Aedes albopictus OVIPOSITION AND LARVAL DENSITY, DEVELOPMENT, AND INTERACTIONS WITH Wyeomyia spp. IN EXOTIC BROMELIADS OF SOUTHERN FLORIDA

By

ROBYN R. RABAN

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by

Robyn R. Raban
To my parents, Bill and Judy Raban and my grandfather, Bill Phillips for their support for all my academic endeavors.
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The monthly mean densities of *Wyeomyia* spp. and *A. albopictus* in *B. pyramidalis* at each site.
Aedes albopictus OVIPOSITION AND LARVAL DENSITY, DEVELOPMENT, AND INTERACTIONS WITH Wyeomyia spp. IN EXOTIC BROMELIADS OF SOUTHERN FLORIDA

By Robyn R. Raban
December 2006

Chair: L. P. Lounibos
Major Department: Entomology and Nematology

Wyeomyia mitchelli and Wyeomyia vanduzeei, are indigenous mosquitoes that inhabit exotic and native bromeliads in southern Florida. In the mid-1980s Aedes albopictus invaded Florida where its immature stages occupied artificial and natural containers, including bromeliads. Previous studies have shown reduced abundance of A. albopictus in exotic bromeliads co-occupied by Wyeomyia spp. immatures, and reduced larval growth and survivorship in the presence of late instar Wyeomyia spp.

Our study examined chemical interference, encounter competition, predation, and egg hatch inhibition as potential interactions between Wyeomyia spp. and A. albopictus in exotic bromeliads. Exposure to waste products of Wyeomyia spp. did not affect the growth or survivorship of A. albopictus. Experimental alterations of surface area to volume ratios and habitat complexity did not affect the decreased growth and survivorship of A. albopictus seen in the presence of Wyeomyia spp. Behavior experiments showed that A. albopictus larvae change their location within a container in the presence of Wyeomyia spp. This change in location may indicate a response of A. albopictus to encounters with Wyeomyia spp. Wyeomyia spp. showed no evidence of predation on A. albopictus, but fourth instar Wyeomyia spp. were shown to inhibit the egg hatch of A. albopictus.
Field surveys and lab experiments were conducted on *A. albopictus* and *Wyeomyia* spp. in *Neoregelia spectabilis* and *Billbergia pyramidalis* bromeliads. Higher densities of *Wyeomyia* spp. were found in *B. pyramidalis*, while higher densities of *A. albopictus* were found in *N. spectabilis*. Correlations between the number of mosquitoes per plant and the volume of water extracted were significant for *Wyeomyia* spp. and *A. albopictus*. Overhead tree canopy type influenced *Wyeomyia* spp. larval densities in bromeliads. *A. albopictus* females oviposited preferentially in *N. spectabilis*, and *A. albopictus* developed faster when exposed to *Wyeomyia* spp. within *N. spectabilis* as compared to the same exposure in *B. pyramidalis*.

Encounter competition with *Wyeomyia* spp. larvae is the most probable mechanism reducing the growth and survivorship of *A. albopictus* in exotic bromeliads in southern Florida, although other interactions may also influence the relative abundances of these mosquito species.
CHAPTER 1
Aedes albopictus AND Wyeomyia spp. IN THE EXOTIC BROMELIADS OF SOUTHERN FLORIDA

With expanding global transport and commerce, humans have increased the distributions of many plant and animal species, often to novel locations. While some of these species fail to establish in these new locations (Mack et al. 2000), some become successful biological invaders (Lounibos 2002).

Aedes albopictus in the United States

Since its establishment in North America in the 1980s (Sprenger and Wuithiranygool 1986), Aedes albopictus has invaded various habitats that have previously been occupied by other mosquito species. It has expanded its range throughout the southeastern and midwestern United States by its transport in used tires (Hawley 1988). In Florida, A. albopictus was first discovered in 1984, and is currently found throughout most of the state (O'Meara et al. 1995a, Juliano et al. 2004). Invasions by A. albopictus have been much discussed (Lounibos 2002), and this mosquito species continues to colonize new areas of western (Aranda et al. 2006) and eastern Europe (Klobucar et al. 2006).

Aedes albopictus is one of the commonest mosquitoes in natural and artificial containers in the eastern United States and Brazil (Lounibos 2002). Aedes albopictus larvae are often found inhabiting man-made containers, but can also be found in natural phytotelmata such as treeholes (Novak et al. 1993) and bromeliads (Lounibos et al. 2003, O'Meara et al. 2003). Displacement by A. albopictus of mosquitoes that traditionally occurred in these habitats, such as Aedes aegypti (Juliano 1998), and Culex pipiens (Carriero et al. 2003) has been seen in some cases.

To date, considerations of the interactions of A. albopictus with its competitors have included mating interference (Nasci et al. 1989), egg hatch inhibition (Edgerly et al. 1993, Edgerly et al. 1991), larval resource competition (Juliano 1998, Braks et al. 2004, Griswold and
Lounibos 2005), predator-mediated competition (Gubler 1971, Lounibos et al. 2001, Griswold and Lounibos 2005), and intraguild predation (Edgerly et al. 1999). Yet, so far competitive exclusion of *A. aegypti* by *A. albopictus* has not occurred mainly due to suspected differences in the egg desiccation tolerances (Costanzo et al. 2005b), or differences in macrohabitat preferences (Lounibos et al. 2001, Costanzo et al. 2005a). In some cases though, local extinction of *A. aegypti* occurred after the arrival of *A. albopictus* (O’Meara et al. 1993).

*Aedes albopictus* have been infected with over 22 species of arboviruses (Moore and Mitchell 1997). In North America, *A. albopictus* have been found infected in nature with LaCrosse (Gerhardt et al. 2001), Eastern Equine Encephalitis (EEE) (Mitchell et al. 1992), and dengue viruses (Ibañez Bernal et al. 1997). Therefore, there are potential health concerns related to *A. albopictus* populations.

**Wyeomyia mitchelli and Wyeomyia vanduzei in the United States**

*Wyeomyia mitchelli* ranges throughout Mexico (Diaz-Najera and Vargas 1973), the Caribbean region (Belkin and Heinemann 1975, Shroyer 1981), central and southern Florida (Darsie and Ward 2005), and Hawaii (Shroyer 1981). This bromeliad specialist mosquito is commonly found co-occurring in Florida with another congeneric bromeliad specialist, *Wyeomyia vanduzei* Dyar and Knab. *Wyeomyia vanduzei* ranges through central and southern Florida (Darsie and Ward 2005), and much of the Caribbean region (Belkin and Heinemann 1975).

Before the introduction of *A. albopictus*, *W. mitchelli* and *W. vanduzei* were the most common day-biting mosquito species in the mixed oak forests of southern Florida (Edman and Haeger 1977). Immatures of these *Wyeomyia* spp. are historically the most common mosquitoes in the epiphytic bromeliad phytotelmata of southern Florida (Fish 1976, Frank 1983, Frank and O’Meara 1985).
Wyeomyia vanduzeei and W. mitchelli are diurnal species with peak diel biting activity occurring just prior to sunset (Edman and Haeger 1977) and oviposition occurring in the late daylight hours (Frank et al. 1985). In a study conducted by Edman and Haegar (1977), both W. vanduzeei and W. mitchelli adults appeared to be generalist feeders, feeding on species of rabbits, deer, and birds. Wyeomyia vanduzeei are facultatively autogenous, while W. mitchelli are not (O’Meara 1979), and their larvae may be slightly segregated as W. vanduzeei lay more eggs in sunny versus shady locations, and these two mosquito species show preferences for different plants (Frank and O'Meara 1985).

Although Wyeomyia spp. have not been found to vector arboviruses in Florida, Venezuelan Equine Encephalitis virus has been found in W. mitchelli (Scherer et al. 1971) and Ilheus and Wyeomyia viruses have been found in W. vanduzeei (Srihongse and Johnson 1965, 1967)

**Bromeliaceae as a Habitat**

Phytotelmata are water bodies that are held in plant structures such as flowers, leaf axils, or tree holes (Maguire 1971, Fish 1983, Clements 1999). The family Bromeliaceae contains many species which impound water in their leaf axils and are referred to as tank bromeliads (Frank 1983). There are over 2000 bromeliad species in the Americas (Frank 1983), with 16 species of bromeliads native to Florida, 7 of these being tank bromeliads that hold water throughout most of the year (Fish 1976).

While bromeliads obtain energy from photosynthesis, the nutrients they sequester for photosynthesis are obtained in multiple ways. Tank bromeliads can absorb nutrients through scales on the shoot called trichomes (Pittendrigh 1948, Benzing et al. 1976, Benzing 2000), or by way of interfoliar roots (Pittendrigh 1948). Frank (1983) classifies nutrient acquisition by tank bromeliads into two categories, dendrophilous nutrition, which is rain throughfall from trees often collected by epiphytic bromeliads, and anemophilous nutrition, which are wind blown
nutrients. Frank (1983) and Benzing (2000) also describe the release of nutrients provided by the wastes of faunal tank inhabitants which may contribute to the nutrients available to the plant.

In bromeliads, mosquitoes may encounter a wide variety of other unrelated species. To date, bacteria, fungi, algae, protozoa, platyhelminthes, rotifers, gastrotrichs, ostracods, nematodes, oligochaetes, crustacea, acarids, Hemiptera, Coleoptera, Culicidae, Psychodidae, Syrphidae, Ceratopogonidae, Chironomidae, Sarcophagidae, Tabanidae, Periscelidae, Tipulidae, Muscidae, Sciaridae, and odonates have all been identified within bromeliad phytotelmata (Maguire 1971, Fish 1976, Frank 1983). Some chironomids, muscids, periscelids, and turbellarians are predatory and feed upon smaller organisms and mosquito eggs, while some psychodids and sciarids feed on the submerged leaf litter in the bromeliad (Fish 1976). Bromeliads vary in their faunal compositions by the species of bromeliad and environmental conditions, such as sunlight and humidity (Laessle 1961, Fish 1976).

Since the popularization of exotic bromeliads as ornamental plants (Edman and Haeger 1977), there has been an increase in exotic bromeliad habitat for *Wyeomyia* spp. in Florida. Yet, with the arrival of the Mexican bromeliad weevil, *Metamasius callizona*, there has been a decline of the native bromeliad *Tillandsia utriculata* (Frank and Thomas 1994), the common native phytotelmata of *W. mitchellii* and *W. vanduzeei* (Fish 1976). Exotic bromeliads, which have become another common habitat of these two species, hold more water than the native bromeliad species and have a large central water-holding tank, uncommon in native bromeliads (O’Meara et al. 2003).

The two bromeliads which were the focus of most of this study are *Billbergia pyramidalis*, the summer torch bromeliad, and *Neoregelia spectabilis*, the painted fingernail plant. *Billbergia pyramidalis* and *Neoregelia spectabilis* are native to Brazil (Frank et al. 1988), and can both live
as either an epiphyte or as a ground dwelling species. *Neoregelia spectabilis* is the larger of the two species and tends to be a dark green to dark purple color with bright purple tips. The inflorescence of *N. spectabilis* is nidulate, meaning it is nested into the central tank of the plant. *Billbergia pyramidalis* is a light green color and has a stalked inflorescence.

**Interactions of *A. albopictus* and *Wyeomyia* spp. in Bromeliad Phytotelmata**

There is evidence that *Wyeomyia* spp. larvae exhibit negative effects on *A. albopictus* within bromeliads. Possibly due to the freezing temperatures, or the absence of native bromeliads in northern Florida, *W. mitchellii* and *W. vanduzeei* are infrequently found north of Orlando (O'Meara et al. 2003). In the absence of *Wyeomyia* spp. in northern Florida, *A. albopictus* has become a common mosquito in bromeliads, but in southern Florida where *Wyeomyia* spp. are abundant, *A. albopictus* individuals are scarce in bromeliads (O'Meara et al. 1995b, Lounibos et al. 2003). A study conducted in Hawaii determined that in the absence of *Wyeomyia* spp., *A. albopictus* dominated leaf axil phytotelmata (Shroyer 1981).

This study attempts to further investigate the results of Lounibos et al. (2003). In that study, 1st instar *A. albopictus* were exposed to varied quantities of leaf litter with 1st or 4th instar *Wyeomyia* spp. in *B. pyramidalis* bromeliads. *Aedes albopictus* experienced a reduction in survivorship with reduced leaf litter and the presence of 4th instar *Wyeomyia* spp., but the presence of *Wyeomyia* spp. accounted for most of the reduction in survivorship. The mean growth stage of *A. albopictus* was only affected by the presence of 4th instar *Wyeomyia* spp. with no effect due to leaf litter. 1st instar *Wyeomyia* spp. had no effect on the growth or survivorship of *A. albopictus*.

Lounibos et al. (2003) hypothesized that the reduction in growth and survivorship of *A. albopictus* in the presence of *Wyeomyia* spp. was attributable to interference competition. Lounibos et al. (2003) suggested that because the addition of leaf litter did not reduce the
negative effects of *Wyeomyia* spp. on *A. albopictus*, this interaction is independent of resources. Interference competition is categorized into overgrowth, chemical, territorial, and encounter (Schoener 1983). Overgrowth interference competition occurs only in sessile organisms, and mosquito larvae are not known to exhibit territorial interference behaviors, so these mechanisms are not considered in the possible competitive interactions between *A. albopictus* and *Wyeomyia* spp..

Chemical interference in mosquitoes results from the buildup of larval waste products that lead to an environmental deterioration which can effect the growth and survivorship of individuals (Bedhomme et al. 2005). *Aedes albopictus* has been found to be subject to chemical interference competition with the mosquito *Tripteroides bambusa* (Sunahara and Mogi 2002). Chemical interference has also been demonstrated in *Aedes sierrensis* (Broadie and Bradshaw 1991), *A. aegypti* (Dye 1984), and *Culex sitiens* (Roberts 1998).

Encounter competition usually occurs when physical contact between organisms causes a reduction in the feeding efficiency of individuals (Anholt 1990). Encounter competition is known among *A. sierrensis* (Broadie and Bradshaw 1991), *A. aegypti* (Dye 1984) and *Culex sitiens* (Roberts 1998). Other mosquitoes such as *Ochlerotatus cantans* (Renshaw et al. 1993) are hypothesized to also exhibit encounter competition effects.

Intraguild predation is another important regulatory mechanism in mosquito populations. Some mosquito species are facultative predators or cannibalistic on smaller instars (Hinman 1934, Reisen and Emory 1976, Koenekoop and Livdahl 1986, Koenraadt et al. 2003, Edgerly et al. 1999, Koenraadt et al. 2004). Egg hatch inhibition occurs when mosquito larvae delay hatch of *Aedes* eggs. Egg hatch inhibition could increase the time to reproduction, which may reduce population growth and increase mortality in the dormant egg stage of some species (Livdahl and

Thus, the purpose of the following experiments on *A. albopictus* and *Wyeomyia* spp. is to determine the mechanism of reduction of growth and survivorship of 1st instar *A. albopictus* in the presence of 4th instar *Wyeomyia* spp. in exotic bromeliads. As mentioned above, chemical interference competition, encounter competition, predation, and egg hatch inhibition are potential competitive mechanisms in mosquito populations. Previously none of these mechanisms have been studied between *Wyeomyia* spp. and *A. albopictus* within bromeliads, and thus these interactions are the focus of a set of experiments in this study.

Furthermore, in attempt to add to the current understanding of the occupancy by *A. albopictus* and *Wyeomyia* spp. of bromeliads in southern Florida (O'Meara et al. 1995b, Lounibos et al. 2003) multiple collections were made from two common ornamental bromeliad species *B. pyramidalis* and *N. spectabilis*. Subsequent oviposition and larval competition experiments were conducted with these two bromeliad species to explain the patterns of mosquito abundance observed from field collections.
CHAPTER 2
INTERFERENCE COMPETITION AS A POTENTIAL MECHANISM FOR THE
REDUCTION OF GROWTH AND SURVIVORSHIP OF Aedes albopictus IN THE
PRESENCE OF Wyeomyia spp. IN THE BROMELIADS OF SOUTHERN FLORIDA.

Introduction

Competitive interactions can be a major factor in determining community structure. Exploitative and interference competition are the two main mechanisms for interactions between individuals within a population (Schoener 1983). Exploitative competition has been the focus of many ecological studies, but interference competition often has a greater influence over species distribution and abundance (Case and Gilpin 1974). Schoener (1983) subcategorized exploitative competition into consumptive competition, which involves food resource competition, and preemptive competition for resource space. The subcategories of interference competition include (i) overgrowth, which involves individuals growing over one another and thus depriving their competitors of resources; (ii) chemical, which deals with toxins produced by some individuals harming other individuals; (iii) territorial, in which individuals aggressively defend units of space to the detriment of the competitors, and (iv) encounter competition in which interactions between mobile competitors cause harm by fighting, predation, or physical interference. Overlap is common in exploitative and interference competition (Schoener 1983). For example, interference competition affects competitors by changing the rate of resource exploitation, or in some cases resulting in injury or death, which in turn affects population growth (Case and Gilpin 1974).

Many plants, such as grasses (Javaid et al. 2005, Singh et al. 2005) and pine trees (Nektarios et al. 2005), release allelopathic chemicals that create an unfavorable environment for possible plant competitors. In some cases, the allelopathic chemicals released by plants are the main determinants of the plant community structure (Rasmussen and Rice 1971), and are
therefore a major competitive influence in the community. Bacteria also release allelopathic chemicals to limit the growth of their neighbors (Riley and Gordon 1999).

Allelopathy has not been identified in animals, but animals experience chemical interference caused by waste materials from other individuals. These waste products, such as ammonia and nitrates, cause a deterioration of the environment leading to a reduction in the growth and survivorship of other individuals (Bedhomme et al. 2005). For instance, intraspecific chemical interference competition has been shown among sea lamprey larvae (Rodríguez-Muñoz et al. 2003), Paramecium (Gill 1972), tadpoles (Griffiths et al. 1991) and larvae of the mosquitoes A. aegypti (Bedhomme et al. 2005, Dye 1984) and Culex pipiens molestus (Ikeshoji et al. 1976).

Density dependent chemical inhibition of larval growth has been demonstrated in other species of Aedes mosquitoes. Broadie and Bradshaw (1991) determined from a laboratory experiment that intraspecific chemical interference competition influenced pupation success, pupal weight and development time in Aedes sierrensis. Chemical interference has also been shown to affect the growth of A. albopictus. Sunahara and Mogi (2002) determined that A. albopictus experienced a reduction in survivorship and pupation success from interspecific chemical interference competition with Tripteroides bambusa in bamboo stumps.

Chemical inhibition of larval growth may be less common in other genera of mosquitoes. Wyeomyia smithii exhibit density dependent increases in development time and reductions in survivorship and pupal weight, but neither encounter competition, chemical competition, nor cannibalism were found to contribute to these density dependent changes in fitness (Broberg and Bradshaw 1995).
While chemical inhibition of growth does occur within larval communities, encounter competition is often found to be the predominant interference mechanism. Dye (1984) found that some strains of *A. aegypti* demonstrated intraspecific chemical interference, but chemical effects were small compared to those of encounter competition. Roberts (1998) also found that chemical inhibition of growth occurred among *Culex sitiens* conspecifics, but that encounter competition was the stronger of the two competitive factors.

Encounter competition was defined by Brian (1956) as the ability of an organism to harm another organism by directly attacking it, or indirectly by damaging its food resources or blocking its access to those resources. Direct attack encounter competition has been found to occur in mosquito larvae and often alters their survivorship. Among some filter-feeding mosquito species, 1st instar larvae may be attacked and often killed by fourth instar conspecifics in laboratory microcosms (e.g., Reisen and Emory 1976, Koenekoop and Livdahl 1986, Koenraadt et al. 2003). Mosquitoes may experience encounter competition when either high densities of individuals cause frequent encounters with one another (Broadie and Bradshaw 1991, Roberts 1998) or larvae frequently encounter larger individuals of later instars (Dye 1984, Broadie and Bradshaw 1991). The combination of high densities and larger individuals can have the biggest effect on early instar mosquitoes (Broadie and Bradshaw 1991).

In many cases, physical contact between organisms can lead to a reduction in feeding efficiency or other metabolic costs (Anholt 1990). Physical contact was implicated as the cause of reduced feeding rates of *A. sierrensis* (Broadie and Bradshaw 1991) and *Anopheles gambiae* (Koenraadt et al. 2003) at high densities.

Reductions in feeding efficiency can cause alterations in life history characteristics by increasing larval mortality and development time and decreasing adult size. *Aedes cantans*
experienced a reduction in adult size and an increase in larval mortality, which were hypothesized to be due to contact competition (Renshaw et al. 1993).

Previous studies have tried to vary the effects of encounter competition by altering the water volume (Dye 1984), the surface to volume ratio (Broadie and Bradshaw 1991, Roberts 1998), or habitat complexity (Broadie and Bradshaw 1991). While experiments with alterations in the surface area to volume ratio did not significantly alleviate the effects of competition by changing the encounter rate between individuals (Broadie and Bradshaw 1991, Roberts 1998), there were successes with the alteration of water volume (Dye 1984) and habitat complexity (Broadie and Bradshaw 1991).

Habitat complexity affects the levels of competition and predation among species (Hixon and Menge 1991, Hixon and Jones 2005). With increasing structural complexity of a habitat, the number of competitive refuges increases (MacArthur and Levins 1967, Finke and Denno 2002), and the number of physical encounters between predator and prey decreases (Murdoch and Oaten 1975). With more physical encounters in habitats of decreased complexity, the occurrence of intraguild predation increases (Marshall and Rypstra 1999, Roda et al. 2000, Finke and Denno 2002). In fact, most studies conducted on the effects of habitat complexity on competitive interactions have involved predator-prey interactions (e.g., Crowder and Cooper 1982, Schneider 1984, Diehl 1992, Babbitt and Tanner 1998, Alto et al. 2005), although some studies have found benefits to competitors as well as prey populations with increasing habitat complexity (Almany 2004). As with predator-prey interactions, an increase in habitat complexity should decrease encounters between potential competitors. Therefore, structural complexity may provide refugia from physical encounters with non-predatory species.
Behavioral changes in organisms that experience encounter competition are common. Anholt (1990) observed noticeable behavioral changes in feeding behavior of damselflies when exposed to different interspecific encounter rates. As mentioned before, physical encounters with other individuals can cause changes in feeding efficiency, and thus there should be a change in feeding behavior of individuals influenced by encounter competition.

Lounibos et al. (2003) found that *A. albopictus* had a reduction in growth and survivorship when exposed as 1st instars to 4th instars of *Wyeomyia* spp. in bromeliads and hypothesized interference competition as the mechanism for this effect. In this chapter, chemical interference competition and encounter competition are examined as potential types of interference to explain the negative effect of *Wyeomyia* spp. on larvae of *A. albopictus*.

In this chapter, one experiment was conducted to determine whether the waste products of *Wyeomyia* spp. reduce the growth or survivorship of *A. albopictus*. Two experiments were conducted to determine whether physical contact with the larger 4th instar *Wyeomyia* spp. affected the growth and survivorship of *A. albopictus*. The first of these experiments varied the surface area to volume ratio of artificial bromeliads in an attempt to change the frequency of encounters between the species. The second experiment varied habitat complexity as an alternative method of changing the frequency of encounters between species. Finally, the last experiment attempted to elucidate changes in feeding location of *A. albopictus* in response to the presence of *Wyeomyia* spp..

**Materials and Methods**

**Chemical interference**

Twenty-five 1st instar colony-raised *A. albopictus* were added to 1000 Dalton, 31 mm diameter Spectra/Por Biotech Cellulose Ester dialysis tubes that contained 25 mL of sieved bromeliad water and 0.1 g of chopped live oak leaves (*Quercus virginiana*). The leaves were
dried at 68 °C for 48 h before weighing. The bromeliad water was collected from approximately 60 plants, and sieved through a 130 μm gauge mesh screen to remove detritus and macroscopic organisms. All sieved water was mixed into one large bucket, and then covered with a plastic bag for 2 days.

The 1000 Dalton dialysis tubing was chosen for this experiment because its pore size allows waste products such as nitrates and ammonia to pass through, while excluding larger compounds such as food particles. Each tube was cut to 11.5 cm in length, which created approx. 8 X 3.1cm of aquatic habitat for the 1st instar larvae, and excess tubing of approximately 3.5 X 3.1cm was left over for folding the ends and for suspending the tubing. A butterfly clip strung with wire was attached to the tubing to suspend it in the center axil of a *Billbergia pyramidalis* bromeliad. The butterfly clip also kept the tubing from sealing at the top, which would have prevented access to the air.

The dialysis tubing had been preserved in 0.1% sodium azide solution, which was toxic to the larvae in preliminary tests. Therefore, a few days before use each dialysis tube was detoxified by soaking in distilled water 3 times for 30 minute intervals, after which it was placed in a 1.0 % sodium benzoate solution to prevent decay. On the day of the experiment, each tube was once again soaked 3 times for 30 min to remove the sodium benzoate, and then closed off at the bottom by folding and then sealing with a plastic-coated wire garbage tie.

To the center axil of 10 of the 20 plants, 30 4th instar *Wyeomyia* spp. were added to 100 mL of sieved bromeliad water containing 0.4 g of chopped oak leaves. One hundred milliters of sieved bromeliad water and 0.4 g of chopped oak leaves were added to the center axil of the ten control plants. Then each plant was randomly assigned to one of five cages where their bases
were secured in a small plastic container and held for 48 h at 26°C with a 12:12 h light dark cycle and approximately 80% relative humidity.

The plants were checked every 12 h for leaking, which was observed in 10 of the twenty plants, a loss of around 30 mL by 24 h. At 24 h, more sieved bromeliad water was added to the leaky plants corresponding to the amount of water loss detected in the plastic container holding the bromeliad.

Ammonia levels and pH of the bromeliad water were measured before and after the experiment. Ammonia concentration was recorded using the Fisher Scientific Accumet Portable AP63 pH/mV/ion meter using an ammonia probe, and pH was recorded using a Corning pH-20 meter.

After 48 h, the water in each bromeliad was removed by a pipette, and rinsed into a metal rearing pan with a spray bottle containing tap water. The number of surviving *A. albopictus* and the instars of each *A. albopictus*, determined by head capsule widths, were recorded for each replicate. An ANOVA was performed in SAS (2002) to determine whether the mean development stage or survivorship of *A. albopictus* differed between experimental (*30 Wyomyia*) and control (no *Wyomyia*) plants.

**Surface Area to Volume Ratio Encounter Competition Experiment**

Four different sized artificial bromeliads were built with circumferences of 4.5, 5.5, 7.0, and 9.0 cm at the 75 mL water level, representing surface area to volume ratios of 1.48, 1.82, 2.48, and 3.83 cm²/mL, respectively. The artificial bromeliads were constructed from 8.5 by 11 inch weatherproof map paper (IGage, Mapping Corporation, Salt Lake City UT), and Perfect Glue No. 1 (Liquid Nails, Cleveland, OH) was used as an adhesive on the outer parts of the plant. The inner parts of the plant were water protected and sealed with non-toxic, waterproof, 100% silicone aquarium tank sealant (All-Glass Aquarium, Franklin, WI).
Twenty-five 1st instar *A. albopictus*, 0.5 g oak leaves, and 75 mL of sieved bromeliad water were added to 15 control bromeliads of each size. The other 15 of each size represented the experimental group and differed from the controls by the addition of 30 4th instar *Wyeomyia* spp.. Each replicate was placed into a plastic container of appropriate size and put onto one of three randomly chosen shelves in a climate controlled rearing room kept at 26°C with a 12:12 light dark cycle and approximately 80% humidity. On the day of setting, each artificial bromeliad was marked with a pencil to indicate the 75 mL waterline. To compensate for evaporation, and the differences in evaporation based on the varied surface area to volume ratios among treatments, on the 4th and 8th day of the experiment each plant was refilled with sieved bromeliad water to its original 75 mL water level. After 10 days the experiment was terminated, and all of the *A. albopictus* in each replicate were counted, and their instars were recorded based on head capsule widths.

Using PROC GLM in SAS (2002) an ANOVA was performed to detect significant variation among the bromeliad sizes and between the presence and absence of *Wyeomyia* spp. in the development rate (average instar) and survivorship (percent alive) of *A. albopictus* immatures.

**Habitat Complexity Experiment**

The habitat complexity experiment used the same artificial bromeliads described in the surface area to volume ratio experiment. In this experiment, the smallest artificial bromeliad with a circumference of 4.5 cm and a surface area to volume ratio of 1.48:1 was excluded because it was too narrow for the habitat complexity manipulation and still allow for larval access to the air. In the other three sizes, 20 1st instar *A. albopictus*, 20 4th instar *Wyeomyia* spp., 0.5 g oak leaves, and 75 mL of sieved bromeliad water were added to 30 bromeliads of each.
Two different types of 3 cm² squares were constructed from weatherproof paper to add habitat complexity to the experiment. The first type of paper square, which represents the low habitat complexity treatment, remained two dimensional (Fig. 2-1). Three of these squares were added to half of each of the three bromeliad sizes. The second type of paper square, which represents high habitat complexity, was cut and folded to make a three dimensional structure (Fig. 2-2). When added to the cone, the low complexity square remained at the top or along the sides, while the high complexity square made a lattice-like structure within (Fig. 2-2).

After 5 days, the experiment was terminated, and the numbers and developmental stage of the remaining 1st instar *A. albopictus* larvae were recorded as described in previous experiments. An ANOVA was performed in SAS (2002) with survivorship and development time of *A. albopictus* as dependent variables and surface to volume ratio of the artificial bromeliad (n=3) and the internal complexity (n=2) of the habitat as the independent variables. Bonferroni-adjusted multivariate pairwise means comparisons followed detection of significant effects by ANOVA.

**Resource Dependent Location of *A. albopictus* and *Wyeomyia* spp.**

To determine whether foraging behavior of *A. albopictus* and *Wyeomyia* spp. changed in the presence of each other, or in different levels of food resources, an experiment was conducted in which three treatments within plastic cups received 0.1 g of dried chopped oak leaves (*Q. virgininia*) as prepared in previous experiments, and the other three treatments received 0.5 g of the same leaves. The leaves were located at the bottom of all containers. As described in prior experiments, 75 mL sieved bromeliad water was also added to each container. Each of the food levels had one treatment with 10 1st instar *A. albopictus* and 10 4th instar *Wyeomyia* spp., one treatment with only 10 1st instar *A. albopictus*, and one treatment with only 4th instar *Wyeomyia* spp.. Each of these treatments was replicated two times. All *Wyeomyia* spp. were collected as
larvae from bromeliads in Vero Beach and Fort Pierce, FL. All *A. albopictus* came from a colony described in the previous experiments.

An instantaneous scan census (Martin and Bateson 1986) was implemented every hour for 7 h starting after a 3 h acclimation period. Treatments with only one species had fewer individuals, so behavioral observations were usually finished in approximately 15 seconds. The treatments with both species had more individuals to examine, so those observations often took as long as 45 seconds. The two replicates for each treatment were sampled consecutively before moving onto the next treatment, and the treatment observed first was changed at each observation. To make observations of 1st instar *A. albopictus* easier, and to prevent disturbing the larvae with light at every observation, treatments were illuminated by a 60 watt desk lamp for the entirety of the experiment.

Because the changes in location within the container were the focus of this experiment, the locations of each *Wyeomyia* spp. and each *A. albopictus* at each time were coded into one of four categories, predetermined from preliminary observations: 1) at the middle of the cup (1 cm from top to 1 cm from bottom); 2) on the bottom of cup (bottom to 1 cm from bottom); 3) at the top of cup (water surface to 1 cm below the water surface with or without siphon extended for breathing) or 4) wandering (swimming without noticeable foraging behavior). Owing to the small size of 1st instar *A. albopictus*, whether the larva was resting, filter feeding or browsing was not recorded because the differences in these behaviors often could not be determined. For instance, during preliminary examinations there were multiple occasions when *A. albopictus* was nearby a surface, so it appeared to be browsing on that surface, but after closer examination at a higher magnification, the larva was found to be filter feeding close to the surface, not browsing upon it.
At the end of the experiment, the raw data in each observation category were converted to a proportion based on the number of individuals of each species that showed the categorical behavior at the observation time. The proportional data were arcsine square root transformed and a multivariate analysis of variance (MANOVA) was performed with the effects of species, food, alone (whether with or without the other species), time, replicate, and the interactions of species x treatment, species x food, and treatment and food. Further analysis of variance (ANOVA) analyses with subsequent Tukey post-hoc tests differentiated the effects found to be significant in the MANOVA.

Results

Chemical Interference

There was no significant difference ($F_{1,19}=1.03, P=0.32$) between the average instars (development rate) of *A. albopictus* in dialysis bags with or without *Wyeomyia* spp. outside the bag (Fig. 2-3). There was also no significant difference ($F_{1,19}=2.94, P=0.10$) in the arcsine square root transformed survivorship between the treatment and the control groups (Fig. 2-4).

The mean values of ammonia, and pH showed that there was little change in these concentrations between pre- and post-experiment (pH: $t_{19}=1.07, P=0.30$; ammonia: $t_{19}=1.34$, $P=0.20$) (Table 2-1).

Surface Area to Volume Ratio Experiment

The *A. albopictus* in the treatments with *Wyeomyia* spp. were found to have a significantly lower average instar (LS mean =2.72 ± 0.07 SE) as compared to the LS mean (3.96 ± 0.08 SE) with *Wyeomyia* spp. (Fig. 2-5). There was no significant effect of bromeliad size on average instar, but there was a significant interaction between the presence of *Wyeomyia* spp. and size (Table 2-2). From smallest to largest size, the average instars (mean and standard error) at each surface area to volume ratio were 3.29 ± 0.17, 3.54 ± 0.16, 3.32 ± 0.16, 3.22 ± 0.12 respectively.
In treatments without *Wyeomyia* spp., *A. albopictus* were found to have a significantly lower survival (Table 2-3) (0.83 ± 0.02 (mean ± SE)) compared to the control (0.89 ± 0.02 (mean ± SE)). The largest three surface area to volume ratios contributed the most to the significant *Wyeomyia* spp. effect on survivorship (Fig 2-6). A Tukey post hoc test determined that the artificial bromeliad with the 6 cm diameter, which was the second to smallest diameter, had a higher mortality rate than the other three sizes, but was only significantly different from the smallest size (Fig. 2-6).

**Habitat Complexity Experiment**

There was no difference in the survivorship of *A. albopictus* between high and low complexity treatments (F\(_{1,89}=0.57\), \(P=0.45\)) or surface area to volume ratios (F\(_{2,89}=0.74\), \(P=0.48\)). There was no significant difference in the development rate of *A. albopictus* among surface area to volume ratios (F\(_{2,89}=1.29\), \(P=0.28\)) but *A. albopictus* developed faster in high complexity treatments compared to the low complexity treatments (F\(_{1,89}=30.02\), \(P<0.01\)) (Fig 2-7) There was no significant interaction between surface area to volume ratio and habitat complexity on developmental rate (F\(_{2,89}=2.54\), \(P=0.08\)), or on survivorship (F\(_{2,89}=0.89\), \(P=0.42\)).

**Resource-Dependent Foraging Location**

MANOVA indicated significant effects of species, presence of other species, and resource levels (Table 2-4). In general, *Wyeomyia* spp. foraged on the sides and on the bottom of containers more than *A. albopictus* (Figs. 2-8 and 2-9). *Aedes albopictus* were found at the top of the container more frequently and wandered more than *Wyeomyia* spp..

There were some significant differences between the locations of the mosquito species at the different food concentrations (Table 2-5 and 2-6). At high food concentrations *Wyeomyia* spp. allocated more time at the water surface (Fig 2-11). *Aedes. albopictus* also spent more time at the water surface at high resource concentrations (Fig. 2-10). At low food concentrations,
Wyeomyia spp. allocated more time on the bottom of the container, while A. albopictus occurred on the sides on the container more frequently (Figs 2-10 and 2-11). There was significant variation in the location of A. albopictus attributable to the presence of the other species and food level (Table 2-5).

In the presence of Wyeomyia spp., A. albopictus spent more time on the top of the container and less at the bottom or on the sides of the containers (Fig. 2-8). There were significant interactions between the presence of the other species (alone effect) and species, and the presence of the other species, and the level of food resources (Table 2-4). Thus, there was a difference in larval behavior of A. albopictus in the presence of Wyeomyia, and the level of food resources also influenced the relative location of these two species.

Discussion

The chemical interference experiment provided no evidence that waste products from Wyeomyia spp. larvae interfere with the growth or survivorship of A. albopictus. The buildup of waste products in experiments that exhibited chemical interference, such as among A. aegypti (Bedhomme et al. 2005, Dye 1984), may be less likely to occur in the bromeliad tank and axil habitat. Billbergia pyramidalis is both an epiphytic and ground dwelling plant (Frank et al. 1988). While there are no specific reports on the nitrogen absorption abilities of B. pyramidalis, in general, epiphytic bromeliads use dissolved nitrogen held in their tank water as their main nitrogen source (Benzing et al. 1976, Raven 1988). Epiphytic bromeliads also assimilate most of their nitrogen from ammonia (Endres and Mercier 2001), which is a common excretory product of mosquitoes (Clements 1999). By the end of the experiment, there was little change in the ammonium concentration or pH of the bromeliad water (Table 2-1), even though 30, 4th instar Wyeomyia spp., and 25 A. albopictus had been added to the tank for 48 hours. Because B. pyramidalis is sometimes epiphytic, it is possible that the plant absorbed the
ammonia excreted by the mosquitoes, thus reducing the probability of chemical interference from nitrogenous wastes in this phytotelm. On the other hand, at 24 hours as much as 30 mL of water was added to some of the plants that had leaked water. This addition of water could have reduced or eliminated the effects of chemical interference of *Wyeomyia* spp. on *A. albopictus* in this experiment.

The dialysis bag used in the chemical interference experiment physically separated *A. albopictus* from *Wyeomyia* spp. Thus, other potential forms of interspecific competition, such as encounter, resource, or space competition were eliminated. In some other experiments of this chapter, *A. albopictus* were in direct contact with *Wyeomyia* spp., which resulted in a significant reduction in growth of *A. albopictus*. In the chemical interference experiment, no significant effects were detected, suggesting that encounter, resource or space competition plays an important role in the reduction of growth and survivorship seen in the other experiments.

Previous experiments where surface area to volume ratios were manipulated in order to influence the impact of encounter competition have often been unsuccessful. Broadie and Bradshaw (1991) altered the surface area to volume ratio of treehole microcosms, but only the density of *A. sierrensis* had an effect on pupation success, larval development time, or pupal weight. Anholt (1990) altered the surface area available to damselfly larvae in cages, which had no effect on density dependent decreases in growth and survival. Damselfly larvae exhibited a behavioral response to the increase of structural complexity inside cages, but habitat complexity did not change the decrease in growth, survival time, and size seen at high densities (Anholt 1990).

In the current study, while the surface area to volume ratio of the plant had no effect on the development rate of *A. albopictus*, there was a significant variation in survivorship of this
species among the different surface area to volume ratios. Mean survivorship in size 2 differed significantly from size 1. The biological significance of this difference between these two sizes is unknown.

Another unexpected result of the surface area to volume ratio experiment was the reduced survivorship of *A. albopictus* in the absence of *Wyeomyia* spp.. The three largest surface area to volume ratios appeared to contribute to most of this reduction in survivorship, although the interaction between plant size and *Wyeomyia* spp. was not significant (Table 2-3).

While the results of the surface area to volume ratio experiment do not explain the effects of *Wyeomyia* spp. on *A. albopictus*, increased habitat complexity in this experiment reduced the development time of *A. albopictus* in the presence of *Wyeomyia* spp.. In the habitat complexity experiment the addition of waterproof paper increased the foraging surface area. This increase provided additional surface area to the developing *A. albopictus*, a factor which was not included in other competition experiments in this thesis. In this experiment, while more foraging area was added to the treatments, the total foraging area between treatments was kept the same, because in both treatments the size of the paper added, and the number of pieces were kept constant, the paper was folded only to increase complexity. By the end of the experiment though, many of the low complexity treatment squares had become appressed to the sides of the artificial bromeliad, thus resulting in a decrease in the foraging surface area between the two habitat complexities. Therefore, habitat complexity may have decreased the development time of *A. albopictus* in the presence of *Wyeomyia* spp., but the difference in foraging surface area between the habitat complexities may have contributed to this decrease in development time. If this effect is due to habitat complexity, its relevance under field conditions is uncertain. When *Wyeomyia* are present in bromeliads, which is the case in most of southern Florida (O’Meara et al.1995b), then
increased complexity could decrease the development time of *A. albopictus*. Whether increased habitat complexity affects the developmental rate of *A. albopictus* in the absence of *Wyeomyia* spp. remains uncertain as no treatments without *Wyeomyia* were used in this experiment.

The results of the behavior experiment indicated that *Wyeomyia* spp. spent most of the time foraging on the bottom of the container, especially during periods of low food concentrations. When in contact with *Wyeomyia* spp., *A. albopictus* spent significantly less time on the bottom of the container, and stayed the furthest away from *Wyeomyia* spp. by spending significantly more time at the top of the container irrespective of food concentration. Such behavior of *A. albopictus* could indicate avoidance of *Wyeomyia* spp.

These experiments do not support chemical interference as a likely competitive mechanism to explain negative effects of *Wyeomyia* spp. on *A. albopictus*. From the habitat complexity experiment, encounter competition is the likely explanation for the increase in development time of *A. albopictus* in the presence of *Wyeomyia* spp., but, as mentioned before, increased foraging area may have also contributed to this effect. Like other surface area to volume ratio habitat experiments performed with mosquitoes, this surface area to volume ratio experiment failed to explain the competitive interactions between *A. albopictus* and *Wyeomyia* spp.. The behavioral experiment provided some indication that *A. albopictus* larvae change location within the container in the presence of *Wyeomyia* spp. These changes in location could be a result of encounter competition causing *A. albopictus* to change their location to avoid encounters with *Wyeomyia* spp. This avoidance of *Wyeomyia* spp. may contribute to the decrease in development rate observed among *A. albopictus* in the presence of *Wyeomyia* spp.
Table 2-1. Means and standard errors for the concentrations of ammonia and pH pre- and post-experiment.

<table>
<thead>
<tr>
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<th>pH (ppm) (mean ± SE)</th>
<th>Ammonia (mean ± SE)</th>
</tr>
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<tbody>
<tr>
<td>Pre-experiment</td>
<td>6.21 ± 0.02</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Post experiment</td>
<td>6.19 ± 0.02</td>
<td>0.21 ± 0.01</td>
</tr>
</tbody>
</table>

Paired t-tests showed no significant differences (alpha= 0.05) between pre- and post-experiment means.
Table 2-2. Analysis of variance for *A. albopictus* average instar based on the presence or absence of *Wyeomyia* spp. and the surface:volume ratio of the artificial plant and the interaction of these two variables.

<table>
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<th>F</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wyeomyia</em></td>
<td>162.38</td>
<td>1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bromeliad Size</td>
<td>2.01</td>
<td>3</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Wyeomyia x Size</em></td>
<td>4.56</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table 2-3. Analysis of variance for *A. albopictus* average survival based on the presence or absence of *Wyemyia* spp., and the surface area:volume ratio of the plant (size) and the interaction of these two variables.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wyeomyia</em></td>
<td>6.48</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Bromeliad Size</td>
<td>5.41</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Wyeomyia</em> x Size</td>
<td>1.56</td>
<td>3</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Table 2-4. Multivariate analysis of variance table for feeding location.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Wilks’ Lambda</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>65.20</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Alone</td>
<td>2.66</td>
<td>6</td>
<td>0.02</td>
</tr>
<tr>
<td>Food</td>
<td>8.30</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time</td>
<td>0.88</td>
<td>42</td>
<td>0.68</td>
</tr>
<tr>
<td>Species x Alone</td>
<td>4.83</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Species x Food</td>
<td>4.68</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Alone x Food</td>
<td>3.84</td>
<td>6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The species effect refers to the location of either *A. albopictus* or *Wyeomyia* spp. Alone refers to whether or not each species is alone or in the presence of the other species. The food category variables were either 0.1 g leaves (low treatment) or 0.5 g leaves (high treatment). Time is the hour of observation from the start of the experiment.
Table 2-5. Analysis of variance for the effects of food level presence or absence of *Wyeomyia* spp. on the location of *A. albopictus*.

<table>
<thead>
<tr>
<th>Condition</th>
<th>F</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combo High Food</td>
<td>15.40</td>
<td>3,63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>A. albopictus</em> only High Food</td>
<td>3.49</td>
<td>3,63</td>
<td>0.02</td>
</tr>
<tr>
<td>Combo Low Food</td>
<td>12.24</td>
<td>3,63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>A. albopictus</em> only Low Food</td>
<td>1.12</td>
<td>3,63</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Combo indicates the treatments that had both *Wyeomyia* spp. and *A. albopictus*. 
Table 2-6. Analysis of variance for the effects of food level and presence or absence of *A. albopictus* on the location of *Wyeomyia* spp.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combo High Food</td>
<td>14.55</td>
<td>3,63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>A. albopictus</em> only High Food</td>
<td>33.87</td>
<td>3,63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Combo Low Food</td>
<td>39.58</td>
<td>3,63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>A. albopictus</em> only Low Food</td>
<td>15.58</td>
<td>3,63</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Combo indicates the treatments that had both *Wyeomyia* spp. and *A. albopictus*. 
Figure 2-1. Construction of the squares for the habitat complexity experiment. A) the low complexity square is unaltered before introduction to the bromeliad. B) the high complexity square was cut along the lines indicated inside the square.
Figure 2-2. The folded shape of the high complexity and low complexity treatments and the orientation of the squares within the cones. A) in the low complexity experiment the squares floated on the top, or laid on the sides of the cone. C) in the high complexity cone, the altered squares create a lattice-like formation on the inside of the cone. B) The diagram in the center shows the shape of the altered square after the cutting and folding described in Fig. 2-1.
Figure 2-3. Average instars of *A. albopictus* after 48 h exposure to *Wyeomyia* spp. through a dialysis membrane and without *Wyeomyia* in the control. The error bars are standard error.
Figure 2-4. Mean survivorship of *A. albopictus* after 48 h exposure to *Wyeomyia* spp. through a dialysis membrane and without *Wyeomyia* spp. in the control. The error bars are standard error.
Figure 2-5. The average instar ± SE of *A. albopictus* in the absence or presence of *Wyeomyia* spp. in four plant sizes. NoWy refers to the treatments with no *Wyeomyia* spp., and Wy refers to treatments with *Wyeomyia* spp.. The size below these symbols refers to the surface area to volume ratio in the artificial bromeliad, proceeding from smallest (1) to largest (4), as quantified in Materials and Methods. Means with common letters above the bars are not significantly different by (*P*<0.05).
Figure 2-6. The average survivorship ± SE of *A. albopictus* in the absence or presence of *Wyeomyia* spp. in four plant sizes. NoWy refers to the treatments with no *Wyeomyia* spp., and Wy refers to treatments with *Wyeomyia* spp.. The size below these symbols refers to the surface area to volume ratio in the artificial bromeliad, proceeding from smallest (1) to largest (4), as quantified in Materials and Methods. Mean values without common letters above the bars are significantly different (*P*<0.05).
Figure 2-7. The average instar (±SE) of *A. albopictus* in two levels of habitat complexity and three levels of surface area to volume ratio. L and H denote the low and high complexity treatments. Numbers correspond to increasing surface area to volume ratios, as indicated in Materials and Methods. Mean values without common letters above the bars are significantly different by a Bonferroni adjusted multivariate pairwise test.
Figure 2-8. Location of *A. albopictus* in the presence and absence of *Wyeomyia*. The bottom and top locations are significantly different between *Wyeomyia* and no *Wyeomyia* treatments (alpha<0.05) by a Tukey means comparison. The bars indicate SE.
Figure 2-9. Location of *Wyeomyia* spp. in the presence and absence of *A. albopictus*. No locations were significantly different between *A. albopictus* and no *A. albopictus* treatments (alpha<0.05) by a Tukey means comparison. The bars indicate SE.
Figure 2-10. Location of *A. albopictus* by food concentration and the presence and absence of *Wyeomyia* spp.. The bars indicate SE. Different letters over the same bar category indicate significantly different means among the food concentrations (high and low) and treatments (*Wyeomyia* spp. and No *Wyeomyia* spp.) for each location (side, bottom, top and wander) (alpha=0.05).
Figure 2.11: Location of *Wyeomyia* spp. by food concentration and the presence and absence of *A. albopictus*. The bars indicate SE. Different letters over the same bar category indicate significantly different means among the food concentrations (high and low) and treatments (*A. albopictus* spp. and No *A. albopictus* spp.) for each location (side, bottom, top and wander) (alpha=0.05)
CHAPTER 3

*Wyeomyia spp.* AS POTENTIAL PREDATORS OF *Aedes albopictus* IN BROMELIADS IN SOUTHERN FLORIDA

**Introduction**

In mosquito populations predation can effect the survivorship (Griswold and Lounibos 2006, Blaustein et al. 1995, Fincke et al. 1997), and development time (Alto et al. 2005, Grill and Juliano 1996) of the prey as predators consume prey or the prey become less active in the presence of predators. These alterations in survivorship and development time can effect the population and community structure of both the predator and prey species (Sih 1985).

Intraguild predation is the killing and consumption of competitors that consume similar resources, which has an immediate energetic benefit and a beneficial reduction in exploitative competition for the predator (Polis et al. 1989). Age and size dependent intraguild predation is common throughout all ecological systems, and many predators are cannibalistic on smaller sized or younger conspecifics (Polis et al. 1989). Often intraguild predation occurs between competitors with the greatest resource overlap, and may be a result of the increased encounter rates due to niche overlap (reviewed by Polis et al. 1989).

Mosquitoes that are predominantly browsers and filter feeders, such as *Aedes* and *Anopheles* species (Clements 1999), may become cannibals (Hinman 1934, Reisen and Emory 1976, Koenekoop and Livdahl 1986, Koenraadt et al. 2003) or facultative predators (Edgerly et al. 1999, Koenraadt et al. 2003) under certain conditions. Koenekoop and Livdahl (1986) and Edgerly et al. (1999) found that cannibalism in *Aedes triseriatus* was resource dependent with lower resource availability leading to higher cannibalism. Koenraadt et al. (2003, 2004) found no increase in cannibalism or predation by *Anopheles gambiae* complex mosquitoes related to resource availability. Increased habitat complexity also reduced predation by *A. triseriatus* on congeners, but the opposite effect was seen with *A. aegypti* (Edgerly et al. 1999). Koenraadt et
al. (2004) also showed that higher densities of anopheline mosquitoes in small spaces increased cannibalistic and predatory behavior.

Although facultative predation may influence the population dynamics of many *Aedes* and *Anopheles* mosquitoes, *W. mitchellii* and *W. vanduzeei* have yet to be examined as potential predators of *A. albopictus* in bromeliads. Because predation is an important mechanism shown to effect the survivorship and development time of mosquito species in other studies, *Wyeomyia* spp. larvae from Florida bromeliads were dissected after exposure to *A. albopictus* to determine whether these *Wyeomyia* spp. may be facultative predators of *A. albopictus*.

**Materials and Methods**

**Testing for Density Dependent Predation by *Wyeomyia* spp.**

In this experiment, three densities (0, 25, 50) of 1st instar *A. albopictus* were placed into plastic containers containing 75 mL sieved bromeliad water and 0.5 g dried (68°C for 48 h), chopped *Quercus virginiana* leaves. Thirty-six containers were used, allowing 12 replicates of each density. To each container thirty 4th instar *Wyeomyia* spp. were added after previously being kept in tap water without food for 36 h prior to the experiment. The lack of food for *Wyeomyia* spp. prior to the experiment was to ensure that all gut contents examined after the experimentation resulted from the food obtained during the experiment, and not prior feedings. After 12 h, 6 containers were removed from each density, and all live 4th instar *Wyeomyia* spp. larvae were dissected. At 24 h, the remaining containers were removed and again all of the live 4th instar *Wyeomyia* spp. larvae were dissected. Dead larvae of *Wyeomyia* spp., and pupae were not dissected.

**Testing for Resource Dependent Predation by *Wyeomyia* spp.**

In a second experiment, two densities of food resources (0.1 g and 0.5 g) of dried (68°C for 48 h), chopped *Q. virginiana* leaves were placed into 24 plastic containers with 75 mL sieved
bromeliad water and 25 1st instar *A. albopictus*. Twenty-five 4th instar *Wyeomyia* spp. were added to each container. Twelve hours and 96 h after the start of the experiment, 6 containers at each food level were removed and their live 4th instar *Wyeomyia* spp. were dissected. As before, dead *Wyeomyia* spp., and pupae were not dissected.

**Predation Observation**

Visual examinations of the behavior of *A. albopictus* and *Wyeomyia* spp. were conducted in plastic containers. Ten 1st instar *A. albopictus* were added with either (1) 0.1 g food and no *Wyeomyia* spp.; (2) 0.1 g food and 10 4th instar *Wyeomyia* spp.; (3) 0.5 g food and no *Wyeomyia* spp.; or (3) 0.5 g food and 10 4th instar *Wyeomyia* spp.. There were two replicates of each combination. The food was dried, chopped *Q. virginiana* leaves as used in the two previously described predation experiments. Two cups at each food level were selected, every hour for 10 h, to be examined for signs of predation by *Wyeomyia* spp. on *A. albopictus*. Predatory behavior was classified as chewing, biting, grabbing or actually consuming another organism. Each examination occurred for 1 min, and since light was necessary for visualization of the 1st instar *A. albopictus*, a desk light kept the cups illuminated for the entire 10 h duration of the experiment.

**Results**

No *A. albopictus* body parts were found in the guts of any of the 941 *Wyeomyia* spp. dissected (Table 3-1). All guts of *Wyeomyia* spp., including the treatment without *A. albopictus*, contained brown organic material, but no signs of head capsules, or any other body parts, of *A. albopictus*. There were also no signs of aggression upon *A. albopictus* by *Wyeomyia* spp. throughout the entire duration of the predation observation experiment.
Discussion

From these experiments, it seems very unlikely that \textit{Wyeomyia} spp. prey upon \textit{A. albopictus}. When field-collected larvae of \textit{W. vanduzeei} were examined by Fish (1976), no remains from any invertebrates were found; only organic particulate matter, protozoans and pollen grains were found in the gut contents. In a study by Broberg and Bradshaw (1995), the pitcher plant mosquito, \textit{Wyeomyia smithii}, was found to not exhibit density dependent cannibalism. Therefore, although other detritivores, such as some \textit{Aedes} and \textit{Anopheles} species, can be cannibalistic or facultative predators, it seems that \textit{Wyeomyia} spp. of North America do not exhibit these characteristics, at least under the density and resource conditions examined in the lab.

As an adaptation to the variable water and resource levels in a bromeliad, \textit{W. vanduzeei} can develop slowly on limited food resources (Frank and Curtis 1977), so the \textit{Wyeomyia} spp. of Florida bromeliads may not have adopted a predation strategy, because they have adapted to a food limited environment. Most \textit{Aedes} and \textit{Anopheles} species that are cannibalistic or facultative predators would starve at food levels that \textit{Wyeomyia} spp. can subsist upon (Barrera and Medialdea 1996).

As with any lab experiment, the applicability of these results to nature comes into question. As mentioned in other chapters, bromeliad phytotelmata vary in size, structure, complexity, water holding capacity, and faunal composition. All of these factors could influence the expression of predation in \textit{Wyeomyia} spp.. Variations of bromeliad phytotelm size, structure, and complexity have been examined to some extent in this study. In this study, whether conducted within an actual bromeliad or an artificial one, there was no significant reduction in survivorship of \textit{A. albopictus} that would indicate predation. In conclusion, if there is predation
by *Wyomyia* spp. on *A. albopictus* it occurs so rarely as to be unimportant in overall survivorship of *A. albopictus*. 
Table 3-1. Number of *Wyeomyia* examined in predation experiments.

<table>
<thead>
<tr>
<th>Time</th>
<th>0 Albo.</th>
<th>25 Albo.</th>
<th>50 Albo.</th>
<th>Low food</th>
<th>High food</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>111</td>
<td>123</td>
<td>128</td>
<td>74</td>
<td>111</td>
</tr>
<tr>
<td>24 h</td>
<td>117</td>
<td>126</td>
<td>151</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>96 h</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>87</td>
<td>158</td>
</tr>
</tbody>
</table>

This table compiles the number of dissected *Wyeomyia* spp. from two different experiments. The time box indicates the number of hours after *A. albopictus* were added that *Wyeomyia* were examined. The next three categories, 0 Albo., 25 Albo., and 50 Albo., correspond to the first experiment in which the *Wyeomyia* were examined in the presence of 0, 25 and 50 1st instar *A. albopictus*. The next two categories, low food and high food, indicate the treatments in the second experiment in which *Wyeomyia* spp. were examined after exposure to 0.1 g or 0.5 g of food in the presence of 25 1st instar *A. albopictus*. 
CHAPTER 4
DO Wyemyia spp.. LARVAE INHIBIT EGG HATCH OF Aedes albopictus?

Introduction

Eggs of Aedes spp. hatch after submersion in water, and low oxygen levels caused by nearby microbial growth stimulate hatching (Gjullin et al. 1941, Judson 1960, Fallis and Snow 1983). Most eggs will hatch in response to the initial stimulus, but some hatching is delayed, awaiting later stimuli (Gillett et al. 1977, Livdahl and Koenekoop 1985, Andreadis 1990). Suppression of egg hatch by larvae occurs when the larvae consume microbes on and near the eggs, thus causing a reduction in the local microbial population, and a subsequent increase in oxygen resulting in inhibition (Gillett et al. 1977, Edgerly and Marvier 1992). There are also abiotic secondary determinants of hatch inhibition, such as temperature (Mallack et al. 1964), photoperiod (Horsfall 1956, McHaffey and Harwood 1970, McHaffey 1972, Shroyer and Craig 1980), and variations in wet and dry periods or humidity (Andreadis 1990, Clements 1999), which will also delay hatch by putting eggs into quiescence or diapause until conditions are favorable for hatching.

Egg hatch inhibition is hypothesized to benefit larvae by delaying hatch when competition for resources is high (Livdahl et al. 1984, Livdahl and Edgerly 1987), risk of predation is high (Koenekoop and Livdahl 1986), or when abiotic conditions are unfavorable for development (Shroyer and Craig 1980, Clements 1999). For many Aedes species, these benefits are thought to outweigh the costs of egg hatch inhibition, which increases the time until reproduction. Increasing the time to reproduction may lead to a reduction in population growth and may increase mortality in the dormant egg stage of aedine mosquitoes (Livdahl and Edgerly 1987).
Because many *Aedes* mosquitoes exhibit some level of hatch inhibition (Edgerly et al. 1993), it is important to examine this role of competition on *Aedes* hatch. Within the exotic bromeliads of southern Florida, larval competition between *Wyeomyia* spp. and *A. albopictus* is thought to influence the mosquito community structure in this system (Lounibos et al. 2003), although the mechanism for this competition has yet to be determined. Field collections from exotic bromeliads in southern Florida show that high densities of 4th instar *Wyeomyia* spp. are common in exotic bromeliads (Raban unpublished data). Additionally, *Wyeomyia* larvae develop slowly, as an adaptation to the variable water and resources levels in a bromeliad (Frank and Curtis 1977), so it is feasible that these large, later instar larvae are present for long durations. Also, *W. mitchellii* and *W. vanduzeei* are found in the larval stage throughout the year, while some *A. albopictus* overwinter as eggs. Thus, even an initial spring *A. albopictus* cohort could experience egg hatch inhibition from the presence of *Wyeomyia* spp. Therefore, a resource dependent egg inhibition experiment was conducted to determine whether older instar *Wyeomyia* spp. inhibit the hatch of *A. albopictus* eggs and whether inhibition varies with larval food level.

**Materials and Methods**

Fifty *A. albopictus* eggs on each of 56 papers inserted individually in plastic cups were submerged in 75 mL of sieved bromeliad water. All *A. albopictus* eggs used in the study were from one oviposition paper, resulting from approximately 3 days of oviposition from a Florida colony of this species supplemented irregularly with wild mosquitoes. All eggs were counted under a dissecting microscope to ensure no hatch or damage before the start of the experiment. Twenty-eight of these cups were randomly assigned to a low food level of 0.1 g of dried, chopped, live oak leaves (*Q. virginiana*), and the remaining 28 were assigned a high food level of 0.5 g of dried, chopped, live oak leaves. All leaves were dried at 41°C for 72 h before the
experiment. At each food level, 20 field collected, 4th instar *Wyeomyia* spp. were added to 14 of the cups. The other fourteen cups contained only the 50 *A. albopictus* eggs and served as controls. After 48 h the experiment was terminated, and the egg hatch rates were recorded for each cup. The number of eggs hatched was determined by counting the number of *A. albopictus* larvae present and the number of eggs that hatched on the paper. Whichever hatch number was higher, number of larvae or the number of eggs hatched on the paper, was considered the hatch number for that cup. In almost all cases, the number of eggs hatched on the paper exceeded or equaled the number of larvae found in the cup.

The bleaching technique of Trpis (1970) was used to determine the viability of the unhatched eggs. Eggs were considered viable if they had a fully developed embryo. Empty eggs and partial or shriveled embryos were considered inviable and were omitted in the data analysis. On the other hand, if eggs were not wholly encased and appeared to be crushed or damaged, these eggs were classified as damaged.

Due to the high number of inviable eggs, an ANOVA was conducted on their incidence to ensure their uniform spread across treatments. To ensure homogeneity of variances, hatch rates were arcsine square root transformed before an ANOVA was performed in SAS (2002) with a subsequent Tukey’s post-hoc test. A Kruskal-Wallis test and a Dunnet’s test was performed on the damaged egg data. Two separate treatments had one replicate each that was omitted from the analysis because they both had a 0.0% hatch rate with only 2 to 4 viable eggs per replicate.

**Results**

Overall, 29.0% ± 2.4% (mean ± SE) of all eggs were inviable, with no difference among treatments (*F*<sub>3,50</sub>= 1.88, *P* = 0.14). There was a significant difference between the hatch rates of the treatments (*F*<sub>3,50</sub>=6.28, *P*=0.02), which was attributed to the high food with *Wyeomyia* spp. treatment being significantly different than both food treatments without *Wyeomyia* spp. (Fig. 4-
1). There was a lower hatch rate for the treatments with *Wyeomyia* spp. as compared to the treatments without *Wyeomyia* spp., although this difference was only significant for the higher food concentration (Fig. 4-1). On the other hand, there was a significant variation ($\chi^2=8.06$, df=3, $P=0.04$) among treatments in the incidence of damaged eggs (Fig. 4-2). Significantly more damaged eggs were observed in the low food treatment with *Wyeomyia* spp. compared to both the food treatments without *Wyeomyia* spp. (Fig. 4-2).

**Discussion**

In this experiment, there was a significantly lower hatch rate of *A. albopictus* in the presence of fourth instar *Wyeomyia* spp at higher food concentrations. These findings are not overly surprising as previous studies by Edgerly et al. (1993) showed reduced hatch of *A. albopictus* in the presence of high densities of larger instar *Aedes* larvae at 24 h. While the hatch rate of *A. albopictus* in the lower resource treatment with *Wyeomyia* spp. was lower than the lower resource control, this difference was not significant, indicating that resource level may influence the egg hatch inhibition in *A. albopictus*.

Even though in this experiment egg hatch inhibition by *Wyeomyia* on *A. albopictus* was found to influence the hatch rate at high food concentrations, the effects on hatch rate were only moderate. In this experiment, treatments with *Wyeomyia* experienced only an approximately 9% decrease in hatch rate at 48 h, and 80% to 90% of viable eggs still hatched in all treatments. Therefore, while there may be egg hatch inhibition, if the hatch rates seen in this experiment are indicative of natural field hatch rates, then the influence of egg hatch inhibition on *A. albopictus* development is not pronounced and does not explain the decrease in development rate of *A. albopictus* in the presence of *Wyeomyia* spp. seen in the surface area to volume ratio experiment, and the larval experiment in Lounibos et al. (2003).
The reduced effects of egg hatch inhibition on *A. albopictus* are not surprising, as Edgerly et al. (1993) found that at higher larval densities *A. albopictus* egg hatch was inhibited, but at reduced rates as compared to other *Aedes* spp. mosquitoes tested. So, in general it is possible that *A. albopictus* does not experience pronounced egg hatch inhibition. Also, this experiment was allowed to run for 48 h, while the Edgerly et al. (1993) experiment was terminated at 24 h, so it is possible that most pronounced egg hatch inhibition occurs in the first 24 h, which was missed in a 48 h experiment.

Livdahl et al. (1984) and Livdahl and Edgerly (1987) found that *Aedes triseriatus* hatch rates are reduced by large densities of late instar conspecifics. These authors hypothesized that large 4th instar larvae inhibit hatch through their elevated grazing intensity, which reduces oxygen-depleting microbes. The significantly larger amount of egg damage seen in the presence of *Wyeomyia* spp. in this experiment may be a result of browsing by the late instar *Wyeomyia* spp. on the eggs of *A. albopictus*. Because the percentage of eggs damaged was highest in the low resource group (but not significantly different from high resources) with *Wyeomyia* spp., it suggests that with fewer leaf resources to graze upon, *Wyeomyia* spp. may have increased their grazing upon *A. albopictus* eggs. The majority of egg damage probably occurred after hatch or did not effect hatch rate, because the high resource with *Wyeomyia* spp. treatment had lower hatch rate than the lower resource with *Wyeomyia* treatment, but the lower resource treatment with *Wyeomyia* spp. had higher egg damage. Likewise, the lower resource with *Wyeomyia* spp. treatment did not have a significantly different hatch rate from either of the controls at each resource level, but it did have significantly more egg damage. Thus, there seems to be no strong relationship between hatch rate and egg damage.
In nature, egg hatch inhibition of *A. albopictus* within bromeliads could differ from the laboratory results. In the typical bromeliad habitat many other organisms could also be influencing the bacterial content of the area around the eggs and, thus, the hatch rate of *A. albopictus* eggs. Many other bacteria- and detritus-consuming macroinvertebrates are known to inhabit bromeliads (Fish 1976, Frank 1983).

Additionally, the bromeliad itself can influence the chemical composition of its contents based on its physiological needs (see bromeliad as a habitat section in Chapter 1 of this thesis). For instance, Fallis and Snow (1983) determined that an increase in water nitrogen induced hatch in *Aedes punctor*, and nitrogen is one of the main chemicals that is absorbed by bromeliads (Benzing 2000), and thus may influence the hatch of *A. albopictus*. Fallis and Snow (1983) also found that the change in oxygen content, and not the concentration of oxygen was the hatching trigger for *A. punctor*, which may also be regulated by the bromeliad. So, although *Wyeomyia* spp. may inhibit the hatch of *A. albopictus* eggs at high resource levels, it is possible that other organisms or processes within the bromeliad can alter egg hatch inhibition in *A. albopictus*.

With more macroinvertebrates in the aquatic community, there could be more grazing and an increase in localized oxygen levels near the eggs, and thus more inhibition. In contrast, Edgerly and Marvier (1992) hypothesized that at a certain density, the number of organisms surrounding eggs can be great enough to deplete the overall oxygen levels in the water, and thus stimulate egg hatch. Although as mentioned before, Edgerly et al. (1993) found that at high densities *A. albopictus* had a low level of hatch inhibition compared to congeners, so higher densities of mosquitoes may have little effect on the overall hatch of *A. albopictus*.

In this experiment the eggs of *A. albopictus* were submerged below the water line in a cluster on the side of the container. In the location study from Chapter 2 of this thesis
Wyeomyia spp. larvae were located more often at the bottom and at the top of the container and less on the sides. If A. albopictus does not oviposit its eggs on the inner axil walls of bromeliads, then this experiment may misrepresent the influence of Wyeomyia spp. on egg hatch inhibition of A. albopictus. There is no evidence that A. albopictus lays its eggs on the sides of the bromeliads, nor is it probable that their eggs are laid in one cluster as in this experiment. Further investigation into the location and distribution of oviposited eggs of A. albopictus within bromeliads is needed to determine the relevance of this experiment to natural conditions.
Figure 4-1. Proportion hatch of viable eggs ± SE in each treatment. High Food refers to high food treatments, and Low food refers to low food treatments. No Wy indicates treatments with no Wyeomyia spp. and Wy indicates treatments with Wyeomyia spp. Treatments with the same letter above the SE bar are not significantly different based on a Tukey test.
Figure 4-2. Proportion eggs damaged ±SE per treatment. High Food refers to high food treatments, and Low food refers to low food treatments. No Wy indicates treatments with no Wyeomyia spp. and Wy indicates treatments with Wyeomyia spp.. Treatments with the same letter above the SE bar are not significantly different based on a Dunnett’s Test.
CHAPTER 5
FIELD STUDIES ON Aedes albopictus AND Wyeomyia spp. IN EXOTIC BROMELIADS OF SOUTHERN FLORIDA

Introduction

In a study conducted by O’Meara et al. (1995b), Wyeomyia spp. were the most abundant mosquitoes in bromeliads throughout southern Florida, but in northern Florida where W. vanduzeei and W. mitchelli are absent, A. albopictus was the most common mosquito species in bromeliads. In the southern sites with Wyeomyia spp., A. albopictus was often found in great numbers in nearby artificial containers. For example, when vases were placed near bromeliads in Vero Beach, Florida, A. albopictus larvae were subsequently collected from all the vases but from only 40 percent of the bromeliads. In the study of Lounibos et al. (2003), A. albopictus was also found in bromeliads in greater numbers in the absence of Wyeomyia spp..

Wyeomyia spp. vary in larval abundance and ovipositional preference based on the species of bromeliad. In collections by O’Meara et al. (1995b), Wyeomyia spp. were less abundant in Neoregelia spectabilis than in Aechmea fasciata. Frank and O’Meara (1985) also found that W. vanduzeei showed an oviposition preference for the native bromeliad Tillandsia utriculata over another native bromeliad, Catopsis berteroniana.

The purpose of the current study was to further explore the differences in abundance and distribution of Wyeomyia spp. and A. albopictus within bromeliads based on the location within the plant (axil or central tank), by bromeliad species, and by macrohabitat. Neoregelia spectabilis and B. pyramidalis are two exotic bromeliad species commonly featured in the residential landscaping of many homes in the cities of Vero Beach and Fort Pierce, Florida. These two plants were chosen for further study because they were common and distinctly different in size, color, and shape, but are often grown in the same location. In the current study,
N. spectabilis and B. pyramidalis were sampled to compare the abundances of Wyeomyia spp. and A. albopictus in these two bromeliad species. Due to the differences in their physical characteristics these bromeliads may have different abundances of mosquitoes. These samples were further divided by location within the plant, i.e. lateral axil or central axil, to determine whether the abundance of Wyeomyia spp. or A. albopictus varied by location within the plant. It is also hypothesized that the abundance of mosquitoes will not vary by location within the bromeliad, because bromeliad studies by Frank and Curtis (1977) have indicated that during rainfall eggs may be washed to other locations within the plant. Thus, if rainfall occurs frequently enough there should be a rather homogeneous distribution of larvae throughout the plant. To further describe habitat preferences of bromeliad-inhabiting mosquitoes, mosquitoes were collected from the field to determine whether mosquito abundance varied between two canopy types, mainly oak tree and palm tree. Frank and O’Meara (1985) determined that W. mitchellii preferred shady habitats and, thus, different canopy types could also provide shade variations that influence the abundance of bromeliad mosquitoes.

Materials and Methods

Differences in the Density of A. albopictus and Wyeomyia spp. within N. spectabilis and B. pyramidalis

The aquatic contents of B. pyramidalis and N. spectabilis bromeliads were collected with a meat baster monthly from September 2005 to July 2006 at eight sites in Vero Beach and Fort Pierce, Florida, and from two sites in Orlando, Florida (Fig 5-1). On a few occasions plants in Tampa Bay and Washington Oaks Park were also sampled (Fig 5-1). At each site, five to twenty-five plants of each species were sampled every 4 to 8 weeks. To avoid taking repeated samples from the same plants, at each sampling the approximate locations of the samples were recorded, although repeated sampling may have occurred at two of the smaller Vero Beach, FL
sites due to a reduced number of plants. The sites where re-sampling of plants was suspected were omitted from all analyses.

From each plant two samples were taken by removing all extractable fluid contents with the baster. One sample consisted of the contents of the central axil of the plant, while a separate sample was taken of the lateral axil contents. The central axil sample was extracted from the area of water held in the center of the plant (photo A in Fig. 2.2), and the lateral axil sample was extracted from the spaces created by the lateral axils of the plant (photo B in Fig. 2.2).

Due to the fact that *B. pyramidalis* with inflorescences held very little, if any, water, only *B. pyramidalis* that were not flowering were sampled in this study. Conversely, since *N. spectabilis* holds very little if any water in its central tank when it is not flowering, only flowering *N. spectabilis* were sampled in this study. The species and development stage of each mosquito immature collected were recorded. To aid in the identification of 1st instar larvae, all samples were initially examined in trays under a dissecting scope.

Studies by Frank et al. (1977) have shown a correlation between bromeliad size and the number of mosquito larvae and pupae. After February 2006, in the current study the amount of water in each bromeliad was also measured to determine whether a correlation exists between the amount of water in a bromeliad and the abundance of either species of mosquito and their total. Thus, the analysis of the data from these collections is broken into two categories 1) all collections, with analyses being based on total mosquitoes per plant, and 2) collections after February 2006, which were analyzed by numbers of mosquitoes per volume of water. ANOVA calculated from type III sums of squares was applied to detect significant variations in the dependent variables (density of mosquitoes per mL and per plant) in relation to the independent variables (bromeliad species). Means comparisons were conducted with Tukey’s tests. The
density means (mos/mL and mos/plant) were also compared by a paired t-test in relation to the location within the plant (lateral vs. central axil). A repeated measures ANOVA, with subsequent means comparisons by Tukey’s tests, was applied to detect significant variations in the dependent density variables by the month of collection. Correlations were performed using Spearman’s ρ between the volume of water in bromeliads and the numbers of *A. albopictus* and *Wyeomyia* spp. found within.

**Canopy Effects on Density**

A study was also conducted on the differences in density of *Wyeomyia* spp. and *A. albopictus* in bromeliads based on the surrounding habitat. Preliminary observations during collections showed that there may be fewer *Wyeomyia* spp. and *A. albopictus* in bromeliads with palm trees overhead versus in bromeliads shaded by oak trees. These habitats differ in amount of sun exposure, leaf litter, and throughfall input into the bromeliads below.

The largest sample site in Vero Beach, FL (VB-5) contained four clumps of interspersed *N. spectabilis* and *B. pyramidalis* under palm trees and seven clumps of interspersed *N. spectabilis* and *B. pyramidalis* under oak trees. In both habitats *B. pyramidalis* was the more common of the two bromeliad species. Once in April 2006 and once in June 2006 two samples, one from the central axil, and one from the lateral axil, of fifteen *B. pyramidalis* and nine *N. spectabilis* were taken from under either an oak tree or a palm tree. In order to avoid sampling the same plants twice, the location of the first collection was different from the location of the second collection.

The mosquitoes collected were counted and recorded as described in the previous section. Significant effects of canopy type on the numbers of *Wyeomyia* spp. in bromeliads were tested by nested ANOVA with bromeliad species (*B. pyramidalis* vs. *N. spectabilis*) nested within canopy type (Oak vs. Palm). *Aedes albopictus* was omitted from ANOVA analysis as no larvae of this species were found in the oak location.
Results

Both mosquitoes per plant (mos/plant) and per unit volume (mos/mL) varied significantly between plant species (Table 5-1 and Table 5-2). *Aedes albopictus* had a higher density (mos/mL and mos/plant) in *N. spectabilis*, but *Wyeomyia* spp. was found in significantly higher densities in *B. pyramidalis* (Tables 5-3 and 5-4). *Aedes albopictus* had higher densities (mos/plant) in the central axils of *N. spectabilis* ($t_{217}= 2.22, P=0.03$), but showed no difference in density between the central and lateral axils in *B. pyramidalis* ($t_{242}= 0.86, P=0.39$) (Table 5-4). *Aedes albopictus* also showed no difference in density (mos/mL) between the central and lateral axils in either *N. spectabilis* ($t_{172}=-0.70, P=0.49$) or *B. pyramidalis* ($t_{193}= 1.00, P=0.32$). *Wyeomyia* spp. showed no differences in densities (mos/plant) between the lateral and central axils of *B. pyramidalis* ($t_{242}= 1.38, P=0.17$) or *N. spectabilis* ($t_{217}= 1.60, P=0.11$). However, *Wyeomyia* spp. did have higher mean densities (mos/mL) in the lateral axils of both *N. spectabilis* ($t_{172}= 2.27, P=0.02$) and *B. pyramidalis* ($t_{193}= 2.52, P=0.01$) (Table 5-4).

The densities (mos/plant) of *Wyeomyia* spp. and *A. albopictus* varied by the month of collection (*W*.- $F_6= 8.74, P<0.01$ and *A. albo*.- $F_6= 12.36, P<0.01$) and by the site of collection (*W*.- $F_8= 10.18, P<0.01$ and *A. albo*.- $F_8= 2.15, P=0.03$). The density of *A. albopictus* in *B. pyramidalis* was relatively constant throughout the year, but in *N. spectabilis* the density at each site of *A. albopictus* increased in September and in April through July (Fig. 5-3). In *B. pyramidalis* the density of *Wyeomyia* spp. increased in September, decreased in November through March, and increased in April and in July. *Aedes albopictus* also experienced similar changes in density (mos/mL) over time, but *Wyeomyia* spp. density (mos/mL) did not differ significantly among months (Tables 5-5, 5-6, Figs. 5-4, 5-5.).

There were also distinct differences in the water holding capacity between the plants (Table 5-7). More water was extracted on average for *N. spectabilis* than *B. pyramidalis* (Table
When the data were analyzed separately by the location within the plant there was significantly more water in *N. spectabilis* than *B. pyramidalis* in both lateral and central axils (Table 5-7). There were positive correlations between the number of *Wyeomyia* spp. ($\rho_{400} = 0.12; P=0.01$), the number of *A. albopictus* ($\rho_{400} = 0.26; P<0.01$), and the total mosquitoes ($\rho_{400} = 0.18 P<0.01$) and the amount of water extracted from the bromeliad.

*Wyeomyia* spp. differed in densities between the palm and oak sites (Table 5-8), and by plant species in the palm and oak sites (Table 5-9). *Wyeomyia* spp. were less abundant in the palm sites, and in both the oak and palm sites *Wyeomyia* spp. was found in a significantly greater densities in *B. pyramidalis* plants (Table 5-9).

**Discussion**

In the current surveillance *Wyeomyia* spp. immatures were denser in *B. pyramidalis* than in *N. spectabilis*. O’Meara et al. (1995b) showed that *Wyeomyia* spp. were more abundant in *Aechmea fasciata* than in *N. spectabilis*. On the other hand, *A. albopictus* had much higher mean densities in *N. spectabilis* than in *B. pyramidalis*. The differences in abundances in each plant could indicate that *A. albopictus* is avoiding contact with *Wyeomyia* spp. by ovipositing in bromeliads not occupied by *Wyeomyia* spp., but in an experiment conducted by Lounibos et al. (2003), *A. albopictus* showed no difference in ovipositional preference between *B. pyramidalis* with *Wyeomyia* spp. present or absent. The Lounibos et al. (2003) experiment indicates that ovipositing *A. albopictus* are not responding to cues from *Wyeomyia* spp. larvae. The Washington Oaks site had the greatest abundance of *A. albopictus*, and the lowest abundance of *Wyeomyia* spp. The studies by O’Meara et al. (1995b) and Lounibos et al. (2003) indicate that more northern sites like Washington Oaks have more *A. albopictus* in their bromeliads than in the southern sites. These two studies also indicated that lower abundances of *Wyeomyia* spp. occurred in northern Florida, which was likely due to the intolerance of *Wyeomyia* spp. to
temperatures below freezing, or to the rarity of native bromeliad phytotlemata in northern Florida.

There were differences in the densities of *Wyeomyia* spp. and *A. albopictus* by location within the plant. *Wyeomyia* spp. had higher densities (mos/mL) in the lateral axils of *B. pyramidalis* and *N. spectabilis*, so either *Wyeomyia* spp. oviposits more frequently in lateral axils, or if *Wyeomyia* spp. oviposit more eggs into the central axil, then some eggs or larvae are washed by rainfall into the lateral axils. Frank et al. (1976) observed that female *W. vandueezi* oviposit more frequently into the central axils of *T. utriculata*, but were still found in the lateral axils of that bromeliad species. In a Frank and Curtis (1977) study, *W. vandueezi* were found to be easily washed out of the central tank of the bromeliad during rainfall. Therefore, it is unknown whether the lateral axils and central axils have the same number of mosquitoes due to oviposition preference or due to egg and larval movement during rainfall.

The Frank et al. (1977) study determined that there was a correlation between the size of the bromeliad and the number of mosquitoes within the bromeliad. The Frank et al. (1977) experiment used the total water holding capacity of the bromeliad as the indicator of the size of the bromeliad. In the current research, the actual amount of water within the bromeliad, not the total holding capacity, was used to measure the densities of mosquitoes per milliliter of extracted fluid. While the quantity of water actually present is probably not as good an indicator of size as the total water holding capacity of the bromeliad, it gives an approximation of the habitat available to the mosquito at the time of sampling.

There was positive correlation between the amount of water present in the bromeliad and the number of mosquitoes extracted, although its strength varied between mosquito species. *Aedes albopictus* had a stronger positive correlation with water volume than *Wyeomyia* spp.,
which is possibly due to the fact that *Wyeomyia* spp. are bromeliad specialists, while *Aedes albopictus* is a container generalist. *Wyeomyia* spp. are probably better adapted to the variable bromeliad environment, and thus can thrive under a variety of bromeliad conditions, including reduced water. For instance, Frank and Curtis (1977) hypothesized that the prolonged larval development of *W. vanduzeei* under reduced food conditions was an adaptation to the variable habitat in bromeliads. *Aedes albopictus* on the other hand, can also have prolonged larval development with reduced resources (Barrera and Medialdea 1996), but probably cannot prolong its life as long as *Wyeomyia* spp. Thus, under variable conditions *A. albopictus* females have to either be more selective for environments with more water for their oviposition, or risk a reduction in survivorship due to desiccation or decreased space. Conversely, ovipositing *Wyeomyia* spp. females do not have to be as selective for the amount of water in the bromeliad habitat, as their larvae can tolerate alterations in water conditions.

In the habitat study *Wyeomyia* spp., but not *A. albopictus*, differed in mean densities under different canopy types. It is possible that with more samples, *A. albopictus* would have also differed in mean densities, because the total number of *A. albopictus* recovered from all plants was small. Since the palm trees offered less protection from the sun, the bromeliads under palms were exposed to more insolation. In a study by Frank and O’Meara (1985), *W. mitchellii* showed a preference for shaded habitats. In the current study, on two occasions the larvae collected from most sites were identified to species. During both examinations about 80-85% of the *Wyeomyia* spp. collected were *W. mitchellii* with the remaining being *W. vanduzeei*. Thus, the decrease in density of *Wyeomyia* spp. in the palm habitat was most likely due to the preference of *Wyeomyia mitchellii* for shaded areas.
Even though *Wyeomyia* spp. had the higher mean density in all plants, *Wyeomyia* spp. had a higher mean density in *B. pyramidalis*, while *A. albopictus* had a higher mean density in *N. spectabilis*. In the following chapter, oviposition and larval competition experiments were conducted to explain the causes of the differing relative abundances of *Wyeomyia* spp. and *A. albopictus* in these two bromeliad species.
Table 5-1. Analysis of variance for the density of mosquitoes per plant based on month (month of collection), site, and bromeliad species (N. spectabilis vs. B. pyramidalis).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F-statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>6,472</td>
<td>0.27</td>
<td>0.53</td>
</tr>
<tr>
<td>Site</td>
<td>7,472</td>
<td>12.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bromeliad species</td>
<td>1,472</td>
<td>7.92</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Note:** A repeated measures ANOVA was applied to the month variable.
Table 5-2. Analysis of variance for the density of mosquitoes per mL based on month (month of collection), site, and bromeliad species (N. spectabilis vs. B. pyramidalis).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F-statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>4,378</td>
<td>4.50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Site</td>
<td>7,378</td>
<td>3.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bromeliad species</td>
<td>1,378</td>
<td>42.92</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

A repeated measures ANOVA was applied to the month variable.
Table 5-3. Analysis of variance for the density of each mosquito species per mL of water in each bromeliad species.

<table>
<thead>
<tr>
<th>Mosquito</th>
<th>Bromeliad</th>
<th>Mean ± SE (mos/mL)</th>
<th>F-Statistic</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. albopictus</td>
<td>N. spectabilis</td>
<td>0.03±0.11</td>
<td>7.16</td>
<td>1,399</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B. pyramidalis</td>
<td>0.0003±0.0003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyeomyia spp.</td>
<td>N. spectabilis</td>
<td>0.09±0.01</td>
<td>42.53</td>
<td>1,399</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B. pyramidalis</td>
<td>0.26±0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These means are representative of the data taken after February 2006. All samples including those with no mosquitoes were included in this analysis.
Table 5-4. Mean densities of mosquitoes (mos/plant) in B.pyramidalis and N. spectabilis based on location within the plant and plant species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>albopictus abundance (mos/plant)</th>
<th>Wy. spp. abundance (mos/plant)</th>
<th>Total Abundance (mos/plant)</th>
<th>Total Wy spp. abundance (mos/plant)</th>
<th>Total mosquito abundance (mos/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Total Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>N. spectabilis</td>
<td>central axil</td>
<td>1.67 ± 0.32</td>
<td>2.53 ± 0.44</td>
<td>2.58 ± 0.39</td>
<td>5.67 ± 0.77</td>
<td>8.30 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>lateral axil</td>
<td>0.92 ± 0.16</td>
<td>3.13 ± 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. pyramidalis</td>
<td>central axil</td>
<td>0.06 ± 0.03</td>
<td>7.87 ± 0.54</td>
<td>0.11 ± 0.04</td>
<td>16.48 ± 1.01</td>
<td>16.60 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>lateral axil</td>
<td>0.05 ± 0.02</td>
<td>8.62 ± 0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1: $F_{1,472}=42.53$, $P<0.01$; 2: $F_{1,472}=18.07$, $P<0.01$; 3: $F_{1,472}=18.51$, $P<0.01$. 
Table 5-5. Analysis of variance table for the effects of month and site on mos/mL and mos/plant.

<table>
<thead>
<tr>
<th></th>
<th>Mosquito</th>
<th>Effect</th>
<th>F-statistic</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mos/mL A. albopictus</td>
<td>Month</td>
<td>9.17</td>
<td>4,378</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>mos/mL Wyeomyia spp.</td>
<td>Month</td>
<td>2.07</td>
<td>4,378</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>mos/plant A. albopictus</td>
<td>Month</td>
<td>12.36</td>
<td>6,472</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>mos/plant Wyeomyia spp.</td>
<td>Month</td>
<td>8.74</td>
<td>6,472</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>mos/plant A. albopictus</td>
<td>Site</td>
<td>2.15</td>
<td>8,472</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>mos/plant Wyeomyia spp.</td>
<td>Site</td>
<td>10.18</td>
<td>8,472</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
Table 5-6. Analysis of variance results for the effect of month and sites on mosquito abundances (mos/plant) by bromeliad species.

<table>
<thead>
<tr>
<th>Bromeliad</th>
<th>Mosquito</th>
<th>Effect</th>
<th>F-statistic</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. pyramidalis</td>
<td>Total Mosquitoes</td>
<td>Month</td>
<td>25.58</td>
<td>6,252</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site</td>
<td>7.53</td>
<td>7,252</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N. spectabilis</td>
<td>Total Mosquitoes</td>
<td>Month</td>
<td>7.14</td>
<td>6,225</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site</td>
<td>13.47</td>
<td>8,225</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B. pyramidalis</td>
<td>Wyeomyia spp.</td>
<td>Month</td>
<td>24.13</td>
<td>6,252</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site</td>
<td>7.30</td>
<td>7,252</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N. spectabilis</td>
<td>Wyeomyia spp.</td>
<td>Month</td>
<td>3.86</td>
<td>6,225</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site</td>
<td>16.86</td>
<td>8,225</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B. pyramidalis</td>
<td>A. albopictus</td>
<td>Month</td>
<td>6.37</td>
<td>6,252</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site</td>
<td>0.64</td>
<td>7,252</td>
<td>0.73</td>
</tr>
<tr>
<td>N. spectabilis</td>
<td>A. albopictus</td>
<td>Month</td>
<td>12.99</td>
<td>6,225</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site</td>
<td>2.02</td>
<td>8,225</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 5-7. Average (±SE) amount of water extracted from each plant species.

<table>
<thead>
<tr>
<th></th>
<th>N. spectabilis lateral axil</th>
<th>B. pyramidalis lateral axil</th>
<th>N. spectabilis central axil</th>
<th>B. pyramidalis central axil</th>
<th>Total water N. spectabilis</th>
<th>Total water B. pyramidalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean water (mL) ± SE</td>
<td>56.04 ± 2.96</td>
<td>39.03 ± 2.36</td>
<td>53.08 ± 3.81</td>
<td>33.28 ± 1.45</td>
<td>109.12 ± 5.80</td>
<td>79.09 ± 2.90</td>
</tr>
<tr>
<td>ANOVA results</td>
<td>$F_{1,198} = 3.02; P&lt;0.01$- by plant species</td>
<td>$F_{1,198} = 47.59; P&lt;0.01$- by plant species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5-8. A nested ANOVA table for effects of Oak vs. Palm canopy and bromeliad species (*B. pyramidalis* vs. *N. spectabilis*) on *Wyeomyia* spp. densities by mos/plant.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wyeomyia</em> spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oak vs. Palm</td>
<td>26.61</td>
<td>1,32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>B. pyramidalis</em> vs. <em>N. spectabilis</em></td>
<td>8.77</td>
<td>1,32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oak vs. Palm</td>
<td>6.73</td>
<td>1,28</td>
<td>0.01</td>
</tr>
<tr>
<td>(<em>B. pyramidalis</em> vs. <em>N. spectabilis</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5-9. Means and SE of densities of *A. albopictus* and *Wyeomyia* spp. by location within plants under oak and palm canopies.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wyeomyia spp. Oak</strong></td>
<td></td>
</tr>
<tr>
<td><em>B. pyramidalis</em> central axil</td>
<td>15.73 ± 3.20</td>
</tr>
<tr>
<td><em>B. pyramidalis</em> lateral axil</td>
<td>7.80 ± 1.16</td>
</tr>
<tr>
<td><em>N. spectabilis</em> central axil</td>
<td>3.85 ± 0.92</td>
</tr>
<tr>
<td><em>N. spectabilis</em> lateral axil</td>
<td>4.08 ± 0.73</td>
</tr>
<tr>
<td><strong>Wyeomyia spp. Palm</strong></td>
<td></td>
</tr>
<tr>
<td><em>B. pyramidalis</em> central axil</td>
<td>1.00 ± 0.50</td>
</tr>
<tr>
<td><em>B. pyramidalis</em> lateral axil</td>
<td>0.75 ± 0.41</td>
</tr>
<tr>
<td><em>N. spectabilis</em> central axil</td>
<td>0.57 ± 0.43</td>
</tr>
<tr>
<td><em>N. spectabilis</em> lateral axil</td>
<td>0.14 ± 0.14</td>
</tr>
<tr>
<td><strong>A. albopictus Oak</strong></td>
<td></td>
</tr>
<tr>
<td><em>B. pyramidalis</em> central axil</td>
<td>0</td>
</tr>
<tr>
<td><em>B. pyramidalis</em> lateral axil</td>
<td>0</td>
</tr>
<tr>
<td><em>N. spectabilis</em> central axil</td>
<td>0</td>
</tr>
<tr>
<td><em>N. spectabilis</em> lateral axil</td>
<td>0</td>
</tr>
<tr>
<td><strong>A. albopictus Palm</strong></td>
<td></td>
</tr>
<tr>
<td><em>B. pyramidalis</em> central axil</td>
<td>0.13 ± 0.13</td>
</tr>
<tr>
<td><em>B. pyramidalis</em> lateral axil</td>
<td>0.38 ± 0.26</td>
</tr>
</tbody>
</table>
Figure 5-1. Map of collection sites. Figure adapted from O’Meara et al. 1995b, Fig. 1, Pg. 218.
Figure 5-2. Location of water samples. The white arrow indicates the location where water samples were taken from the central axil (Photo A) and the lateral axils (Photo B) of a *N. spectabilis*. 
Figure 5-3. The monthly mean densities of *Wyeomyia* spp. and *A. albopictus* in *N. spectabilis* and *B. pyramidalis* at each site. Error bars represent SE of the means at each site. For *B. pyramidalis* n= 243 plants (12.88 ± 1.58 plants sampled per month at each site (mean ±SE)), and for *N. spectabilis* n= 218 plants (9.53 ± 1.00 plants sampled per month at each site (mean ± SE)).
Figure 5-4: The monthly mean densities of *Wyeomyia* spp. and *A. albopictus* in *N. spectabilis* at each site. Error bars represent SE of the means at each site. Means without a common letter written above indicate significant differences for *Wyeomyia* spp.. Means without a common letter written below indicate significant differences for *A. albopictus*.
Figure 5-5. The monthly mean densities of *Wyeomyia* spp. and *A. albopictus* in *B. pyramidalis* at each site. Error bars represent SE of the means at each site. Means without a common letter written above indicate significant differences for *Wyeomyia* spp.. Means without a common letter written below indicate significant differences for *A. albopictus*.
CHAPTER 6
OVIPOSITION AND LARVAL DEVELOPMENT OF Aedes albopictus IN TWO EXOTIC SPECIES OF BROMELIAD IN SOUTHERN FLORIDA

Introduction

Oviposition behavior by the female determines the aquatic habitat of the mosquito’s larval stages (Clements 1999). Thus, it is not surprising that most ecological theories on oviposition selection are generally based on the assumption that adults choose oviposition sites that maximize the survivorship and reproductive output of their offspring and minimize their development time (Srivastava and Lawton 1998). At least for some herbivorous insects, there is some indication that fitness considerations of ovipositing females are also important in choice of oviposition sites (Mayhew 2001). Whatever the ecological significance of oviposition preference, numerous experiments have explored the cues that insects use to select oviposition sites.

Culicids select oviposition sites based on visual, olfactory and tactile cues (Bentley and Day 1989). The most common visual ovipositional stimuli of studied culicids are color cues. Members of the genus Toxorhynchites are often attracted to black colored containers (Hilburn et al. 1983, Jones and Schreiber 1994, Collins and Blackwell 2000), as are many species of the genus Aedes (Beckel 1955, Wilton 1968). Culex mosquitoes are most often found to be attracted to black and red (Dhileepan 1997), although color preference cannot be generalized by genus. Aedes triseriatus are attracted to darker colors in the blue spectrum (Williams 1962, McDaniel et al. 1976), and A. aegypti responded most strongly to black artificial bromeliads (Frank 1985).

There are also many chemical ovipositional cues originating from the habitat and from conspecifics, predators, parasites and other species. There are numerous studies which indicate that mosquitoes orient to cues from the organic material within the habitat (reviewed by Clements 1999). For instance, in a laboratory study conducted by Wilton (1968), A. triseriatus
oviposited significantly more eggs in water collected from a treehole than in distilled water of similar color. *Culex* spp. have also been found to respond to habitat specific chemical cues derived from bacteria or the organic matter of the habitat (Ikeshoji et al. 1967, Beehler et al. 1994). Species of other mosquito genera also orient to chemical oviposition cues derived from the habitat (Ikeshoji and Mulla 1970b, Bentley et al. 1979, Millar et al. 1992, Beehler et al. 1994). Habitat humidity (Kennedy 1942), salinity (Navarro et al. 2003), or algal content (Bond et al. 2005) also influenced the oviposition site selection of mosquitoes.

Mosquitoes often respond to the presence of conspecifics and other species when choosing oviposition sites. Some of the first olfactory studies conducted on Culicidae involved the discovery of chemical oviposition cues in the egg rafts *Culex* spp. (Hazard et al. 1967, Ikeshoji et al. 1967, Osgood 1971, Bruno and Laurence 1979, Laurence and Pickett 1982). In the *Culex* egg raft experiments, female mosquitoes were attracted to oviposition sites that contained conspecific eggs. Other mosquito species have shown a preference for ovipositing in the presence of conspecific larvae (Soman and Reuben 1970, Bentley et al. 1976, Allan and Kline 1998, Mokany and Shine 2003), or larvae of other species (Bentley et al. 1976, Allan and Kline 1998). Mosquitoes are hypothesized to be attracted to oviposit in habitats with conspecifics, or other species, because the presence of other mosquitoes is a possible indication of a successful larval habitat (Clements 1999).

Some culicids have ovipositional preferences related to the presence or absence of predators. In a study conducted by Munga et al. (2006), *Anopheles gambiae* avoided ovipositing in containers that had been preconditioned with the waste of either of two predators. Avoidance of oviposition in the presence of predators has also been seen for *Ochlerotatus australis*.
(Mokany and Shine 2003), Culiseta longiareolata (Blaustein et al. 2004), and Aedes taeniorhynchus (Ritchie and Laidlawbell 1994).

Once eggs are laid, the conditions within the habitat influence larval development. Since *A. albopictus* is a container-inhabiting mosquito, variations in natural and artificial container types occupied by this species influence larval development and survivorship. In treehole communities, resource quantity within the habitat can influence larval development and survivorship (Fish and Carpenter 1982, Leonard and Juliano 1995, Walker et al. 1997) and also influence the community structure (Srivastava and Lawton 1998). The permanence of the habitat also influences the development of larvae (Blaustein and Chase 2007), with drying of the habitat often decreasing larval survivorship.

In southern Florida, *A. albopictus* is common in artificial or natural containers (O'Meara et al. 1995a) and is occasionally found inhabiting bromeliads (O'Meara et al. 1995b). Within these bromeliad habitats *A. albopictus* often co-occurs with other species such as *W. mitchellii* and *W. vanduzeei*, and occasionally with *A. aegypti*, *Aedes bahamensis*, *Culex quinquefasciatus* and *ulex biscaynesis* (Frank 1985, O'Meara et al. 1995b, O'Meara et al. 2003). Past studies on oviposition by *A. albopictus* have demonstrated a preference for darker colors (Gubler 1971, Yap 1975), and a lack of response to the presence of the bromeliad specialists *W. vanduzeei* and *W. mitchellii* (Lounibos et al. 2003). *Wyeomyia vanduzeei* showed a preference for oviposition into flowering Tillandsia utriculata (Frank and O’Meara 1985).

During the field survey discussed in Chapter 5 of this thesis, there was a significant difference between the densities of *A. albopictus* larvae in different species of bromeliads. Of the two species of bromeliads that were the focus of the field survey, *N. spectabilis* had significantly higher densities of *A. albopictus* than *B. pyramidalis*. In order to understand the
differences in mosquito densities in these two plant species, an oviposition experiment was implemented to determine whether oviposition was a factor that contributed to the higher densities of *A. albopictus* in *N. spectabilis*, and a larval development and survivorship experiment was conducted to see whether the growth and survivorship of *A. albopictus* differed between *N. spectabilis* and *B. pyramidalis*.

**Materials and Methods**

**Oviposition Experiment**

To determine whether *A. albopictus* preferentially oviposits into *N. spectabilis* in comparison to *B. pyramidalis*, an experiment was conducted in which 15 gravid *A. albopictus* females were added by mouth aspirator to each of ten cages containing both one *N. spectabilis* and one *B. pyramidalis* bromeliad.

In accordance with approved animal-care protocol (VB-17 project of the University of Florida), each female had been bloodfed on a chicken five days before the start of the experiment and held without an oviposition site. Only mosquitoes that appeared fully engorged were removed from the main colony cage, and then transferred to a cage kept at 26°C in a climate-controlled insectary, with access to a 20% sucrose-water solution for the five days prior to the start of the experiment. The colony from which the females were selected originated from collections from southern Florida.

The *N. spectabilis* plants in the experiment were collected from one residence in the city of Gotha in central Florida, and the *B. pyramidalis* plants were collected from multiple residences in Vero Beach and Fort Pierce, Florida. The *N. spectabilis* plants chosen for the experiment all contained partially submerged, nidulate inflorescences in the central tank (Benzing 2000) and, thus, held larger amounts of water than non-flowering individuals. Because *N. spectabilis* with inflorescences were the plants sampled in the field survey conducted in
Chapter 5, using flowering individuals for experiments may help to explain some of the findings in that chapter. On the other hand, the *B. pyramidalis* in this experiment did not have inflorescences, as their formation often destroys the water holding capacity of this species of bromeliad. Non-flowering *B. pyramidalis* were also the only *B. pyramidalis* sampled in the field surveys of Chapter 5.

Each plant was thoroughly washed by first rinsing in a bucket of water and then by spraying with a high powered hose. Due to the fact that it is very difficult to remove all of the leaves and organisms from the bromeliads, all plants were allowed to dry in an air-conditioned laboratory from 8 to 10 days to ensure the death of all organisms due to desiccation. The plants were then rewashed with a hose to remove any remains resulting from the drying.

Each replicate was enclosed in a 1.0 m wide and 0.76 m high pyramidal cage located within a outdoor screened enclosure constructed for studies of mosquito flight behavior (Bidlingmayer 1977). Within each cage, specimens of each bromeliad species were paired, albeit the *N. spectabilis* were always slightly bigger. 1.0 g of chopped, dried (48 h at 75°C) *Q. virginiana* oak leaves, and 150 mL of sieved bromeliad water collected from both *B. pyramidalis* and *N. spectabilis* bromeliads from the Vero Beach and Fort Pierce, Florida areas were added to the central tank of each bromeliad. The water of each species was pooled, so the water added to each plant was a mixture from both bromeliad species. The water was sieved through a 130 μm mesh to remove macroinvertebrates and detritus. The number of water holding leaves was counted as an estimate of plant water holding capacity.

The experiment was run for 7 days, after which the remaining water in each bromeliad was removed by a pipette, and each leaf containing water was rinsed with tap water into a metal rearing pan. Each pan was kept for 10 days at 26°C in a climate-controlled rearing room and
covered with a sheet of glass to prevent possible oviposition from stray mosquitoes in the rearing room. On each day, the pans were checked for newly hatched *A. albopictus*, and the sums of hatched larvae in the two bromeliad species were regarded as products of recent oviposition and compared by a paired t-test.

**Larval Development and Survivorship Experiment**

Fourteen plants, 7 *B. pyramidalis* and 7 *N. spectabilis*, from the previous experiment were washed in the manner as described above, and 1.0 g of chopped oak leaves (*Q. virginiana*), and 150 mL of sieved bromeliad water were added to the central tank of each bromeliad. Then after 20 4th instar *Wyeomyia* spp. and 20 1st instar *A. albopictus* had been added into the center tank, the plants were placed into metal cages in a 26°C climate controlled rearing room. After 3 days, 50 mL of water was added to the plants to compensate for evaporation. After 6 days, all of the contents of the bromeliads were rinsed into a pan in the manner as the oviposition experiment. Variations between plant species in the number and developmental stage of surviving *A. albopictus* were analyzed by ANOVA in SAS (2002). The survivorship data was arcsine square root transformed to meet the homogeneity of variances assumption of ANOVA.

**Results**

**Oviposition Experiment**

*Aedes albopictus* oviposited significantly more (*t*$_9$= 2.95, *P*<0.01) eggs into *N. spectabilis* (mean ± SE=33.50±6.38) than *B. pyramidalis* (mean ± SE=15.80 ± 5.29).

*Neoregelia spectabilis* had significantly more (*t*$_9$= 3.33, *P*<0.01) (mean ± SE= 9.60 ± 0.70) water-holding axils than *B. pyramidalis* (mean ± SE= 7.1 ± 0.46).

**Larval Development and Survivorship Experiment**

*A. albopictus* had a significantly higher (*F*$_{1,12}$= 67.41, *P*<0.01) average instar number within *N. spectabilis* (mean ± SE=3.96 ± 0.22) than in *B. pyramidalis* (mean ±
The mean survivorships of *A. albopictus* in *N. spectabilis* (0.56 ± 0.08 (SE)) and *B. pyramidalis* (0.67 ± 0.04 (SE)) were not significantly different (F1,12= 1.43, P=0.25).

**Discussion**

While there was significantly more oviposition by *A. albopictus* in *N. spectabilis*, this experiment did not consider the cause of this ovipositional preference. The difference could be based on coloration differences between *N. spectabilis* and *B. pyramidalis*. *N. spectabilis* is dark green with dark purple tips as compared to the entirely light-green *B. pyramidalis*. In an experiment conducted by Frank (1985), other species of mosquitoes occasionally found in bromeliads, *C. quinquefasciatus*, *A. aegypti*, and *Toxorhynchites rutilus*, preferentially oviposited in darkly colored artificial bromeliads, as opposed to lighter green artificial bromeliads. In this same study, *Wyeomyia* spp. preferred to oviposit in the lighter green artificial bromeliads. Therefore, it seems possible that *A. albopictus*, like other container-generalist mosquito species, has a preference for the darker colored bromeliad *N. spectabilis*.

Another visual cue besides color that may have affected the experiment was the size of the plants. Generally in the field, and in this experiment as well, *N. spectabilis* is a horizontally larger plant with much longer leaves. *B. pyramidalis* tends to be smaller overall with shorter leaves and a more vertically dominant stature than horizontally dominant. The paired t-test demonstrated that the *N. spectabilis* also has more water holding axils per plant than the *B. pyramidalis* plants in this experiment.

Bentley and Day (1989) stated that specialist mosquitoes, those which have more restricted ovipositional sites, such as crab holes, bromeliads and natural or artificial containers, tend to rely strongly on visual cues to aid in the identification of ovipositional sites. While this statement may also favor the color difference hypothesis for the observed ovipositional preference of *A. albopictus* for *N. spectabilis*, the fact that there was a difference in the size of the
two plants could also be a factor. *Neoregelia spectabilis*, the larger of the two bromeliad species, may be visually more attractive to *A. albopictus*, regardless of color. This larger size is not an artifact of the experiment, because in nature *N. spectabilis* are commonly larger than *B. pyramidalis*. Therefore, although the difference may be due to size, there are still important ecological implications to this difference.

On the other hand, visual cues may be of little importance to the ovipositional decision of *A. albopictus* in bromeliads. *Aedes albopictus* could be responding to habitat-based chemical cues unique to each plant species. For instance, the submerged nidulate inflorescence of *N. spectabilis* may provide additional nutrients or chemical compounds to the central tank water, while *B. pyramidalis* does not receive this input, because it creates tall, stalky inflorescenses. Since the water from both species was pooled, each species received water originating from both *B. pyramidalis* and *N. spectabilis*. Therefore, it is unlikely that chemical compounds within the water contributed to the oviposition preference of *A. albopictus* for *N. spectabilis* in this experiment. The experiment ran for 7 days, so only chemical cues that accumulated in this short period of time could have affected the preference of *A. albopictus* for *N. spectabilis*.

The larval competition experiment within *N. spectabilis* and *B. pyramidalis* showed that *A. albopictus* can develop at different rates based on the species of bromeliad. The multitude of structural differences in size and shape between the two plant species could contribute to the developmental differences of *A. albopictus* within the two plants. In Chapter 2 of this thesis, and in treehole microcosms study by Broadie and Bradshaw 1991, surface area to volume ratio was not a factor influencing the development rate of *Aedes* mosquitoes. So, it is possible that other structural differences account for the increased development rate of *A. albopictus* in *N. spectabilis*. 

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Neoregelia spectabilis has a sunken inflorescence which was decaying during this experiment, while B. pyramidalis did not have this decaying inflorescence. Therefore, it is possible that this sunken inflorescence provided additional nutrients that increased the food resources available to A. albopictus. Further experiments are necessary to elucidate the causes of the developmental differences of A. albopictus seen in these two bromeliad species.

This experiment shows the importance of bromeliad species in the distribution of A. albopictus. So far in North America, A. albopictus has been found to be infected with LaCrosse (Gerhardt et al. 2001), Eastern Equine Encephalitis (EEE) (Mitchell et al. 1992), and dengue viruses (Ibañez Bernal et al. 1997), and transmits Dengue (DEN) in other areas of the world (Hawley 1988). As this mosquito species is a health concern, then the understanding of its ovipositional preferences within bromeliads can be beneficial to determining the risk of transmission of these viruses to human populations. In Brazil, bromeliad eradication projects have been implemented to eliminate these supposed development sites of dengue vectors (Benzing 2000). With the higher populations of A. albopictus in northern Florida where Wyeomyia spp. is absent (O’Meara et al. 1995b), further studies on the ovipositional preferences of A. albopictus in bromeliad species could help reduce risk by applying control measures only to bromeliad species that are of highest concern.
CHAPTER 7
CONCLUSIONS

In aquatic container habitats, high densities of mosquito larvae can increase development time and reduce survivorship and pupal weight (e.g. Frank and Curtis 1977, Mogi 1984, Livdahl 1982, Broadie and Bradshaw 1991). In the surface area to volume ratio experiment of Chapter 2, and a larval competition experiment conducted by Lounibos et al. (2003), A. albopictus suffered reduced growth and survivorship in the presence of larger 4th instar Wyeomyia spp..

Resource competition is common among mosquito larvae, and interference competition in the forms of chemical interference (Dye 1984, Sunhara and Mogi 2002, Bedhomme et al. 2005), encounter competition (Dye 1984, Roberts 1998, Broadie and Bradshaw 1991), and facultative or intraguild predation (e.g. Reisen and Emory 1976, Koenekoop and Livdahl 1986, Koenraadt et al. 2003) also influence larval mosquito communities. The results of this study suggest that encounter competition, not chemical interference or predation, causes the reduced growth and survivorship of early instar A. albopictus in the presence of 4th instar Wyeomyia spp. in bromeliads. Increasing the habitat complexity in an artificial bromeliad increased the developmental rate of A. albopictus in the presence of Wyeomyia spp.. However, because the effect of habitat complexity on the development time of A. albopictus in the absence of Wyeomyia spp. was not explored, the relationship of habitat complexity to A. albopictus development may be unrelated to interactions with Wyeomyia spp.

Even though large numbers of mosquitoes were used in the chemical interference experiments, excretory products of Wyeomyia spp. did not affect the growth or survivorship of A. albopictus in bromeliads. Neither the pH nor ammonia concentration of bromeliad water differed before and after the experiment. Because bromeliads assimilate ammonia from their
central tank and axils (Benzing 2000), the likelihood of chemical interference caused by mosquito waste products may be reduced in this phytotelmata.

The dissections of over 1200 *Wyeomyia* spp. larvae exposed to multiple prey density and resource dependent conditions, and observations on the behavior of *A. albopictus* and *Wyeomyia* spp., provided no evidence of predation by *Wyeomyia* spp. upon *A. albopictus*. If *Wyeomyia* spp. larvae are facultative predators of *A. albopictus*, then the occurrence of predation is probably very rare and does not greatly effect the dynamics of these species.

Egg hatch inhibition is a competitive mechanism demonstrated in laboratory studies of *Aedes* mosquitoes (Edgerly et al. 1993). Egg hatch inhibition could change the population dynamics of affected species by changing the rate at which larvae enter a habitat and, thus, might cause changes in larval species composition (Livdahl and Edgerly 1987). In this study, *Wyeomyia* spp. larvae inhibited the egg hatch of *A. albopictus* at high resource levels, but only moderately reduced the overall hatch rate. Thus, in bromeliad habitats the rate at which *A. albopictus* larvae hatch may be altered by high densities of *Wyeomyia* spp., but this effect is probably minor among competitive interactions between these two species.

In field collections, the densities of *Wyeomyia* spp. and *A. albopictus* differed between two species of exotic bromeliad. *Aedes albopictus* larvae were more common in *N. spectabilis*, and *Wyeomyia* spp. were more common in *B. pyramidalis*. Experiments determined that *A. albopictus* deposited more eggs and developed more quickly in *N. spectabilis*, the bromeliad species with the higher densities of larvae of this species in field collections. The choice of oviposition in *N. spectabilis* over *B. pyramidalis* is probably not due to the ability of the *A. albopictus* females to detect *Wyeomyia* spp. within the plant, because Lounibos et al. (2003) determined that *A. albopictus* did discriminate between *B. pyramidalis* without or without
Wyeomyia spp. Therefore, the oviposition preference of A. albopictus is likely due to chemical or visual differences between the two bromeliad species.

While A. albopictus larvae developed faster in N. spectabilis when exposed to Wyeomyia spp. than under the same conditions in B. pyramidalis, the cause of this difference is unknown. There are many structural differences between the two host plant species, one being differing surface area to volume ratios, but in the encounter competition experiments conducted in this study, there was no effect of varying surface area to volume ratio of the plant on survivorship or growth of A. albopictus in the presence of Wyeomyia spp. However, this encounter competition experiment was conducted in an artificial bromeliad, so the outcome of competition between A. albopictus and Wyeomyia spp. based on the surface area to volume ratio may be different under field conditions with actual bromeliads. As there are many differences between the two plant species, further investigations are needed to determine the causes of the more rapid developmental rate of A. albopictus within N. spectabilis.
LIST OF REFERENCES


Blaustein, L. and J.M. Chase. 2007. Interactions between mosquito larvae and species that share the same trophic level. Annual Review of Entomology **52**: 489-507.


BIOGRAPHICAL SKETCH

Robyn Raban was born in Lewiston, Maine, and then at the age of twelve moved to Phoenix, Arizona. After graduating from Mountain Pointe High School, she went on to the University of California, Berkeley where she earned a degree in environmental science. During her experiences at UC Berkeley she was able to study tropical ecology in Costa Rica, and work with the Smithsonian Institute on mangrove ecology research in Panama. Through her experiences in central America, she became interested in mosquito biology, and medical entomology. She will continue to pursue her interests in mosquito biology with her next degree in mosquito genetics and arbovirology.