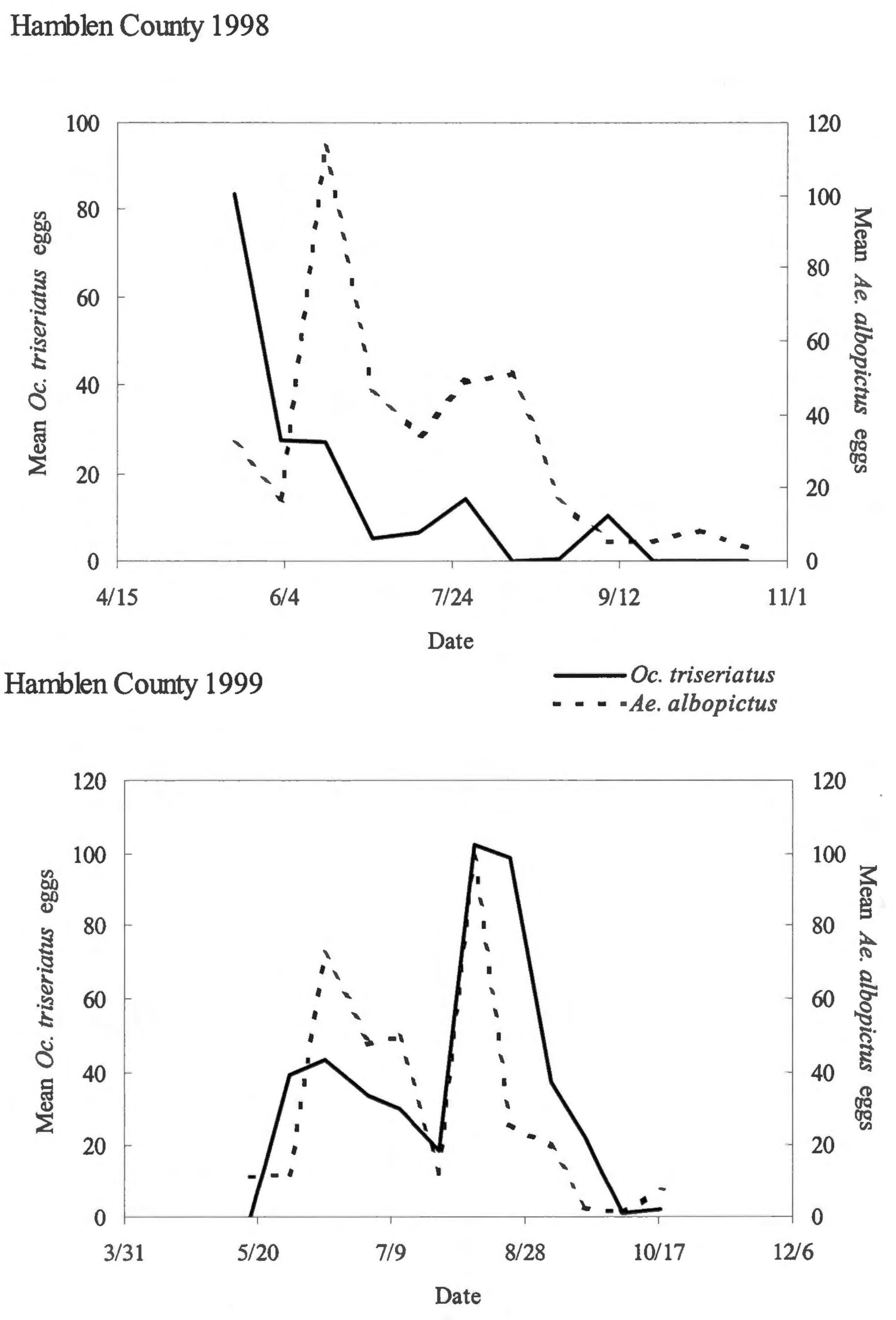
Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Lousiville, Blount County, from 13 March - 17 October 2000 and 30 March - 14 October 2001.



Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Bean Station, Grainger County, from 2 June - 2 November 1998 and 3 May - 1 November 1999.

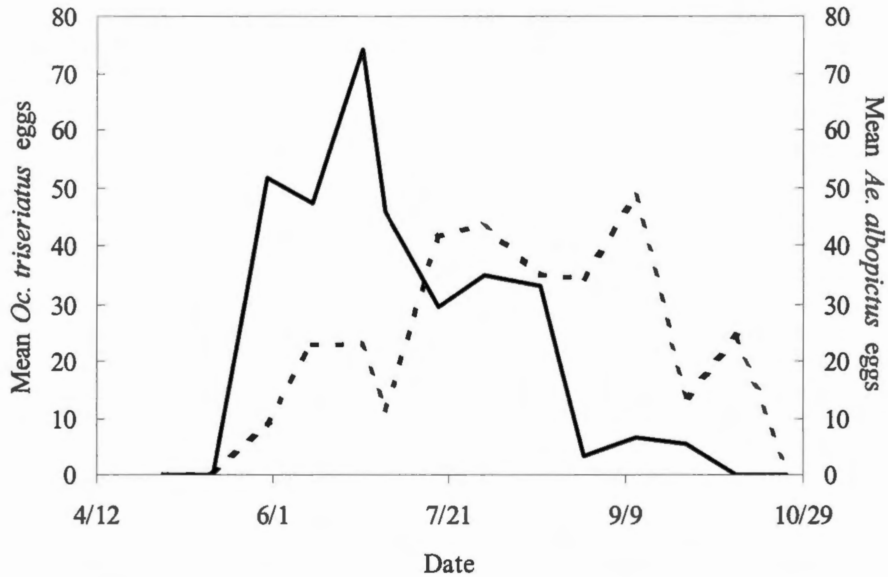


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Bean Station, Grainger County, from 21 March - 24 October 2000 and 21 March - 22 October 2001.

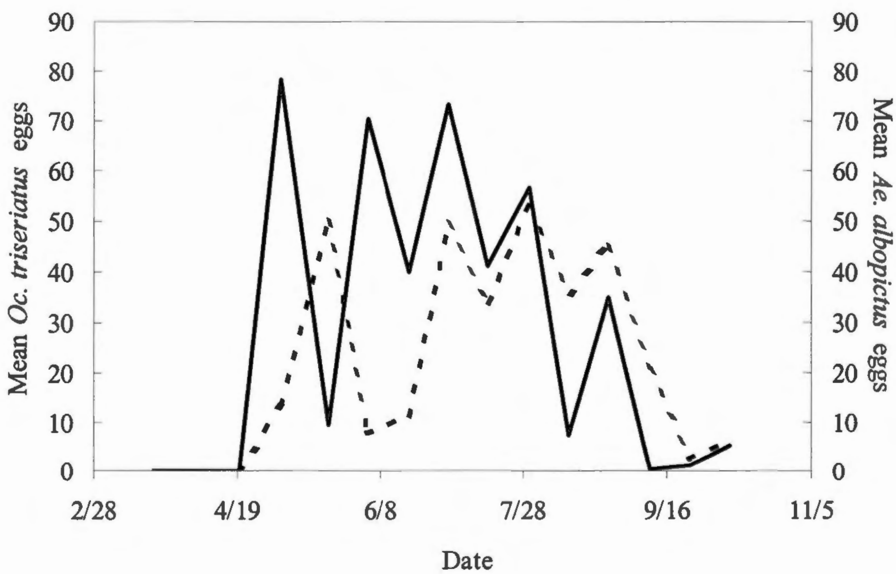


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Morristown, Hamblen County, from 2 June - 2 November 1998 and 3 May - 1 November 1999.

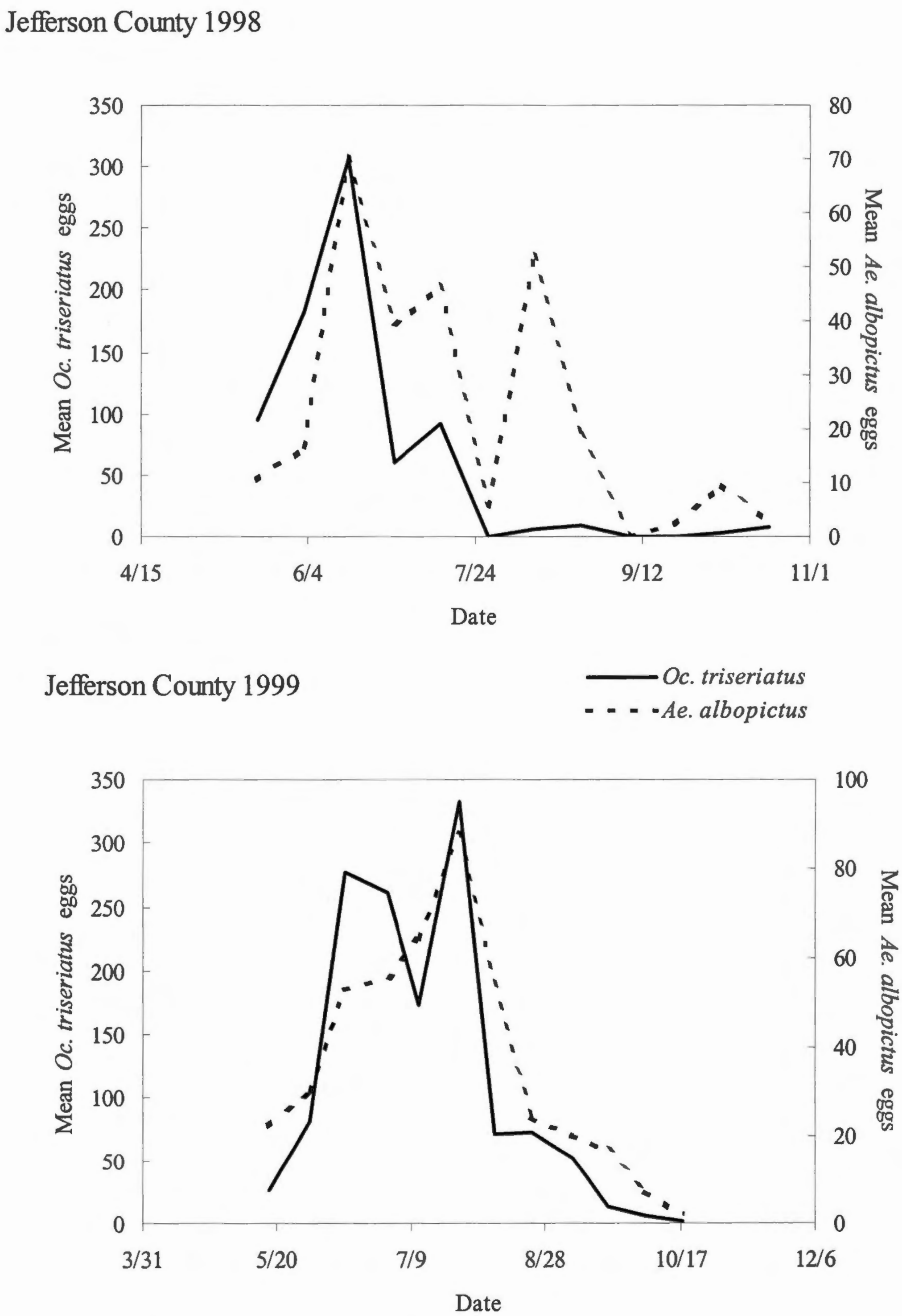
Hamblen County 2000



Hamblen County 2001

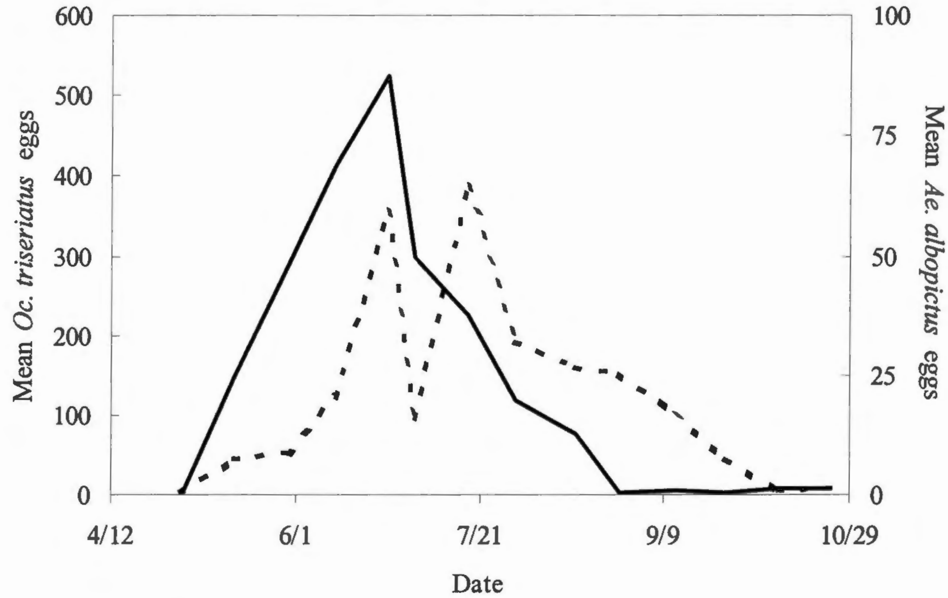


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Morristown, Hamblen County, from 21 March - 24 October 2000 and 21 March - 22 October 2001.

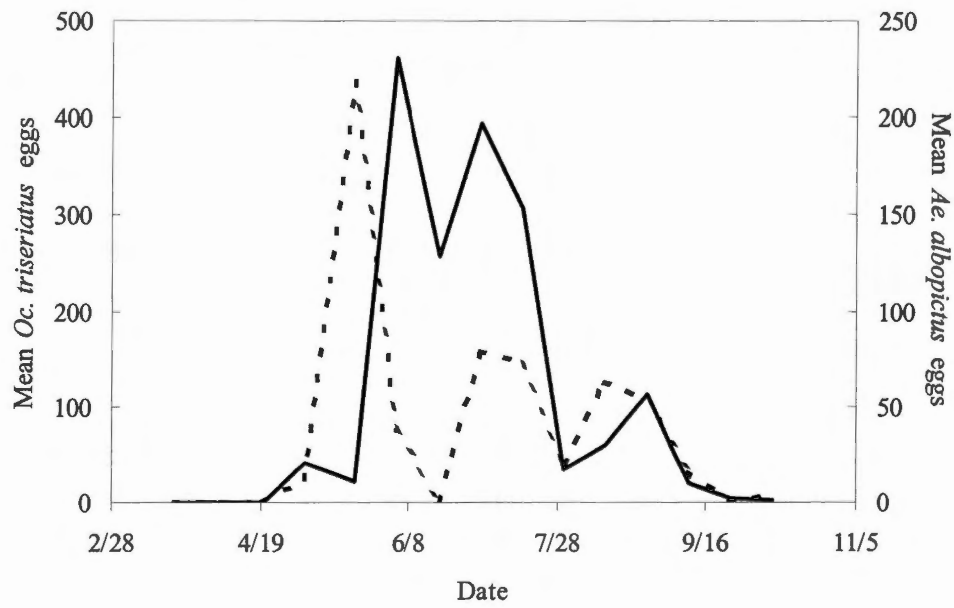


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovtiraps from Dandridge, Jefferson County, from 2 June - 2 November 1998 and 3 May - 1 November 1999.

Jefferson County 2000

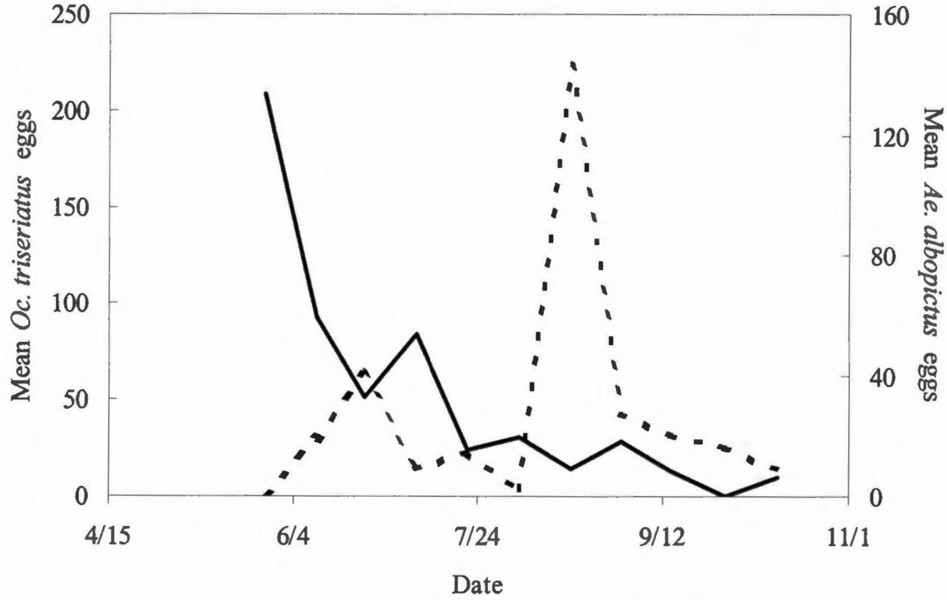


Jefferson County 2001

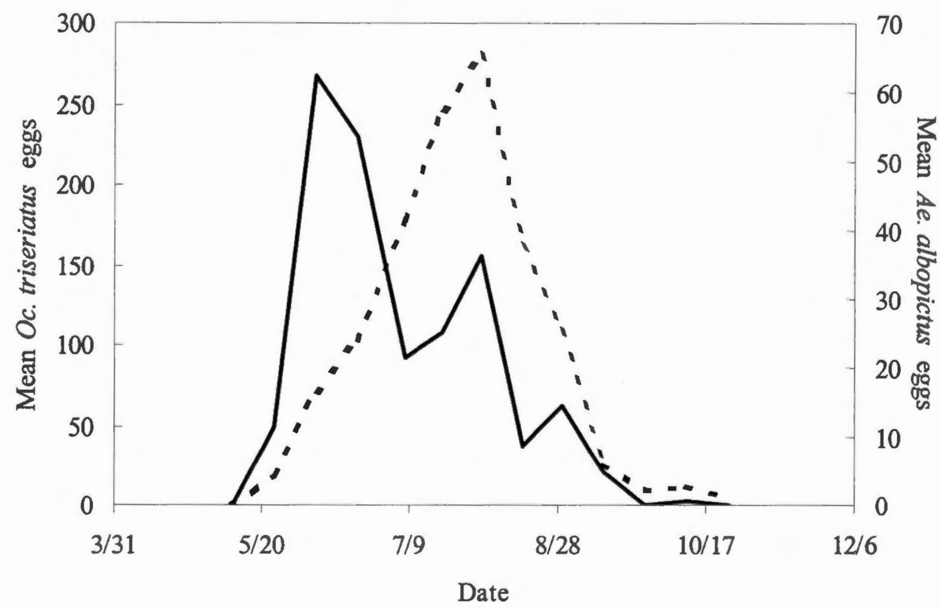


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovtiraps from Dandridge, Jefferson County, from 21 March - 24 October 2000 and 21 March - 22 October 2001.

Knox County 1998

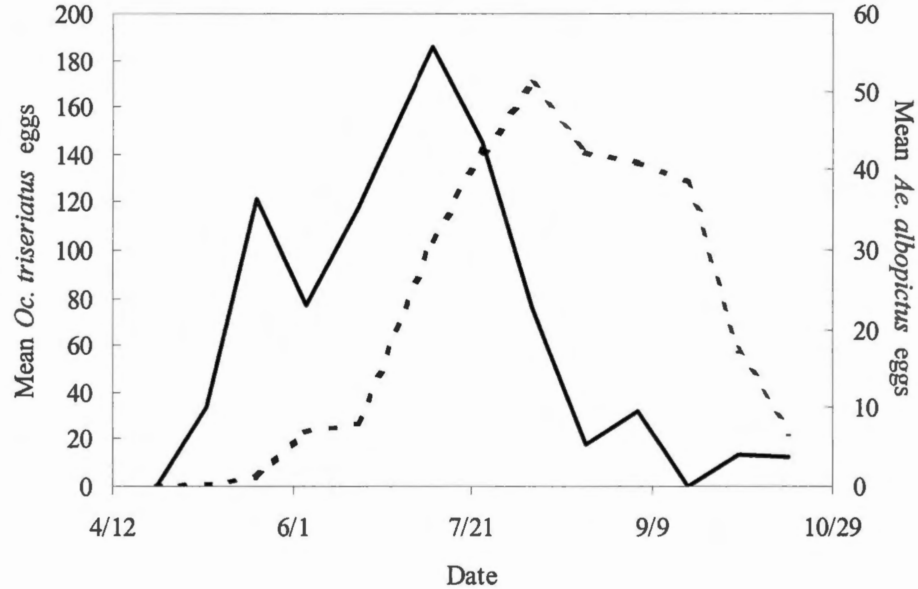


Knox County 1999

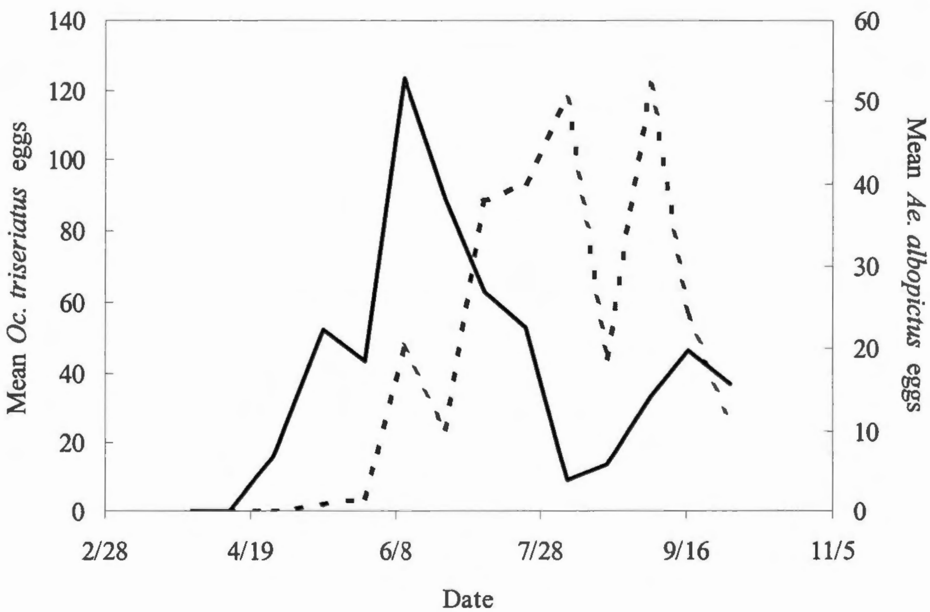


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Island Home, Knox County, from 9 June - 26 October 1998 and 26 April - 25 October 1999.

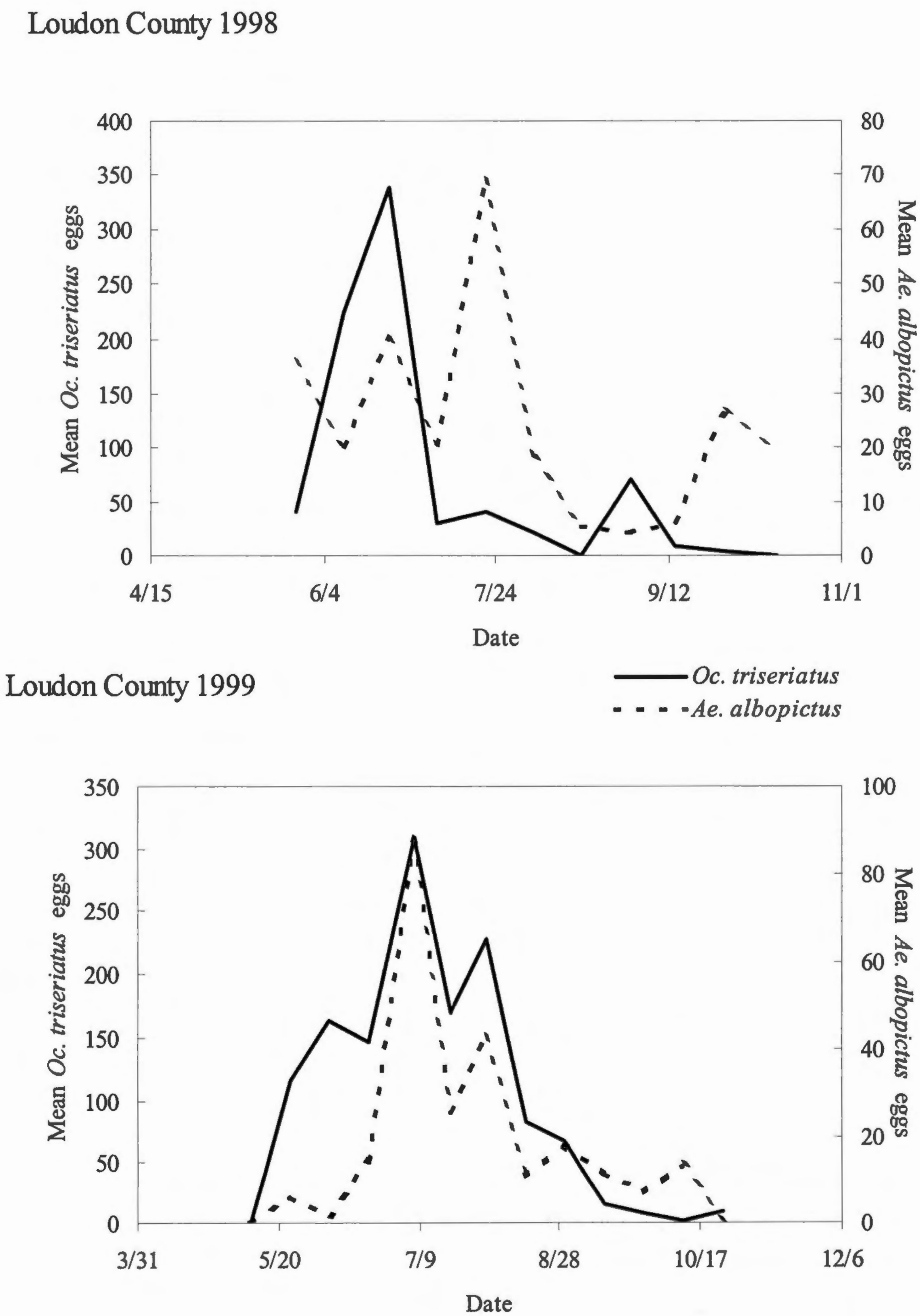
Knox County 2000



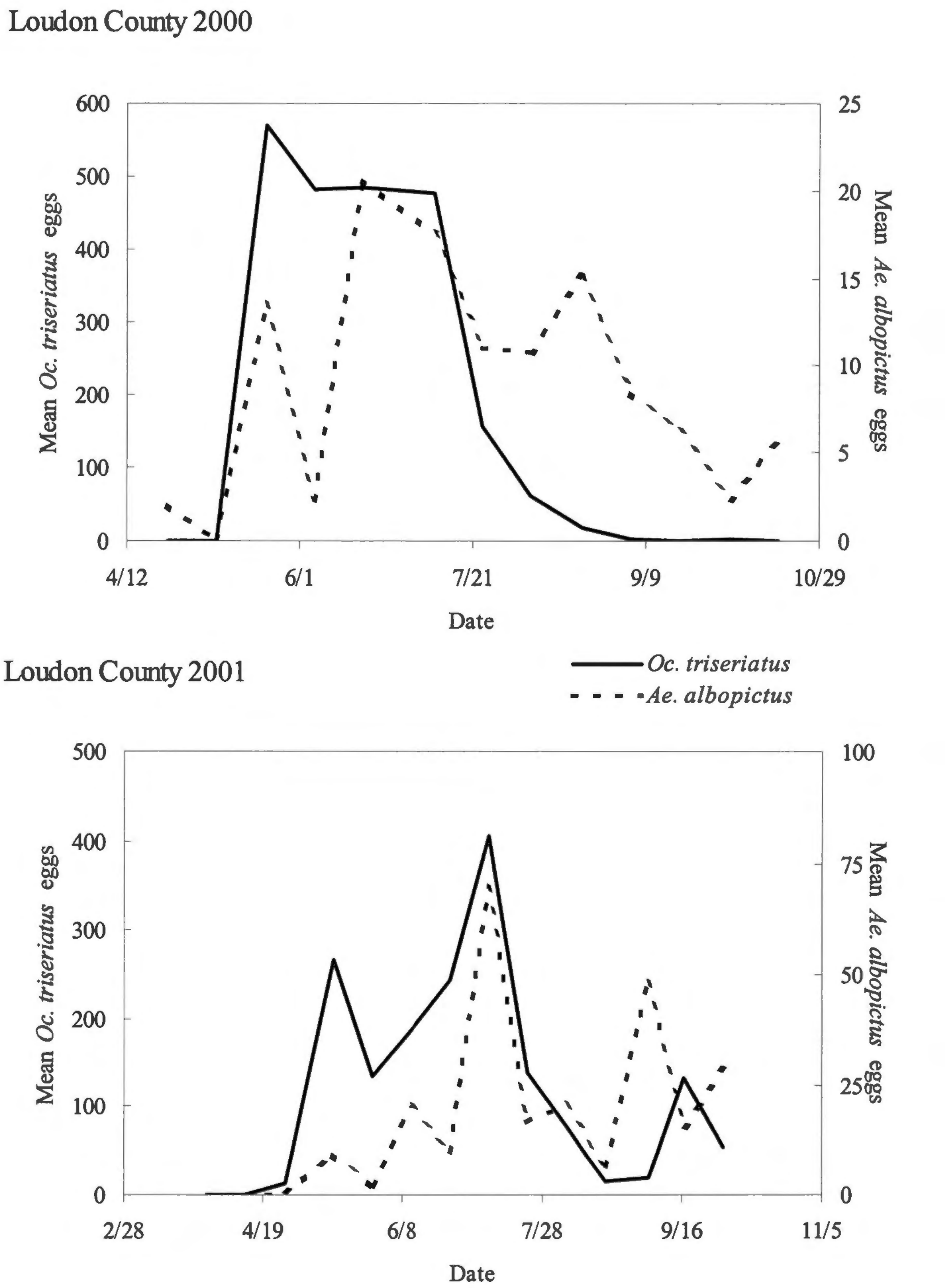
Knox County 2001



Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Island Home, Knox County, from 13 March - 17 October 2000 and 30 March - 14 October 2001.

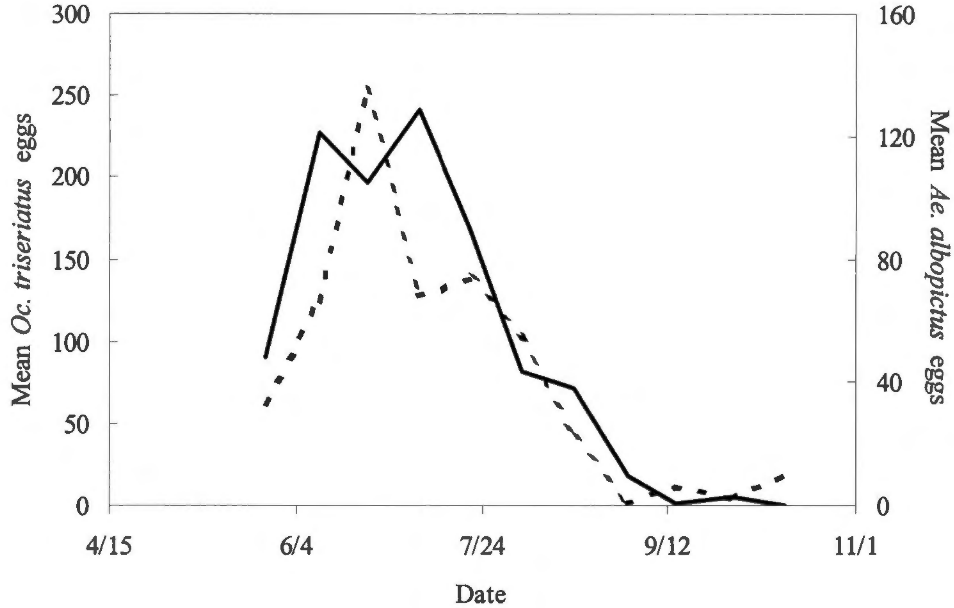


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Lenoir City, Loudon County, from 9 June - 26 October 1998 and 26 April - 25 October 1999.

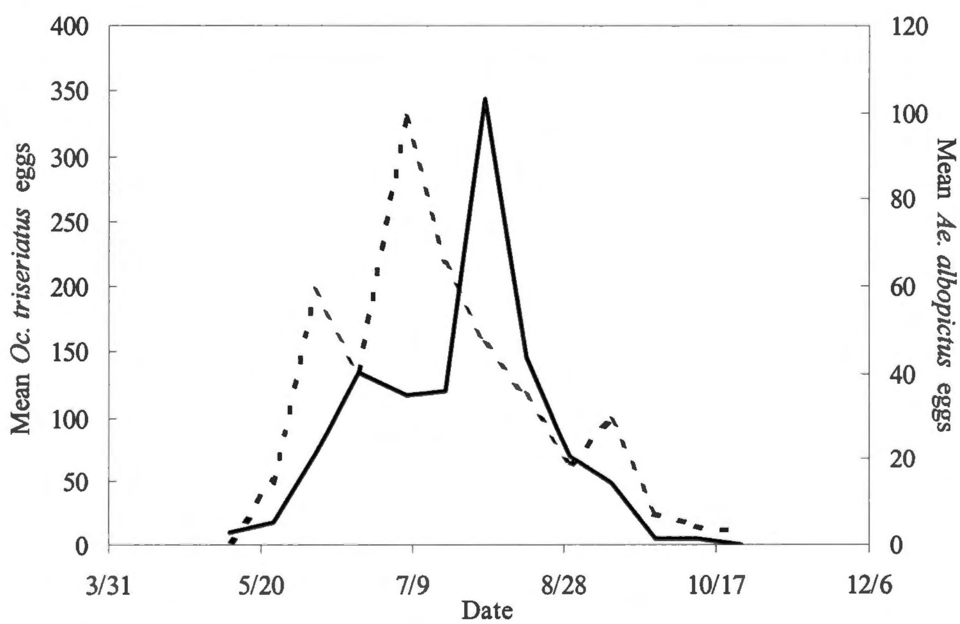


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Lenoir City, Loudon County, from 13 March - 17 October 2000 and 30 March - 14 October 2001.

Roane County 1998

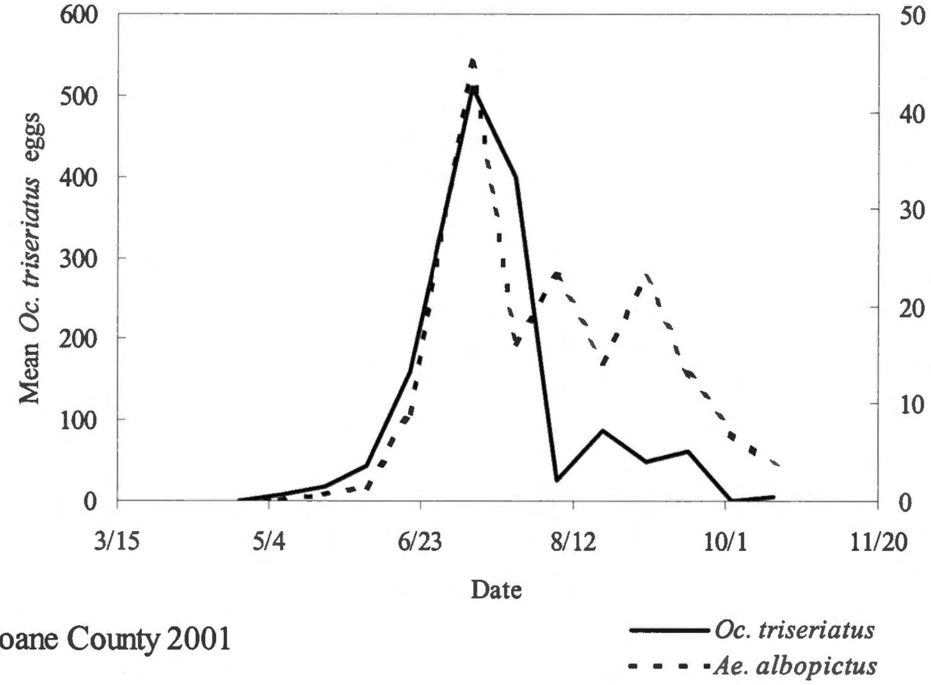


Roane County 1999

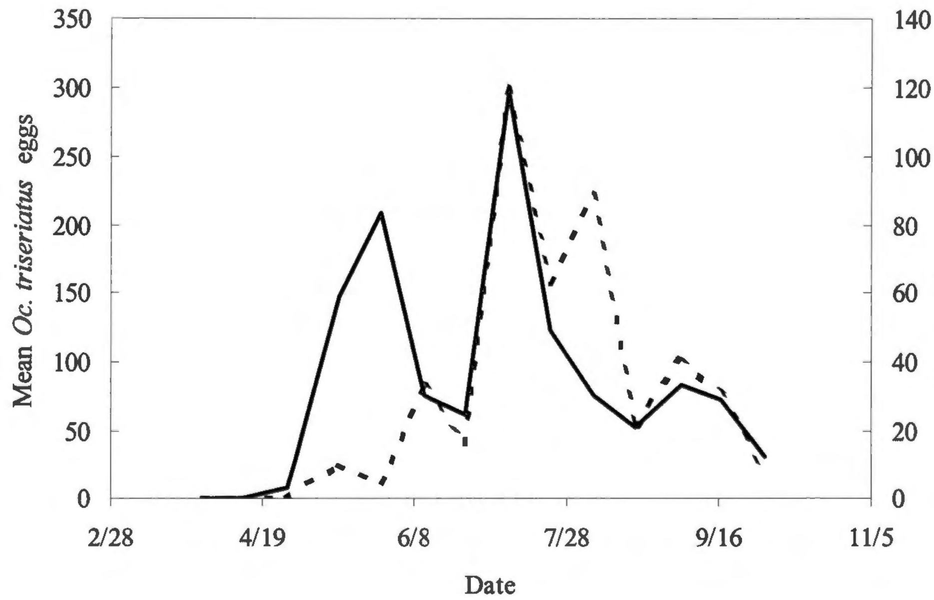


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Kingston, Roane County, from 9 June - 26 October 1998 and 26 April - 25 October 1999.

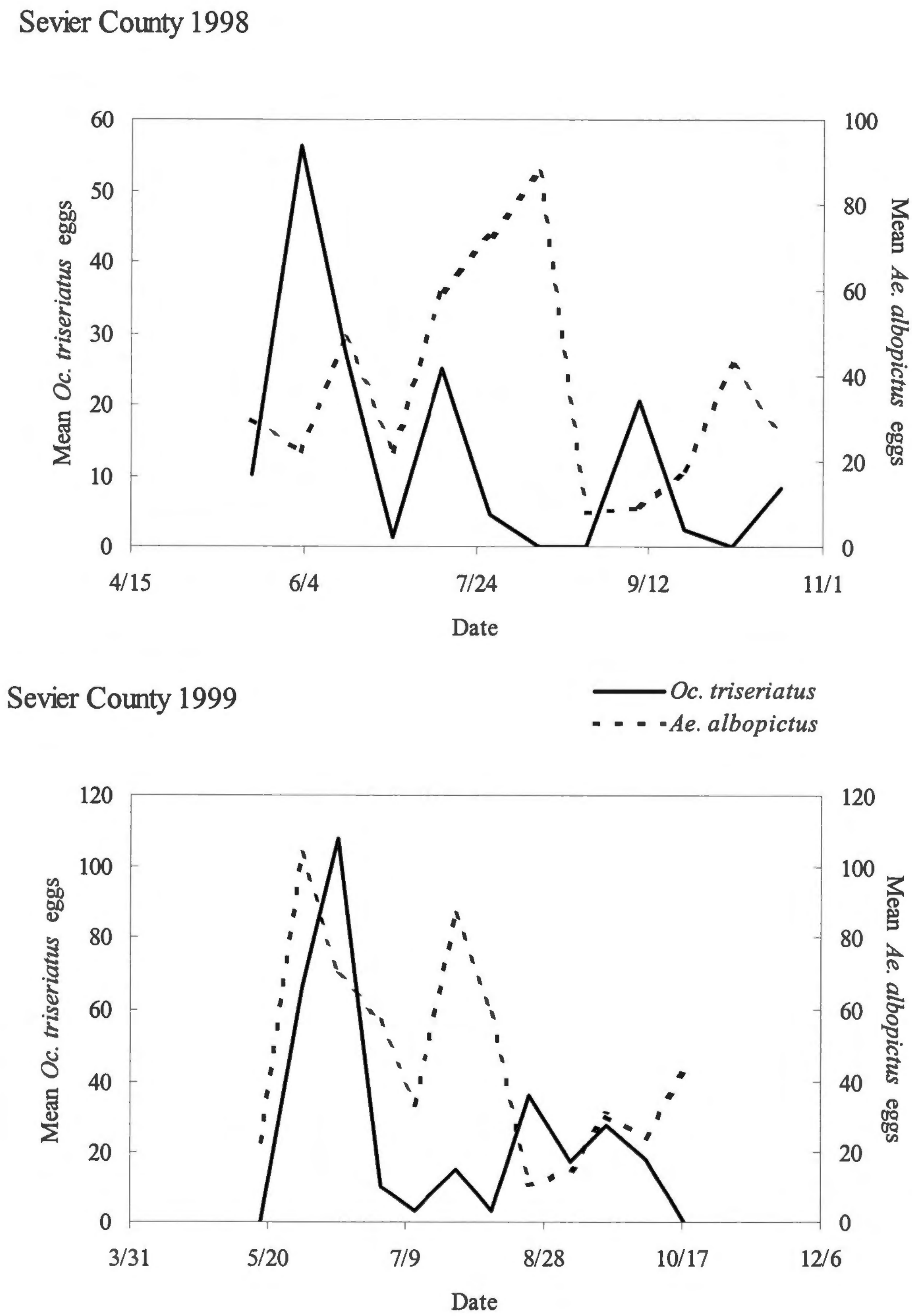
Roane County 2000



Roane County 2001

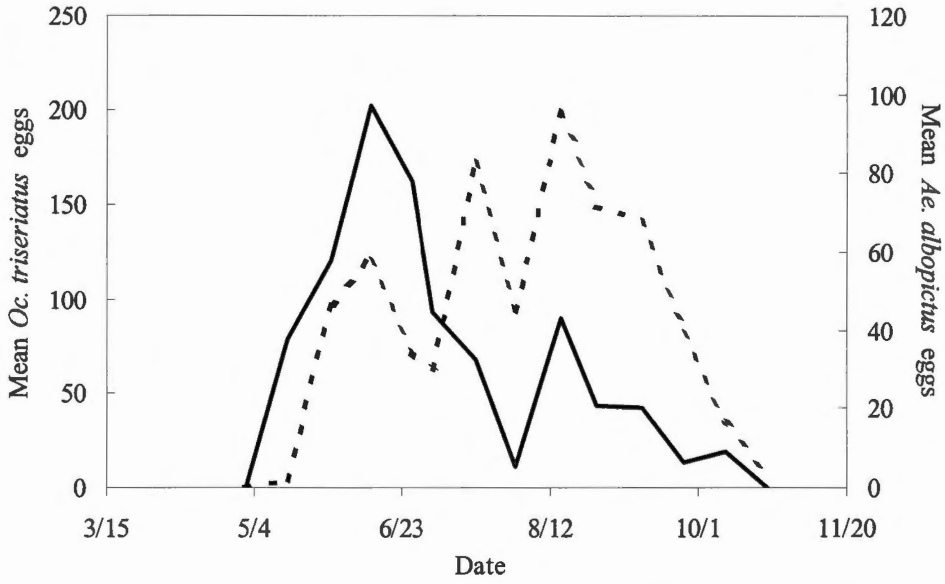


Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Kingston, Roane County, from 13 March - 17 October 2000 and 30 March - 14 October 2001.



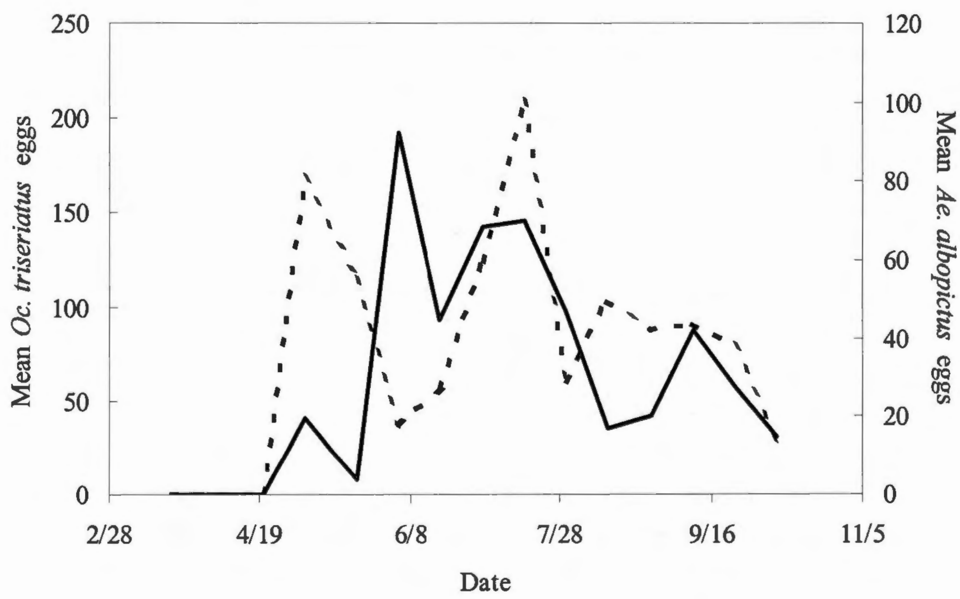
Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Sevierville, Sevier County, from 2 June - 2 November 1998 and 3 May - 1 November 1999.

Sevier County 2000



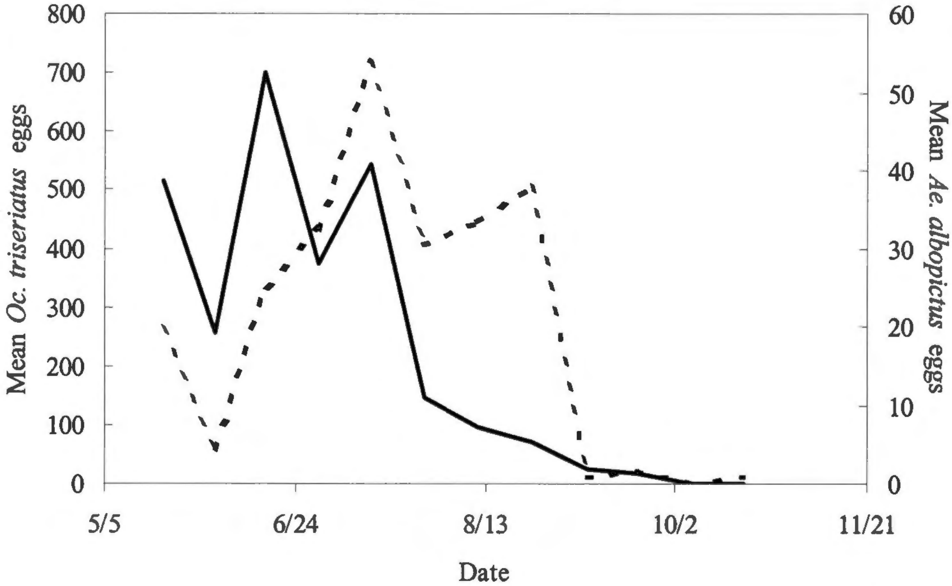
Sevier County 2001

Oc. triseriatus
Ae. albopictus



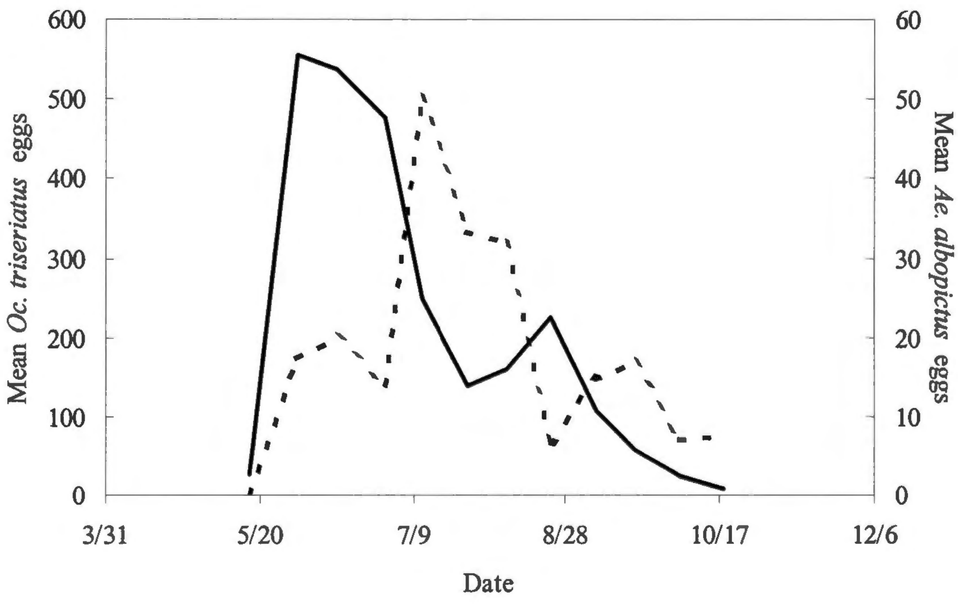
Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Sevierville, Sevier County, from 21 March - 24 October 2000 and 21 March - 22 October 2001.

Union County 1998

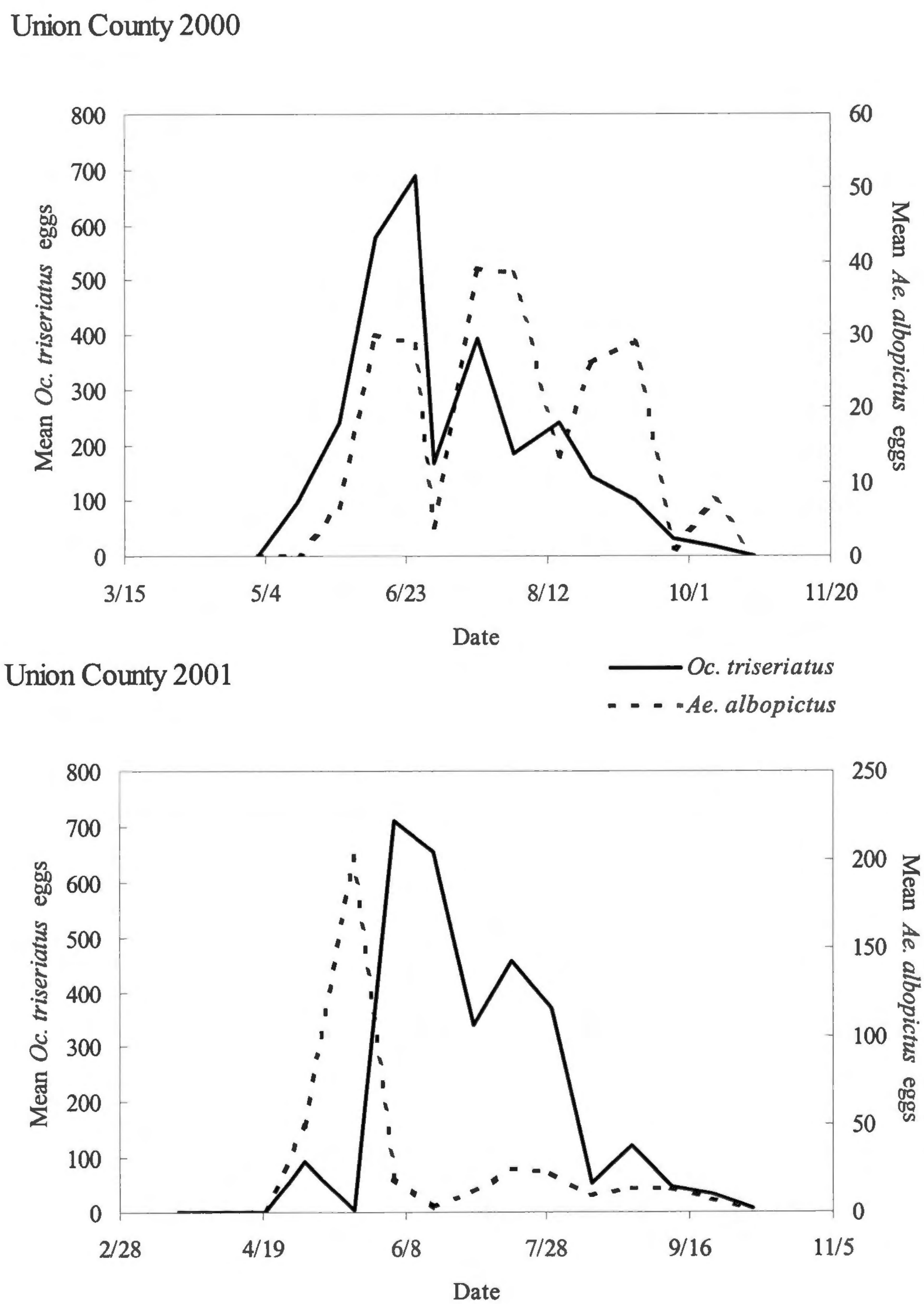


Union County 1999

Oc. triseriatus
Ae. albopictus



Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Maynardville, Union County, from 2 June - 2 November 1998 and 3 May - 1 November 1999.



Mean number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in 5 ovitraps from Maynardville, Union County, from 21 March - 24 October 2000 and 21 March - 22 October 2001.

APPENDIX 2. Four Year Adult Mosquito Data for Tennessee Valley Authority Collections

The Anderson County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

		Ochlerotatus			*Aedes*		*Culex*		*Anopheles*		
Year	*triseriatus*	*trivittatus*	*unknown*	*sticticus*	*albopictus*	*vexans*	*pipiens*	*restuans*	*punctipennis*	*quadrimaculatus*	Total
1998	0	27	59	76	5	40	0	0	24	0	231
1999	88	17	0	0	6	31	0	0	15	1	158
2000	13	121	1	4	3	17	3	0	12	4	178
2001	16	0	0	0	2	21	0	2	16	0	57
Total	117	165	60	80	16	109	3	2	67	5	624

	Psorophora	*Uranotaenia*	*Culiseta*	
Year	*ferox*	*sapphirina*	*inornata*	Total
1998	16	0	0	16
1999	1	0	0	1
2000	20	4	4	28
2001	0	0	0	0
Total	37	4	4	45

The Blount County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

			Ochlerotatus			*Aedes*			*Culex*		
Year	*triseriatus*	*trivittatus*	*unknown*	*sticticus*	*solicitans.*	*albopictus*	*vexans*	*pipiens*	*restuans*	*erraticus*	Total
1998	0	0	0	0	0	5	5	22	0	1	33
1999	4	4	4	7	0	14	19	13	7	11	83
2000	3	1	1	1	0	27	5	3	2	0	43
2001	9	0	0	0	1	20	12	19	0	0	61
Total	16	5	5	8	1	66	41	57	9	12	220

		Anophles			*Psorophora*		*Culiseta*	
Year	*punctipennis*	*quadrimaculatus*	*crucians*	*ferox*	*cyanescens*	*unknown*	*inornata*	Total
1998	12	3	0	1	0	0	0	16
1999	33	18	4	3	1	1	4	64
2000	17	1	1	0	0	0	1	20
2001	15	3	1	1	1	0	0	21
Total	77	25	6	5	2	1	5	121

The Grainger County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

			Ochlerotatus			Aedes		Culex		
Year	*triseriatus*	*trivittatus*	*sticticus*	*sollicitans*	*unkown*	*albopictus*	*vexans*	*pipiens*	*restuans*	Total
1998	23	2	0	0	5	36	5	41	0	112
1999	18	0	0	0	0	40	0	6	8	72
2000	15	0	0	0	1	14	6	6	3	45
2001	11	0	1	3	0	14	8	19	4	60
Total	67	2	1	3	6	104	19	72	15	289

		Culex		Anopheles		Psorophora	Culiseta	
Year	*salinarius*	*territans*	*unkown*	*punctipennis*	*quadrimaculatus*	*ferox*	*inornata*	Total
1998	0	0	0	15	0	2	0	17
1999	1	0	0	2	0	0	7	10
2000	0	0	1	5	0	0	0	6
2001	1	1	2	6	1	0	0	11
Total	2	1	3	28	1	2	7	44

The Hamblen County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

	Ocherotatus		*Aedes*		*Culex*			*Anopheles*		
Year	*triseriatus*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	*unknown*	*punctipennis*	*unknown*	Total
1998	154	7	789	30	66	0	0	7	0	1,053
1999	150	1	783	10	14	5	0	1	0	964
2000	47	1	384	15	1	5	1	4	0	458
2001	78	0	422	5	1	4	0	9	1	520
Total	429	9	2,378	60	82	14	1	21	1	2,995

Year	*Coquilletidia perturbans*	*Psorophora horrida*	*Culiseta inornata*	Total
1998	0	1	0	1
1999	0	0	1	1
2000	0	0	0	0
2001	1	0	0	1
Total	1	1	1	3

The Jefferson County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

			Ochlerotatus			*Aedes*		*Culex*		
Year	*triseriatus*	*trivittatus*	*sticticus*	*sollicitans*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	Total
1998	29	5	8	0	0	183	17	35	0	277
1999	34	2	0	0	2	160	4	11	8	221
2000	44	1	1	0	0	74	2	1	1	124
2001	41	0	0	1	2	155	5	0	4	208
Total	148	8	9	1	4	572	28	47	13	830

	Culex		*Anopheles*		*Psorophora*		
Year	*erraticus*	*unknown*	*punctipennis*	*quadrimaculatus*	*ferox*	*horrida*	Total
1998	0	0	9	1	1	1	12
1999	1	0	9	0	0	0	10
2000	0	1	2	0	0	0	3
2001	0	2	2	1	0	0	5
Total	1	3	22	2	1	1	30

The Knox County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

			Ochlerotatus				*Aedes*		*Culex*		
Year	*triseriatus*	*trivittatus*	*canadensis*	*mitchellae*	*sticticus*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	Total
1998	11	3	0	0	2	1	58	16	15	0	106
1999	12	6	1	3	0	0	27	6	0	1	56
2000	19	2	0	0	0	0	20	3	6	1	51
2001	5	0	0	0	0	0	31	17	5	7	65
Total	47	11	1	3	2	1	136	42	26	9	278

		Culex		*Anopheles*			*Psorophora*		*Culiseta*	
Year	*erraticus*	*salinarius*	*unknown*	*punctipennis*	*quadrimaculatus*	*ferox*	*cyanescens*	*horrida*	*inornata*	Total
1998	0	0	0	6	2	1	0	2	0	11
1999	2	0	0	10	0	0	0	0	1	13
2000	0	1	2	4	0	1	0	0	0	8
2001	0	7	6	4	1	0	1	0	0	19
Total	2	8	8	24	3	2	1	2	1	51

The Loudon County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

	Ochlerotatus				*Aedes*		*Culex*			
Year	*triseriatus*	*trivittatus*	*sticticus*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	*erraticus*	Total
1998	6	29	1	4	28	10	3	0	0	81
1999	21	322	0	1	48	16	0	9	1	418
2000	9	1	0	3	28	3	1	1	0	46
2001	21	0	0	0	38	15	0	3	0	77
Total	57	352	1	8	142	44	4	13	1	622

	Culex		*Anopheles*		*Psorophora*		*Culiseta*	
Year	*territans*	*unknown*	*punctipennis*	*unknown*	*ferox*	*cyanescens*	*inornata*	Total
1998	0	0	14	0	1	0	0	15
1999	0	1	25	1	4	2	0	33
2000	0	2	10	0	0	0	1	13
2001	1	0	11	0	4	1	0	17
Total	1	3	60	1	9	3	1	78

The Roane County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

	Ochlerotatus			Aedes		Culex			
Year	*triseriatus*	*trivittatus*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	*unknown*	Total
1998	6	0	0	60	5	11	0	1	83
1999	6	3	2	38	1	2	2	0	54
2000	11	0	2	50	9	4	4	2	82
2001	21	0	2	145	11	0	7	3	189
Total	44	3	6	293	26	17	13	6	408

	Anopheles		Culiseta	
Year	*punctipennis*	*quadrimaculatus*	*inornata*	Total
1998	2	1	0	3
1999	2	0	2	4
2000	6	1	0	7
2001	7	0	3	10
Total	17	2	5	24

The Sevier County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

		Ochlerotatus				*Aedes*			*Culex*			
Year	*triseriatus*	*trivittatus*	*sticticus*	*sollicitans*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	*erraticus*	*salinarius*	Total
1998	4	17	5	0	6	151	95	84	0	0	0	362
1999	1	10	9	0	5	72	29	66	17	30	3	242
2000	14	13	3	0	9	118	119	16	15	0	0	307
2001	12	0	0	1	4	125	139	8	6	0	2	297
Total	31	40	17	1	24	466	382	174	38	30	5	1,208

	Culex			*Anopheles*		*Psorophora*		*Culiseta*	*Toxorhynchites*	
Year	*tarsalis*	*unknown*	*punctipennis*	*quadrimaculatus*	*crucians*	*ferox*	*columbiae*	*inornata*	*r. seprontrialis*	Total
1998	1	0	0	22	0	3	0	0	0	26
1999	0	0	0	31	0	7	0	0	0	38
2000	0	3	17	1	0	0	1	3	1	26
2001	0	4	38	0	1	1	0	0	0	44
Total	1	7	55	54	1	11	1	3	1	134

The Union County host seeking adult collections from 1998 - 2001, including data from Gottfried (1999), for 1998 and part of 1999.

	Ochlerotatus			*Aedes*		*Culex*				
Year	*triseriatus*	*trivittatus*	*unknown*	*albopictus*	*vexans*	*pipiens*	*restuans*	*erraticus*	*unknown*	Total
1998	18	1	2	18	4	2	0	0	0	45
1999	9	1	2	45	1	0	1	1	0	60
2000	8	0	0	39	3	3	2	0	1	56
2001	23	0	0	17	1	2	1	0	0	44
Total	58	2	4	119	9	7	4	1	1	205

	Anopheles		*Psorophora*		
Year	*punctipennis*	*quadrimaculatus*	*ferox*	*unknown*	Total
1998	0	6	1	2	9
1999	0	5	0	0	5
2000	7	2	0	0	9
2001	7	0	0	0	7
Total	14	13	1	2	30

VITA

Alycia Erin Chapman graduated in December of 1998, from Northland College, Ashland, Wisconsin, with a Bachelor of Science degree in biology. In August of 1999, she entered the Department of Entomology and Plant Pathology at the University of Tennessee as a graduate research assistant. In December of 2001, she was awarded a Master of Science degree with a major in entomology.

The author is a member of Entomological Society of America and the Agricultural Honor Society, Gamma Sigma Delta and plans to continue with a career in the natural sciences.