



Testing predictions of displacement of native *Aedes* by the invasive Asian Tiger Mosquito *Aedes albopictus* in Florida, USA

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Abstract

The Asian Tiger Mosquito *Aedes albopictus* arrived in the USA in 1985 in used automobile tires from Japan and became established in Texas. This species has since spread to become the most abundant container-inhabiting mosquito in the southeastern USA, including Florida, where it has reduced the range of another non-indigenous mosquito, *Aedes aegypti*. To assess the accuracy of predictions that *A. albopictus* would competitively exclude the native Eastern Treehole Mosquito *Aedes triseriatus* from tires but not from treeholes (Livdahl and Willey (1991) Science 253: 189–191), we extensively monitored the abundances of mosquito immatures before and after the Asian Tiger invaded these habitats in south Florida. These field data failed to demonstrate exclusion of *A. triseriatus* from treeholes following the establishment of *A. albopictus* in this microhabitat in 1991. However, *A. albopictus* had significantly higher metamorphic success and showed a significant increase in mean crowding on *A. triseriatus* in treeholes monitored from 1991 to 1999. In urban and suburban sites, *A. triseriatus* was uncommon in abandoned tires even before the arrival of *A. albopictus*. In some wooded sites, there is evidence for a decline in numbers of *A. triseriatus* in used tires and cemetery vases, but the native species has not been excluded from these habitats. Overall, the negative effect of *A. albopictus* on *A. triseriatus* has been less severe than that on *A. aegypti*. Experiments outdoors in surrogate treeholes showed that *A. albopictus* was more successful than *A. triseriatus* in survival to emergence in the presence of predatory larvae of the native mosquito *Toxorhynchites rutilus* when first instar predators encountered both prey species shortly after their hatch. Eggs of *A. albopictus* also hatched more rapidly than those of *A. triseriatus*, giving larvae of the invasive species an initial developmental advantage to escape predation. Biological traits that may favor *A. albopictus* are offset partly by greater treehole occupancy by *A. triseriatus* and the infrequency of the invasive mosquito species in undisturbed woodlands, which mitigates against displacement of the native mosquito in these habitats.

Introduction

In 1985 the Asian Tiger Mosquito, *Aedes albopictus*, was recognized as established in the USA near the port of Houston, Texas (Sprenger and Wuithiranygool 1986). This container-inhabiting species most likely

entered the country in shipments of used automobile tires (Reiter and Sprenger 1987). The probable origin of *A. albopictus* in the USA was deduced from diapause responses to be temperate Japan (Hawley et al. 1987). This species spread rapidly north and east from Texas, especially along the interstate highway system

(Moore and Mitchell 1997), to become the most common container-inhabiting mosquito throughout most of the southeastern USA (Moore 1999). Mosquito species which occupy such man-made containers as water-holding tires and cemetery vases are derived from evolutionary predecessors that occur in plant-held waters, such as leaf axils or treeholes (Frank and Lounibos 1983), which also support the immature stages of *A. albopictus* in Florida (O'Meara et al. 1993).

In the Oriental region *A. albopictus* is an important vector of dengue and is competent to transmit many other viruses (Shroyer 1986). Although the public health consequences of *A. albopictus* in the USA remain unclear (Moore and Mitchell 1997), this species has been captured infected with eastern equine encephalitis virus in Florida (Mitchell et al. 1992), LaCrosse encephalitis virus in North Carolina and Tennessee (Gerhardt et al., 2001), and dengue virus in Mexico near the Texas border (Ibañez Bernal et al. 1997).

Aedes albopictus was first discovered in Florida in 1986 near Jacksonville and has since spread southward so that by 1994 it was found throughout the state, except in the Florida Keys (O'Meara et al. 1993, 1995). In many areas of Florida, as elsewhere in the southeastern USA, the abundance and range of the Yellow Fever Mosquito, *Aedes aegypti*, in man-made containers declined in association with the *A. albopictus* invasion (Nasci et al. 1989; McHugh 1991; Hobbs et al. 1991; O'Meara et al. 1992, 1995). The Yellow Fever Mosquito is not native to the USA, but rather was introduced from Africa in the 15–17th centuries in water containers aboard slave or trade ships (Tabachnick 1991). Experiments conducted in south Florida in shaded tires identified larval resource competition as the probable mechanism for the displacement of *A. aegypti* by *A. albopictus* (Juliano 1998).

The indigenous mosquito species most likely to be affected by *A. albopictus* is the Eastern Treehole Mosquito, *Aedes triseriatus*, which co-occurs in natural and artificial containers with *A. albopictus* throughout the current range of the invading species in the USA. Based upon larval competition experiments in laboratory microcosms, Livdahl and Willey (1991) predicted that *A. albopictus* would completely exclude *A. triseriatus* from tire habitats, but that the two species would co-exist stably in treeholes. These authors postulated that larval resources in tires are used much more effectively by *A. albopictus* than by *A. triseriatus*, but treehole fluid contains resources that each species

exploits differentially. Further studies in laboratory conditions have confirmed that *A. albopictus* outcompetes *A. triseriatus* when larval resources are limiting (Novak et al. 1993; Teng and Apperson 2000), and that the invasive species from Asia may differentially inhibit egg hatch of co-occurring container *Aedes* (Egerly et al. 1993).

Scanty field research has been reported to determine whether the predictions of Livdahl and Willey (1991) have been borne out following the establishment and spread of *A. albopictus* in the eastern USA. In South Carolina, *A. triseriatus* declined in abundance in oviposition trap collections following the *A. albopictus* invasion, but larval habitats of the two species were not monitored (Mekuria and Hyatt 1995). O'Meara et al. (1993) purported to demonstrate a decline in *A. triseriatus* frequency in Florida cemetery vases following colonization by *A. albopictus*, but observations were based only on presence–absence data and reported for only two years. Moreover, *A. triseriatus* occurs preferentially in wooded habitats (Sinsko 1976), but many of the cemeteries sampled by O'Meara et al. (1993) have few trees.

The larval competition experiments of Livdahl and Willey (1991) did not consider the effects of the native predatory mosquito *Toxorhynchites rutilus*, known to reduce the abundance of *A. triseriatus* in Florida treeholes (Bradshaw and Holzapfel 1983; Lounibos et al. 1997). Differential predation on either *Aedes* species could potentially affect the outcome of competition in container habitats. This paper evaluates the predictions of Livdahl and Willey (1991) against extensive quantitative pre- and post-invasion surveys of treeholes and artificial container habitats in southern Florida. We also present results of experiments in semi-natural conditions to assess whether predation by *T. rutilus* might influence the outcome of larval competition between *A. triseriatus* and *A. albopictus* and whether differential hatching responses of the two prey species might affect their susceptibility to predation.

Materials and methods

Censuses and surveillance

The four container types sampled in this study are representative of the most abundant natural or artificial microhabitats occupied by *A. triseriatus* and *A. albopictus* in Florida.

Treeholes

The aquatic fauna of five treeholes in Southern Live Oak, *Quercus virginiana*, trees in a protected 10-acre, oak-palm woodland at the Florida Medical Entomology Laboratory (FMEL) in Indian River County (27°35' N, 80°22' W) were extracted fortnightly as part of a sampling-with-replacement program maintained without interruption since 1978 (Lounibos 1983; Lounibos et al. 1997). Mosquito immatures extracted in aquatic contents with a suction hose were staged and identified before being returned to their respective holes in reconstituted samples, less than 4 h after removal. For purposes of the present study, pupae recorded on any sample date had emerged as adults prior to the next census. Therefore, annual pupal counts are used as relative estimates of a treehole's production of *Aedes* spp., although these counts underestimate the absolute pupal production per hole.

At Indrio (27°31' N 80°24' W), approximately 15 km southwest of FMEL, the aquatic contents of treeholes were sampled destructively at quarterly intervals beginning in 1990. These treeholes, some of which had been used for previous experiments and surveillance (e.g., Lounibos 1983), were in *Q. virginiana* trees in a roadside habitat where the oaks border a heavily traveled highway. Owing to tree coppicing and the decay of some holes over the course of the ten-year observation period, only 12 of the original 20 holes remained in 1999. Fluid volume in each hole was measured, and mosquitoes were counted, staged, and identified. Since the aquatic contents of extracted samples were not returned to treeholes at Indrio and the average developmental time of both species is much less than the three-month intervals between samples, the censuses were regarded as independent for data analyses.

Tires

Only two abandoned automobile tires were found at the Indrio treehole site, and these were sampled quarterly for mosquito immatures from 1992 to 1994 and once subsequently in 2000. The entire aquatic contents were extracted with a turkey baster, and collected mosquitoes were staged and identified in the laboratory.

Records from peninsula-wide surveys of water-containing auto tires for 1990–1999 were analyzed for Florida's southernmost counties (Figure 1), which *A. albopictus* had not yet colonized as of 1990 (O'Meara et al. 1993, 1995). The typically urban and suburban habitats of these used tires included

dump areas (aka 'landfills'), auto salvage yards, service stations, and used tire retail shops. Although some sites were re-sampled in successive years, within-county comparisons before and after the establishment of *A. albopictus* usually surveyed different tires, because of the transient nature of most such habitats. Fluid was extracted from tires with a cup or turkey baster until samples yielded no mosquitoes. Larval and pupal mosquitoes were sorted in the field in white trays before transfer in plastic bottles to the laboratory for identifications.

Cemetery vases

Among many cemeteries in southern Florida surveyed for mosquitoes since 1990 (O'Meara et al. 1992, 1993), four were selected where *A. triseriatus* had been recorded prior to the arrival of *A. albopictus*: Oak Hill in Polk County, Cycadia in Pinellas County, Ft. Denaud in Hendry County, and Joshua Creek in DeSoto County (Figure 1). Each of these cemeteries has several hundred vases accessible for colonization by container-inhabiting mosquitoes. Sample dates in which ≥ 10 vases were positive for *Aedes* were used to compare temporal profiles of mean densities per species per vase. In the field, the entire fluid contents of removable vases were poured into a white tray from which mosquito larvae and pupae were removed and transferred to bottles with a pipette. For vases permanently affixed to tombstones, the fluid contents were extracted with a turkey baster. Identifications of mosquitoes to species were made at FMEL with keys to the immature stages.

Oviposition traps

Oviposition by container-inhabiting *Aedes* is frequently monitored in the field by collecting eggs on moistened, removable substrates inserted in a dark vessel with water (e.g., O'Meara et al. 1989). For this purpose, we lined the interior of black plastic cups of 250 ml capacity with brown germination paper, then added 150 ml of distilled water and five dried leaves. Drainage and suspension holes were drilled on the lips of cups, which were hung from nails on trees in native woodlands at three localities (Figure 1).

The oviposition papers were collected and replaced at 3–6-week intervals. At the time of collection, all larval and pupal mosquitoes in cups were also removed and returned to the laboratory for identifications. Eggs on papers were dried gradually for 5–7 days to ensure embryonation, then immersed in nutrient-enriched

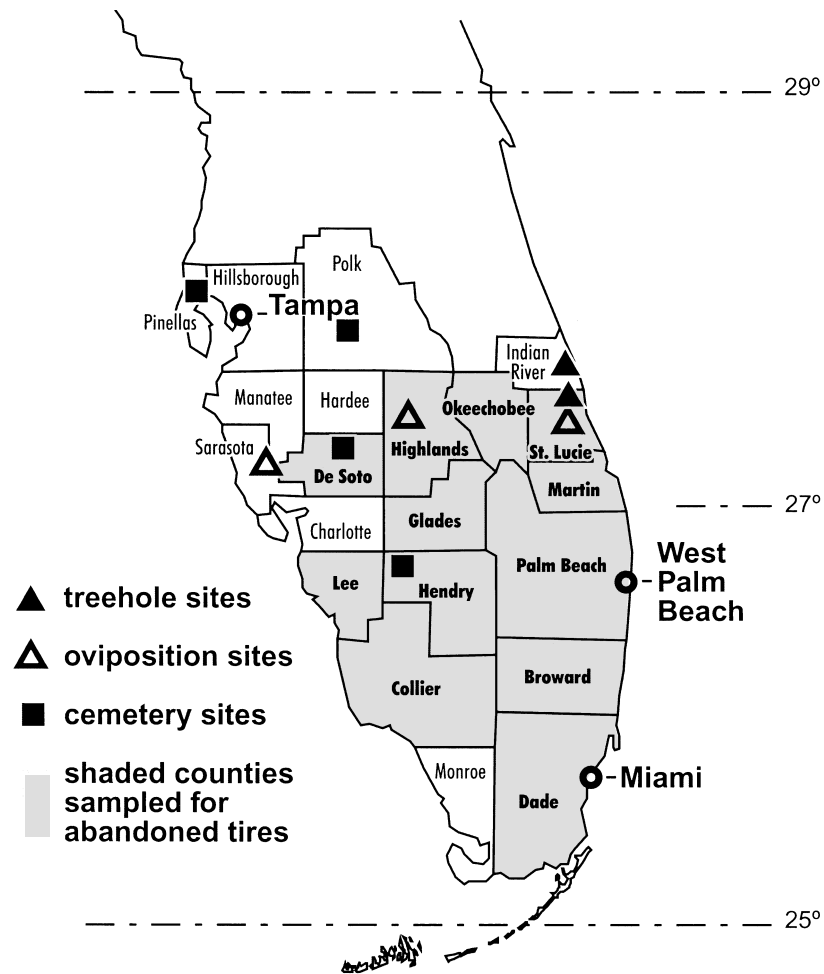


Figure 1. Map of southern Florida showing counties, major cities, and locations of study areas that were sampled or censused repetitively.

or deoxygenated water to hatch larvae, which were identified with keys. Although egg and larval-pupal collections were initially kept separate, identifications from the two collection types were pooled beginning in mid-1997.

The oviposition traps were placed within or on the edges of three temperate, broad-leaved evergreen forests (Platt and Schwartz 1990) in southern Florida, with three collection sites used at each forest. Sherwood Hammock ($27^{\circ}23' N$, $80^{\circ}20' W$), the smallest, is a privately owned oak-palm woodland of approximately 40-acres on the St. Lucie River south of Ft. Pierce in St. Lucie County (Figure 1). Highlands Hammock State Park ($27^{\circ}28' N$, $81^{\circ}32' W$) is a 8140-acre preserve in Highlands County, and Myakka River State Park ($27^{\circ}15' N$, $82^{\circ}19' W$) consists of 28,875-acres in Sarasota County (Figure 1). At

Sherwood Hammock, 5–7 traps each were set within the hammock (HA), near two houses (HO), and along an ecotone (EC) bordering on a clearing for electric utility lines. At Highlands Hammock, traps were placed at localities open to visitors known as Concession Circle (COC), Wild Orange Trail (WOT), and Ancient Hammock (AH). At the Myakka River Park, traps were located at Campfire Circle (CAC), Nature Trail (NT), and Ranch House Road (RHR). HA, WOT, AH, and NT locations were not accessible by auto and relatively more forested than the other five. Based on these criteria, HA, WOT, AH, and NT were classified as undisturbed sites whereas HO, EC, COC, CAC, and RHR were considered disturbed. The two State Parks were sampled from the spring of 1997 through 1999, but Sherwood Hammock was monitored only in 1997.

Mosquito species

From surveillance and census records we report here only the abundances and frequencies of *A. albopictus*, *A. triseriatus*, and *A. aegypti*. *Aedes bahamensis* was the only other congener encountered and occurred only in tires in Dade and Broward Counties (O'Meara et al. 1989). Other mosquito species identified in our container samples were *Culex quinquefasciatus*, *Culex restuans*, *Culex nigripalpus*, *Orthopodomyia signifera*, *T. rutilus*, and *Anopheles crucians*. All non-*Aedes* mosquito species occurred less frequently or abundantly than the common *Aedes* spp.

Experiments

Susceptibility to predation

To determine whether the success of *A. albopictus* relative to *A. triseriatus* might be impeded by the presence of the native larval predator *T. rutilus*, we conducted container experiments outdoors at FMEL. Bamboo internodes, which in the tropics support a mosquito fauna similar to treeholes (e.g., Lounibos 1981), were cut in sections and dried for use as surrogate treeholes. We used an experimental design similar to Nannini and Juliano (1997), differing in that 50 larvae of each of the two species were exposed together to a single predator, instead of 100 larvae of one species.

Eighteen bamboo sections were divided into three groups of six each and inoculated with 1 g of dried (80 °C for 48 h) chopped oak leaf litter and 300 ml of water from woodland tires, pre-frozen and sieved to remove macroinvertebrates and large detritus. Each of the six bamboos in a group represented one replicate of six different treatments. The bamboos were covered with plastic screen of 0.76 mm mesh, which allowed rainfall but not ovipositing mosquitoes to enter, and suspended by hooks in the oak-palm woodland at FMEL for three days prior to the onset of an experiment. The day before the experiment, eggs of *A. triseriatus* and *A. albopictus*, derived from F1 colonies of these species collected locally, were hatched in deoxygenated water and separated into aliquots of 50 first instar larvae. On the following day, each bamboo received 50 first instars of both species, and three bamboos, one in each of three groups, received one first instar *T. rutilus*, hatched from a laboratory colony (Lounibos et al. 1998). At three-day intervals, three additional bamboos received a first-instar *T. rutilus*, up to 12 days after the initial inoculation with *Aedes* larvae. A final three bamboos,

one in each of the three groups, received no predators and served as controls.

Beginning 10 days after the introduction of first instar *Aedes*, each bamboo was checked daily with a flashlight for the emergence of adult mosquitoes, which were extracted with a mouth aspirator through a plugged perforation in the screen covering the bamboo opening. Adult mosquitoes were identified to species in the laboratory. Once per week after the onset of *Aedes* emergence, the fluid contents of each bamboo with a predator was poured into an enamel tray to ascertain survival of *T. rutilus*.

Two runs of the experiment with three-day predator introductions were conducted, one in the spring and one in the fall of 1996. A second experiment was conducted in the summer of 1997 in which the scheduled introductions of first instar *T. rutilus* were made at seven successive one-day intervals; this experiment used 24 bamboo sections instead of 18 and, thus, was set as three groups of eight bamboos. Mean (\pm SD) ambient temperatures in the hammock, based on weekly calculations of $(\max + \min)/2$ during the experimental periods, were $24.6^\circ \pm 0.7$ and $23.9^\circ \pm 2.7$ for the spring and fall, respectively, of 1996 and $26.4^\circ \pm 0.7$ for the summer of 1997.

The survivorship to adulthood of adults of the two species in the predator introduction experiments was analyzed by MANOVA (PROC GLM, SAS Institute, Inc., 1985) following methods described by Scheiner (1993). Eighteen of a possible 60 predators died prior to completion of *Aedes* emergence, and these bamboos were discarded from analyses. Pairwise multivariate contrasts were used to compare predator treatments to controls to detect differential effects of time of introduction of *T. rutilus*. Standardized canonical coefficients were used to determine relative contributions of the response variables (*A. triseriatus*, *A. albopictus* adults) to significant differences.

Hatching rates

Certain species of *Aedes* are known to achieve temporal segregation in treeholes by differential responses to hatching stimuli (Lounibos 1981), but hatching responses of *A. albopictus* and *A. triseriatus* have not been compared. Three- to four-week-old F1 eggs from local colonies of both species were submersed in separate, 250 ml vessels in pre-frozen, thawed tire water, and hatchlings counted and removed at regular intervals. Counts were made hourly up to 10 h after submersion, and a final count was made at 24 h. Eggs

remaining unhatched after 24 h were bleached according to methods of Trpis (1970) to evaluate embryonation. Infertile eggs were removed from calculations of percentage hatch. This experiment was conducted twice on eggs derived from different females.

Results

Censuses and surveillance

Treeholes

Aedes albopictus was first recognized in treehole censuses at FMEL in 1991, when it produced slightly more pupae per liter than *A. triseriatus* in hole #9 (Figure 2A). Between 1992 and 1999 annual pupal counts from hole #9 showed no clear pattern favoring either species, and in two other holes with long-term records, pupae of *A. albopictus* were rare (#6) or not observed (#2) (Figure 2A). In two smaller holes at FMEL censused only since 1992, pupal numbers per liter varied greatly from year to year, numerically favoring *A. albopictus* in hole #12 and *A. triseriatus* in hole #13 (Figure 2B).

At the more disturbed monitoring site in Indrio, *A. aegypti* was identified in small percentages of treehole samples through 1995, but pupae of this species were recovered only in 1992 (Figure 3). *Aedes albopictus* was first observed at Indrio as larvae in 1991 and as pupae in 1992. Despite a general increase between 1991 and 1999 in percentage of wet holes occupied by *A. albopictus*, the annual percent of holes with *A. triseriatus* was consistently higher than that of the invasive species. However, between 1994 and 1999 more total pupae of *A. albopictus* were recovered than of *A. triseriatus* (Figure 3B). For the years 1990–1999, total annual pupal production of *Aedes* in Indrio holes was significantly correlated with the number of samples containing water ($r = 0.72$, $P < 0.05$, 8 df). For the years 1992–1999, the correlation between pupal numbers of *A. triseriatus* and *A. albopictus* was not significant ($r = 0.63$, $P > 0.10$, 6 df).

To assess the potential for interspecific larval competition between *A. albopictus* and *A. triseriatus* in Indrio treeholes, we calculated the mean crowding (Lloyd 1967) of one species upon the other per unit resource (Hurlbert 1978) for each sample date in the 1992–1999 period. The mean crowding of *A. triseriatus* on *A. albopictus* is interpretable as the average number of the former species per liter of treehole fluid

encountered by the average individual of *A. albopictus*. Although no linear trend was apparent in the mean crowding of *A. triseriatus* on *A. albopictus* versus sample date, the mean crowding of the invasive species on the native mosquito increased significantly over time during the 1992–1999 observation period (Figure 4).

We further compared the metamorphic success of the two species in the 11 Indrio treeholes in which *A. albopictus* and *A. triseriatus* co-occurred five or more times in the 1992–1999 period. The Williams mean, a modification of the geometric mean denoted by M_w (Williams 1937; Haddow 1960), where $\log(M_w + 1) = [\sum \log(n + 1)]/N$ was calculated, where n is the number of pupae per total number of immatures in a series of N treehole samples. M_w is a useful measure of the central tendency in which zero values occur (Haddow 1960). By this measure, metamorphic success was higher for *A. albopictus* in 10 of 11 examined holes, and mean metamorphic success of *A. albopictus* was significantly higher when evaluated across holes with a paired t -test (Figure 5).

Tires

Only *A. triseriatus* and *A. aegypti* were collected from Indrio tires in April and June, 1992 (Figure 6). In collections that began in 1993 when *A. albopictus* appeared in this habitat, *A. aegypti* was no longer present. Densities of *A. triseriatus* exceeded those of *A. albopictus* throughout the 1992–1994 observation period of the Indrio tires. In June 2000 these two and one additional tire were quantitatively surveyed after a six-year hiatus. On this occasion, the density (SE) per tire was 15.3 (7.3) for *A. triseriatus* and 141.7 (85.0) for *A. albopictus*.

Used automobile tires surveyed in 12 south Florida counties (Figure 1) were occupied predominantly in 1990–1991 by *A. aegypti*, which occurred in 95.4% of all *Aedes*-positive samples (Table 1). When these same counties were surveyed in 1996–1999, *A. albopictus* predominated in tires and was recovered in 49.8% of *Aedes*-positive samples. The 38.8% incidence of *A. aegypti* in 1996–1999 was significantly less ($G = 250.4$, $P < 0.001$) than in 1990–1991. Significant heterogeneity in *A. aegypti* presence among counties was attributable primarily to three counties in 1990–1991 and eight counties in 1996–1999 (Table 1). Although *A. triseriatus* was found in only 4.6% of tires in the 1990–1991 period, it was completely absent (0%) in 1996–1999, and these frequencies are significantly different ($G = 21.2$, $P < 0.001$).

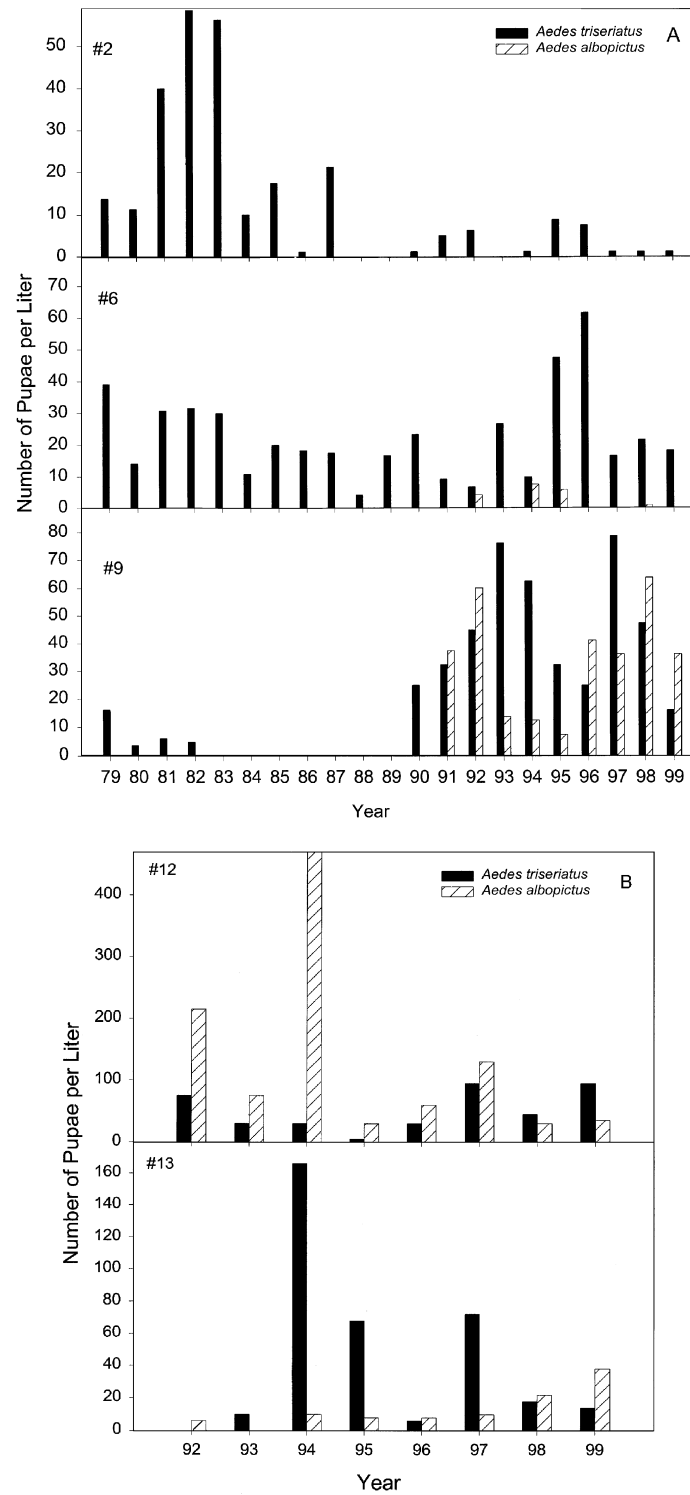


Figure 2. Annual pupal production per liter of *A. triseriatus* and *A. albopictus* from five treeholes at FMEL censused fortnightly from 1979 to 1999 (A) or 1992 to 1999 (B). Hole contents were replaced after each census. Maximum volumes in liters, used to calculate annual pupal densities, are based on 1997–1999 records: #2 = 0.8, #6 = 1.2, #9 = 0.8, #12 = 0.2, and #13 = 0.5.

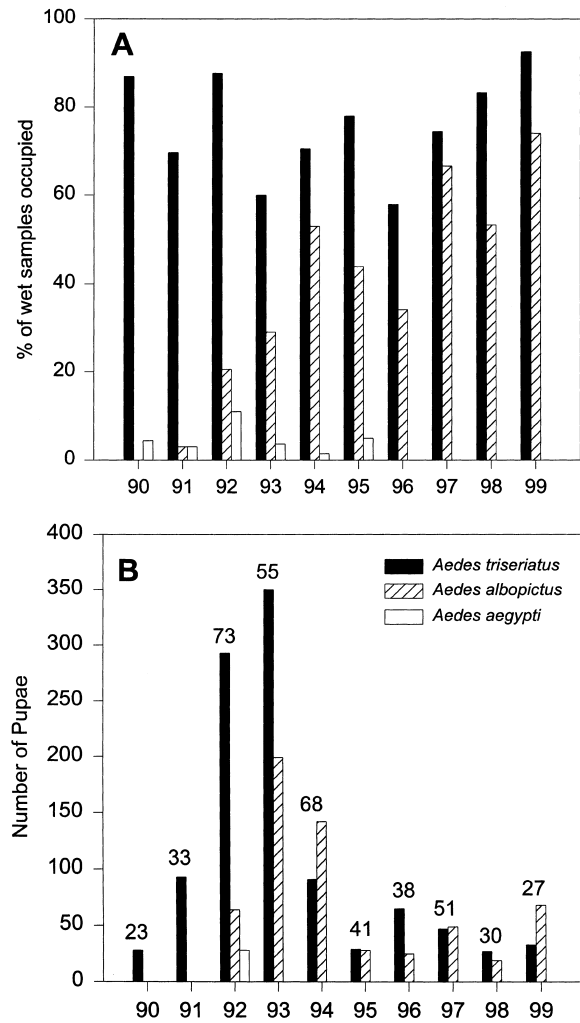


Figure 3. (A) Annual proportion of wet holes occupied by three *Aedes* species at Indrio (St. Lucie County) Florida during quarterly destructive censuses in 1990–1999. (B) Annual numbers of pupae produced in these holes. Annual numbers of wet holes surveyed per year, which declined because of tree coppicing and drought in latter years of the study, are denoted above bars in bottom histogram.

Cemetery vases

Aedes albopictus was absent and *A. aegypti* and *A. triseriatus* were present in vases at the onset of our surveillances in 1991 (Figure 7). In all four cemeteries, *A. aegypti* disappeared in 1992 or 1993 coincident with the establishment and increases in abundance of *A. albopictus*. *Aedes triseriatus*, by contrast, followed different trajectories during the nine-year observation period. At Joshua Creek, this species was present in all but one census. At Oak Hill and Ft. Denaud, 3–5 successive samples in the 1993–1997 period did not yield

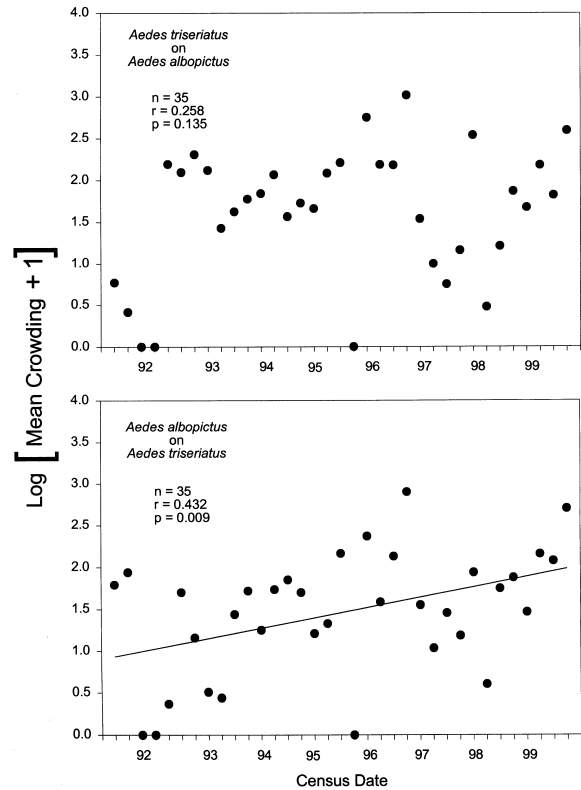


Figure 4. Mean crowding per unit resource of *A. triseriatus* on *A. albopictus* and vice versa for each sampling date of treeholes at Indrio, transformed by $\log(n + 1)$ to stabilize variances. Following Hurlbert (1978), if A_i and T_i are the numbers of individuals of *A. albopictus* and *A. triseriatus*, respectively, then the mean crowding of *A. albopictus* on *A. triseriatus* is $[\sum(A_i T_i / V_i)] / \sum(A_i)$, where V_i is the actual volume in the i th treehole. The regression line in the bottom panel is $\log(\text{mean crowding} + 1) = 0.031(\text{interval number}) + 0.903$, where equally spaced intervals ranged from 1 to 35 and represent the quarterly samples from 1992 to 1999.

this species, which reappeared in these cemeteries in 1997–1998. At Cycadia, where ten mosquito-positive vases were recorded only five times, *A. triseriatus* was not collected between 1997 and 1999.

Oviposition traps

In the 1997 survey for eggs, the frequency of occurrence of *A. albopictus* was significantly higher than that of *A. triseriatus* in HO and EC, two disturbed habitats on the edge of Sherwood Hammock (Table 2). By contrast, in the undisturbed WOT site, the incidence of *A. triseriatus* was significantly higher than that of *A. albopictus*. All other egg frequency comparisons between the two species were non-significant.

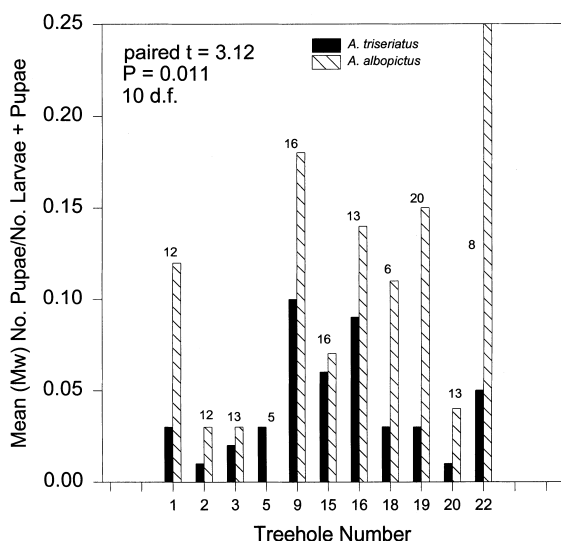


Figure 5. Williams Mean (M_w) number of pupae per total immatures for two *Aedes* species in all Indrio treeholes in which these two co-occurred \geq five times in 1992–1999 censuses. Numbers of samples for each hole are indicated beside histogram bars. M_w is obtained by subtracting one from the antilog of the quantity $[\sum \log(n+1)]/N$, where n is the number of pupae per total immatures in a series of N treehole samples.

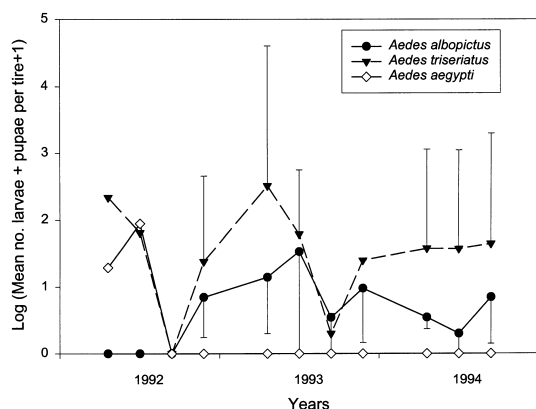


Figure 6. Log mean numbers (+1) of three container *Aedes* in two discarded auto tires at Indrio from April 1992 through September 1994. Standard errors are unidirectional to avoid overlaps and were not calculated for the first two samples, which were pooled.

Abundances of species in 1997–1999 Park samples, based on identifications of all immatures, followed trends similar to egg frequencies. Owing to high variances among collection dates, only the mean abundances at the WOT site were significantly different, favoring *A. triseriatus* (Table 2).

Experiments

Susceptibility to predation

Under both schedules of introduction of *T. rutilus*, *A. triseriatus* was usually less successful than *A. albopictus* in survival to emergence (Figure 8). MANOVA detected a significant run effect in the predator introductions at three-day intervals (Table 3) attributable primarily to the greater overall survivorship of *A. triseriatus* in run one vs two (0.12 vs 0.02) and lesser survivorship of *A. albopictus* in run one vs two (0.14 vs 0.32). A significant treatment effect in the three-day experiment was mainly a consequence of predators introduced at days 0 and 3, which yielded the only significant pairwise contrasts with controls (Table 3). Canonical coefficients (Table 3) for the overall treatment effect, and for the two significant pairwise contrasts with controls, were large and positive for both species, indicating that these significant effects resulted from differences for both species and that those differences tended to be similar in direction (i.e., lower survival when predators are introduced early, Figure 8A) for both species [see Scheiner (1993) for further details on interpreting canonical coefficients]. The absence of significant pairwise differences from controls of later predator introductions indicates that a prey head start of six days or more results in escape from predation.

The single run of one-day predator introductions yielded a significant overall effect of treatments (Table 3). Canonical coefficients (Table 3) indicated that *A. albopictus* survival contributed most to overall treatment effects, which is evident in Figure 8B, as bivariate means spread over a much greater range of values for *A. albopictus* than for *A. triseriatus*. Although no pairwise contrasts with controls were significant (Table 3), trends in the one-day experiment were similar to those in the three-day experiment, insofar as predators introduced early tended to reduce prey survival relative to controls to a greater extent than did predators introduced later (Figure 8, Table 3). In contrast to the three-day introductions, survival of controls without predators in the one-day experiment was lower than that observed in several treatments with predators (Figure 8B).

Hatching rates

In two separate runs, *A. albopictus* was more responsive than *A. triseriatus* to a hatching stimulus of tire water (Figure 9). The greatest discrepancy in hatching rates between the two species occurred in the first four

Table 1. Pre- and post-invasion occurrences of container *Aedes* in urban and suburban used tires in southern Florida.

County	1990–1991 ^a				1996–1999				G_H^c
	Number of sites (collections)	Containers positive		G_H^c	Number of sites (collections)	Containers positive		G_H^c	
		<i>Aedes</i> spp.	<i>A. albopictus</i>			<i>A. aegypti</i>	<i>A. triseriatus</i>		
Broward ^b	8 (8)	51	0	48	2	0.2	12	0	22.7***
Collier	1 (1)	5	0	5	0	0.5	21	17	30.8***
Dade ^b	7 (7)	16	0	13	0	4.2*	200	85	91.6***
DeSoto	6 (7)	31	0	31	0	2.9	10	9	18.9***
Glades	2 (4)	20	0	20	0	1.9	3	3	2.9
Hendry	1 (2)	8	0	8	0	0.2	15	10	0.2
Highlands	2 (4)	9	0	9	0	0.9	2	1	3.8
Lee	1 (1)	2	0	2	0	0.2	11	4	14.0***
Martin	2 (2)	12	0	12	0	1.1			
Okeechobee	4 (5)	51	0	51	0	4.9*	38	37	5.9*
Palm Beach	4 (4)	12	0	12	2	1.1	58	17	90.8***
St. Lucie	5 (10)	20	1	15	7	9.6**	4	3	7.6**
Total	43 (55)	237	1	226	11	27.7***	374	186	289.2***

^aThrough August 1991.^b*Aedes bahamensis*, known in Florida only from Dade and Broward Counties (O'Meara et al. 1989), accounts for instances where the number of *Aedes* spp. exceeds the sum of numbers of the three named species.^c*G*-test for heterogeneity for presence of *A. aegypti*. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

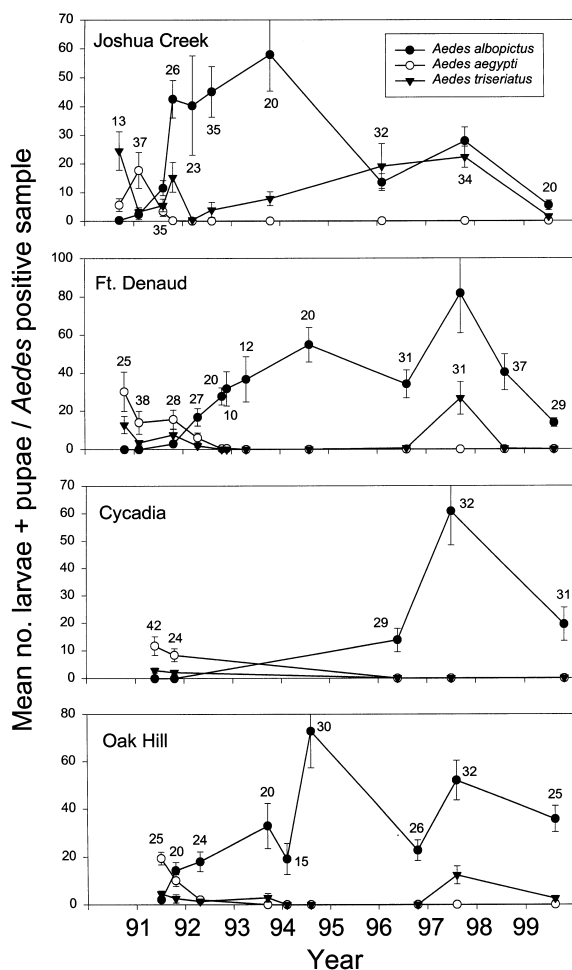


Figure 7. 1991–1999 mean numbers (SE) of container *Aedes* per vase at four cemeteries in south Florida that harbored *A. triseriatus* before the establishment of *A. albopictus*. For each sample date numbers of *Aedes*-positive samples are indicated above or below the mean value.

hours after eggs were inundated. The cumulative frequency distributions of hatch of the two species were marginally significantly different in run one, but not in run two (Figure 9).

Discussion

Our results demonstrate co-existence as predicted for *A. albopictus* and *A. triseriatus* in treeholes of south Florida, albeit for somewhat different reasons than those cited by Livdahl and Willey (1991). In treeholes where the two species co-occur, a measure of metamorphic success favored *A. albopictus* (Figure 5),

supporting laboratory evidence (Novak et al. 1993; Teng and Apperson 2000) that this species is superior in larval resource utilization. However, *A. triseriatus* consistently occupied more treeholes than *A. albopictus* at Indrio (Figure 3), and the invasive species produced few or no pupae in two treeholes at FMEL (Figure 2). The rarity of *A. albopictus* compared to *A. triseriatus* at the undisturbed WOT site indicates that the invasive species occurs infrequently in some intact forests yet is significantly more common than the native species in disturbed habitats, such as EC and HO at Sherwood Hammock (Table 2). Thus, as has been demonstrated for other treehole mosquito species (e.g. Lounibos 1981), *A. triseriatus* and *A. albopictus* are spatially segregated to some degree in south Florida by different macrohabitat preferences that are maintained by female oviposition choices. Some undisturbed forests provide habitat for *A. triseriatus* where larval competition with *A. albopictus* would not play an important role in the population dynamics of the native species. However, at the roadside Indrio site, the significant temporal trend in mean crowding (Figure 4) indicates that the average individual of *A. triseriatus* is encountering increasingly higher densities of *A. albopictus* in treeholes where the two species co-occur.

Livdahl and Willey (1991) predicted that *A. albopictus* would competitively exclude *A. triseriatus* from tires, but our surveys show that urban and suburban tires in south Florida were rarely occupied by *A. triseriatus* before the invasion of *A. albopictus* (Table 1). Thus, any effect of *A. albopictus* on *A. triseriatus* in urban and suburban tires is weak. Mark-recapture experiments have shown that *A. triseriatus* rarely flies far from trees (Sinsko 1976), and the absence of trees in most urban and suburban tire sites would explain the rarity of *A. triseriatus* in these containers.

In tires near Indrio treeholes, *A. triseriatus* was more common than *A. albopictus* in the first three years after local establishment of the latter species (Figure 6), although *A. albopictus* predominated in the lone survey of 2000. In tires located in the small woodland at FMEL, *A. albopictus* was 10× more common than *A. triseriatus* in the guts of dissected *T. rutilus*, while *A. triseriatus* was 3× more common than the invading species in the diet of this predator extracted from treeholes (Campos and Lounibos 2000). Quantitative pre-invasion measures of *A. triseriatus* abundance in these tires are not available to assess whether the native species has declined in numbers or is now simply

Table 2. Frequency of occurrence of *A. albopictus* and *A. triseriatus* in ovitraps set in three wooded, protected areas in south Florida during 1997–1999.

	Frequency (no. pos. traps ^b /total)			Abundance (Mean no. ^c (SE)/trap)		
	<i>A. triseriatus</i>	Significance	<i>A. albopictus</i>	<i>A. triseriatus</i>	Significance	<i>A. albopictus</i>
<i>Disturbed sites</i> ^a						
HO	5/18	*	11/18			
EC	4/18	*	10/18			
COC	5/18		10/18	6.0 (3.0)		16.8 (6.5)
CAC	9/12		8/12	9.7 (5.1)		13.2 (5.6)
RHR	8/12		7/12	6.3 (2.7)		15.2 (5.2)
<i>Undisturbed sites</i>						
HA	8/21		8/21			
AH	7/21		3/21	9.3 (2.8)		5.4 (3.0)
WOT	10/15	***	1/15	12.0 (3.9)	**	0.3 (0.2)
NT	10/12		11/12	18.7 (5.3)		21.8 (6.8)

^aSites in Sherwood Hammock, Highlands Hammock, and Myakka River, as described in ‘Materials and methods’.

^bFractions are numbers of positive oviposition traps over total based on three collections at each location in 1997.

*** $P < 0.001$, * $P < 0.05$ by G -test, 1 df; all other intrasite comparisons are non-significant.

^cMeans (SE) are for eggs, larvae, and pupae pooled, per trap collection, for 11 collections at Highlands and 10 at Myakka, 1997–1999. Intrasite (paired) comparisons tested by Mann–Whitney, ** $P < 0.01$.

outnumbered by *A. albopictus* in this habitat. The overall evidence suggests a negative effect of *A. albopictus* on *A. triseriatus* in south Florida, but the impact depends on the location and type of container.

Cemetery vases are another artificial container habitat for which evidence to support displacement of *A. triseriatus* by *A. albopictus* is also weak, although the invasive species was usually more common where the two species have co-occurred since 1992 (Figure 7). In summary, evidence for displacement of *A. triseriatus* is less compelling than that for *A. aegypti*, whose range in Florida has been demonstrably diminished as a consequence of the *A. albopictus* invasion (O’Meara et al. 1995). The displacement effect of *A. albopictus* on *A. aegypti* varies geographically in south Florida, as demonstrated by the high heterogeneity of 1996–1999 presence/absence records of this species in tire samples (Table 1).

Differential responses of prey species to predators are also known to promote regional co-existence (e.g. Holt 1984). In Florida, the predatory mosquito *T. rutilus* has been shown to maintain the treehole mosquito community below its carrying capacity in native woodlands (Bradshaw and Holzapfel 1983). We compared the survival to emergence of *A. triseriatus* and *A. albopictus* exposed jointly to the predator in an experimental design that mimicked time lags observed in nature between *Aedes* hatch and *T. rutilus* oviposition (Nannini and Juliano 1997). Despite its long evolutionary history with this predator species,

A. triseriatus was less successful than *A. albopictus* in survival to adulthood in the presence of *T. rutilus* (Figure 8, Table 3). Predators reduced prey survival most prominently when introduced into containers shortly after prey hatched. Indeed, when predators were introduced into bamboos more than three days after prey, *Aedes* spp. survival was not significantly reduced compared to controls in all experiments (Table 3). This observation, combined with the slower hatching response of *A. triseriatus* (see below and Figure 9), suggests that it may be *A. albopictus* that more readily escapes this predator by rapidly hatching in newly filled containers prior to the arrival of *T. rutilus*. Survivorship of *A. triseriatus* was inexplicably low in the one-day headstart experiment (Figure 8B), which contributed to the absence of significant differences in pairwise contrasts (Table 3B). The more rapid development of *A. albopictus* larvae (Teng and Apperson 2000) is already apparent as soon as eggs are exposed to a hatching stimulus (Figure 9). The accelerated hatching rate of *A. albopictus* contrasts with a decreased sensitivity of *A. triseriatus* to hatch stimuli, reported by Edgerly et al. (1993) in a three-species comparison.

Interest in displacement of native *Aedes* by invasive *A. albopictus* began in the Pacific region where the latter species was introduced accidentally on islands during or after World War II (e.g. Reeves and Rudnick 1951; Rozeboom and Bridges 1972), leading to the testing of competitive exclusion as a method for vector control (Gubler 1970; Lowrie 1973; Rosen et al. 1976).

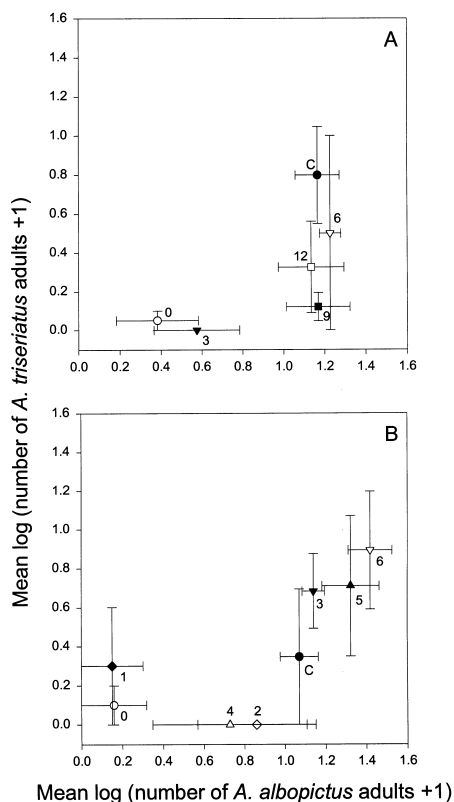


Figure 8. Comparative survivorship to adulthood of *A. albopictus* and *A. triseriatus* exposed jointly in bamboo sections to scheduled introductions of the predator *T. rutilus*. In A, means (\pm SD) are based on two runs with three replicates per treatment, but only one run was conducted for B, which used an alternative schedule of predator introductions. Numbers beside points indicate days headstart for prey (C = control with no predator). Although a significant run effect was detected in A (Table 3), trends were similar, justifying pooling for this figure.

In the past two decades, invasions by this species have spread into the Americas (e.g. Forattini 1986, Marques et al. 1998, Rossi et al. 1999, Broche and Borja 1999), Europe (Mitchell 1995, Romi et al. 1999) and Africa (Savage et al. 1992), although recent studies of interspecific displacements by *A. albopictus* have been limited to the USA.

Container mosquitoes of the genus *Aedes* have proven themselves efficient invaders of North America. In addition to *A. aegypti* and *A. albopictus*, *Aedes togoi* colonized northwestern coastal USA from Asia several decades ago (Belton 1980), *A. bahamensis* took up residence in south Florida in the 1980s, and *Aedes japonicus* has recently settled in the northeastern USA from Japan (Peyton et al. 1999). Transport of used tires has been a major mechanism for the introduction and

Table 3. MANOVA^a results for survival to adulthood^b of *A. albopictus* (ALB) and *A. triseriatus* (TRI) exposed jointly from day of hatch to scheduled introductions of a *T. rutilus* first instar in bamboo sections situated in an oak-palm woodland.

Standardized canonical coefficients				
Source of variation	Pillai's trace	Survival ALB	Survival TRI	
<i>(A) Predator introduced at 3-day intervals (2 runs)</i>				
Run	0.2979	-0.883	1.012	$F_{2,19} = 4.03$, $P = 0.035$
Treatment	0.9525	1.088	0.795	$F_{10,40} = 3.64$, $P = 0.002$
<i>Pairwise contrasts^c</i>				
C vs 0 ^c	0.581* ^d	1.00	0.89	
C vs 3	0.481*	0.82	1.04	
C vs 6	0.052	-0.30	1.27	
C vs 9	0.348	-0.07	1.30	
C vs 12	0.173	0.08	1.30	
<i>(B) Predator introduced at 1-day intervals (1 run)</i>				
Treatment	1.041	1.355	0.388	$F_{14,30} = 2.33$, $P = 0.026$
<i>Pairwise contrasts^c</i>				
C vs 0	0.405 ^d	1.52	0.06	
C vs 1	0.361	1.56	-0.17	
C vs 2	0.080	0.74	0.93	
C vs 3	0.063	0.10	1.15	
C vs 4	0.125	1.10	0.68	
C vs 5	0.099	0.84	0.90	
C vs 6	0.191	0.78	0.90	

^aPROC GLM of SAS Institute, Inc. (1985).

^bLog ($n + 1$).

^cC = Control; numbers are day of predator introduction.

^dStatistical significance ($*P < \alpha$) determined using Bonferroni correction for multiple comparisons, where $\alpha = 0.05/k$ and k = number of comparisons, i.e. $\alpha = 0.010$ for A and 0.007 for B.

spread of these container *Aedes* and will likely remain so because of the inefficiency of inspection or treatment procedures (Reiter 1998). Although the evidence to date in support of competitive displacement in the USA of pre-existing container *Aedes* is strong only for effects of *A. albopictus* on *A. aegypti*, improved pre- and post-invasion monitoring is needed to evaluate more subtle effects. Further, many years may be needed to observe significant changes. Local habitat modifications, such as the cutting of woodlands which are refuges for treehole *Aedes*, may make *A. triseriatus* more vulnerable to gradual population declines, as may be occurring in roadside treeholes and associated tires at Indrio (Figures 4 and 6), attributable in part to displacement by *A. albopictus*.

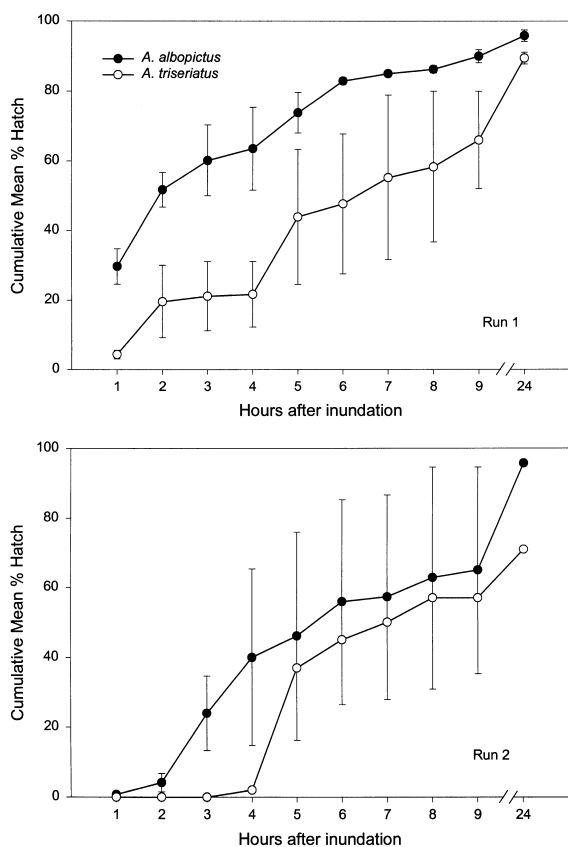


Figure 9. Cumulative hatch (mean%±SD) of eggs of two container *Aedes* exposed in separate vessels to the same hatching stimulus. In run one, batches of *A. albopictus* had 118 and 122 viable eggs; batches of *A. triseriatus* had 90 and 98 eggs. In run two, only one batch of *A. triseriatus* with 96 eggs was exposed; the *A. albopictus* egg batches had 75 and 68 viable eggs. Cumulative frequency distributions examined with K-S tests were marginally significantly different in run one ($D = 60$, $P = 0.055$) but not in run two ($D = 30$, $P = 0.76$).

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