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FAILURE OF *AEDES ALBOPICTUS* TO OVERWINTER FOLLOWING INTRODUCTION AND SEASONAL ESTABLISHMENT AT A TIRE RECYCLING PLANT IN THE NORTHEASTERN USA

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ABSTRACT. In July 2006, an introduction of the Asian tiger mosquito, *Aedes albopictus*, was documented for the first time at a commercial tire recycling plant in northeastern Connecticut, USA. The introduction likely occurred via transport of infested tires originating from northern New Jersey or metropolitan New York City. Efforts were made to determine seasonal establishment and overwintering success by assessing adult biting and oviposition activity in the surrounding woodlands. The first adult female was collected in a CO_2-baited Mosquito Magnet® Liberty trap within the confines of the tire plant during the week of July 28. Additional females were collected intermittently thereafter through October 16. Host-seeking female *Ae. albopictus* attempting to alight on human subjects and larvae hatching from eggs collected in ovitraps placed in the woodlands surrounding the tire plant were detected weekly from August 21 through October 2, denoting seasonal establishment in the adjoining woodlands. However, no larvae of *Ae. albopictus* were recovered from eggs collected in ovitraps that were placed in the surrounding woodlands or in traps placed 1.0–1.6 km away, nor were any host-seeking females detected by human subjects the following season (July to October 2007), indicating that the species did not survive winter conditions to enable successful colonization. The failure of *Ae. albopictus* to overwinter and establish itself in the forested woodlands following several weeks of seasonal breeding and oviposition during the summer and early fall were most likely due to winter egg mortality, interspecific competition from *Aedes triseriatus* and *Aedes japonicus*, and/or other ecological barriers. Permanent establishment of *Ae. albopictus* in New England is unlikely despite the recurring importation of infested used tires into recycling facilities. However, continued monitoring of such facilities for potential reinvasion is warranted especially in urban/suburban environs where global warming and milder winter temperatures may provide more suitable conditions in the future for colonization.

KEY WORDS *Aedes albopictus*, invasive species, introduction, seasonal establishment, tire recycling plant, Connecticut

INTRODUCTION

Aedes albopictus (Skuse) is presently recognized as the most invasive mosquito in the world having spread from its native range in Southeast Asia to at least 36 countries over the last 3 decades (Benedict et al. 2007, Enserink 2008). This has occurred primarily through the worldwide trade in used tires and more recently via containerized shipments of infested ornamental "lucky bamboo" plants (Madon et al. 2002, Roiz et al. 2008). In the USA, established populations of this species have been reported from 866 counties in 26 states in the eastern half of the country extending from southern Florida and Texas to Chicago, IL, the northernmost infestation (Moore 1999, CDC 2008). In the northeast, *Ae. albopictus* has invaded much of New Jersey, portions of southern Pennsylvania, and the New York City metropolitan area (Fig. 1) (Kulasekera et al. 2001, CDC 2008). However, although a few individuals have been occasionally collected in surveillance activities in the neighboring New England states and reported to CDC through the ArboNet surveillance system (Andreadis et al. 2005, Moore 2008), no established infestations have been identified in this region.

Using distribution records for *Ae. albopictus* in Asia and comparative climatological data, Nawrocki and Hawley (1987) conservatively estimated the northern overwintering and summer expansion ranges for this species in the eastern USA to be the 0°C and −5°C daily mean January temperature isotherms, respectively (Fig. 1). A similar estimate of the northern distribution limit was predicted by Benedict et al. (2007) using an ecological niche model, the Genetic Algorithm for Rule Set Production (GARP) (Stockwell and Peters 1999), which analyzed distribution information in relation to specific ecological characteristics. Both estimates included the State of Connecticut as well as southeastern Massachusetts, which are located between the two isotherms and possess suitable habitat and climatic conditions for potential colonization (Fig. 1).

In July 2006, an introduction of *Ae. albopicutus* was detected for the first time at a commercial tire recycling plant in northeastern Connecticut. Upon this discovery, field and laboratory investigations were initiated to 1) determine the extent of the infestation within the immediate vicinity and 2) evaluate overwintering success pursuant to its establishment within the region. The results of this investigation are reported herein.

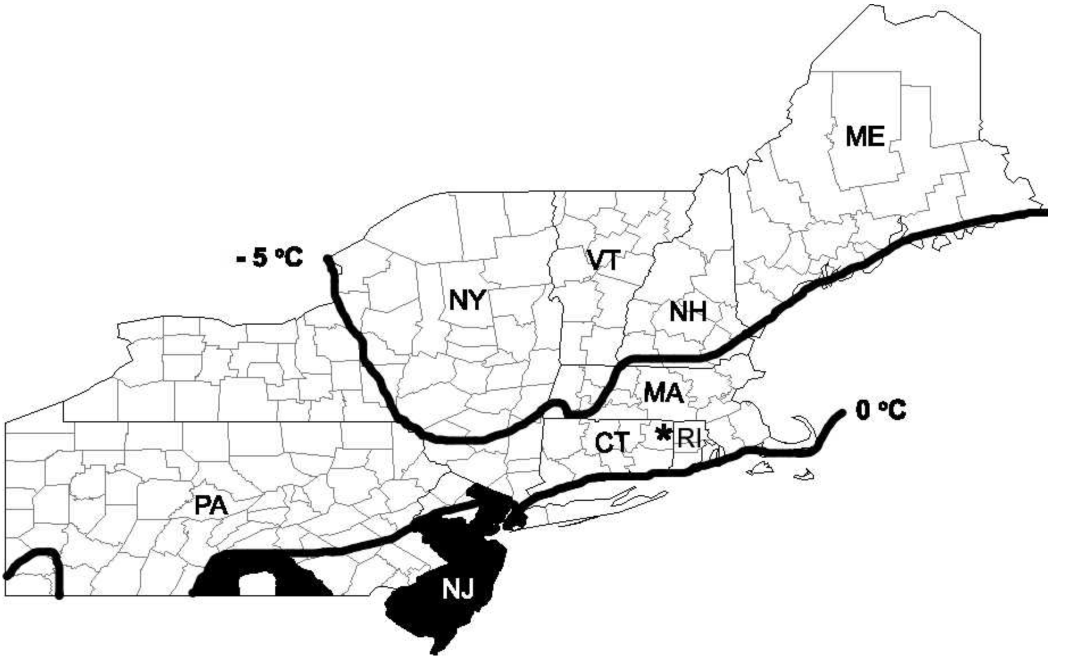

Fig. 1. County map of the northeastern USA showing location of the tire recycling plant in Sterling, CT (*), reported distribution of *Aedes albopictus* (shaded), and the 0°C and −5°C daily mean January temperature isotherms. Redrawn from Nawrocki and Hawley (1987) and Moore (2008).

MATERIALS AND METHODS

Study area

The study was conducted at a dedicated 40-ha tire-to-energy facility in Sterling, Windham County, CT (41°42′45″N, 71°49′18″W) (Fig. 1). This facility serves as a major scrap tire market for used tires generated in New England, the eastern corridor of New York State including the New York City metropolitan area, and northern New Jersey. The plant burns mainly whole tires and processes approximately 10 million tires per year. It is surrounded by deciduous forest. Land use land cover classification from digital Landstat satellite imagery data for the municipality includes 72.3% forest, 14.8% agriculture/turf and grass, 7.1% developed, 4.9% wetland and water, and 0.8% barren (http://clear.uconn.edu/projects/landscape/local/town.asp).

Mosquito sampling

Detection and establishment in 2006: Initial monitoring for detection of adult *Ae. albopictus* was conducted with the aid of a CO_2-baited Mosquito Magnet® Liberty trap (Woodstream Corporation, Lititz, PA) that was placed on the perimeter of the facility adjacent to woodlands. A single trap was operated daily by plant personnel from June 1 through mid-October and collections were submitted weekly to staff at The Connecticut Agricultural Experiment Station for identification. Specimens were identified with the aid of a stereo microscope (90×) based on morphological characters and descriptive keys of Tanaka et al. (1979) and Darsie and Ward (2005).

Following the detection of adult *Ae. albopictus* in the confines of the tire plant, efforts were made to determine seasonal establishment by qualitatively assessing adult biting and oviposition activity in the surrounding woodlands. These studies were initiated on August 14 and continued through October 2. For the oviposition studies, ovitraps where placed at 8 different locations in the adjacent woodlands, approximately 20 m apart along a 180-m perimeter (10–40 m from the edge) surrounding the tire plant. At each site, one ovitrap was placed directly on the ground and another attached to a tree approximately 2 m above ground level. Each ovitrap comprised a 900-ml black plastic cup filled with 500 ml of tap water to which an infusion of ground-up leaves and soil was added. Cups were lined with brown seed-germination paper (Anchor Paper Company, St. Paul, MN), which served as an oviposition substrate. Traps were inspected weekly at which time the oviposition papers and contents of the cup were removed and brought to the laboratory for examination. At the same time, traps were replenished with water, infusion, and oviposition paper. Larvae in the ovitrap samples were identified to species at the time of collection. Oviposition papers were inspected for eggs, placed in plastic bags to prevent desiccation,

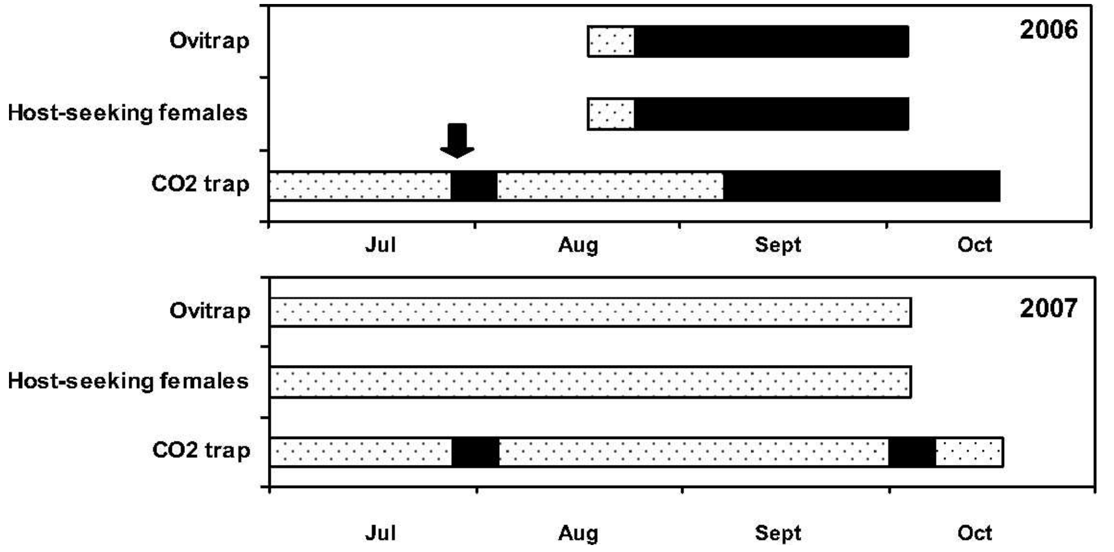

Fig. 2. Schematic representation of weekly sampling (shaded bar) and detection (black bar) of eggs and biting adults of *Aedes albopictus* in 2006 and 2007 through CO_2-baited trapping within the confines of the tire plant, ovitrapping, and human bait collections of host-seeking females in the adjacent woodlands. ➡ = initial detection.

and held at 27°C for 2 wk, following which they were inundated with 2-day-old tap water to induce egg hatch. Resulting larvae were reared in 100-ml culture dishes at 27°C and identified to species as 2nd to 4th instars.

Human bait collections of host-seeking females were made in concert with the oviposition studies at the same locations in the surrounding woodlands. Female mosquitoes that attempted to land on any part of the body of individuals that were attending traps were captured with a handheld battery-powered aspirator, transported to the laboratory and identified to species as described above. These collections were made for approximately 30 min in the morning between 1000 and 1100 h.

Evaluation of colonization in 2007: Efforts to evaluate overwintering success and establishment of *Ae. albopictus* within the immediate vicinity of the plant and dispersal into the neighboring region were made the following year. Adult biting and oviposition activity were assessed at each of the same 8 locations monitored in 2006, and at 6 additional sites that were located in nearby woodlands 1.0–1.6 km from the tire plant in 6 equidistant directions. The trapping methodology was similar to that employed in 2006 with the following modifications: 1) ovitrap and human bait collections of adults were made every 2 wk, 2) ovitraps were placed at ground level only, and 3) the number of larvae hatching from eggs collected in ovitraps was quantified for all mosquito species. Trapping began on July 2 and continued through October 1 for a total of 7 collection dates. Monitoring of adult *Ae. albopictus* within the confines of the tire plant was similarly conducted by plant personnel as in 2006 with the aid of a CO_2-baited Mosquito Magnet Liberty trap placed on the perimeter of the facility adjacent to woodlands.

Meterological data and analysis

Climatological data were obtained from the National Oceanic and Atmospheric Administration Climatological Data Center publications for New England (http://www7.ncdc.noaa.gov/IPS/cd/cd.html). Monthly average temperatures and deviations from normal for each of the 2 years were computed from 9 official recording stations located in the central region of the state (Connecticut Division 2) that included the tire recycling plant. Combined averages from the 9 sites were used for comparative analyses.

Chi-square analysis using Yates correction for continuity and the Fisher Exact Test (SigmaStat 2.0 for Windows; Jandel Corporation, San Rafael, CA) were used to compare and contrast the number of larvae hatching from eggs collected in ovitraps for each species in 2007.

RESULTS

Detection and establishment in 2006: The first adult female *Ae. albopictus* was detected from collections made with a CO_2-baited Mosquito Magnet Liberty trap placed within the confines of the tire recycling plant during the week of July 28 to August 4, 2006 (Fig. 2). No additional *Ae. albopictus* were collected in these traps until the

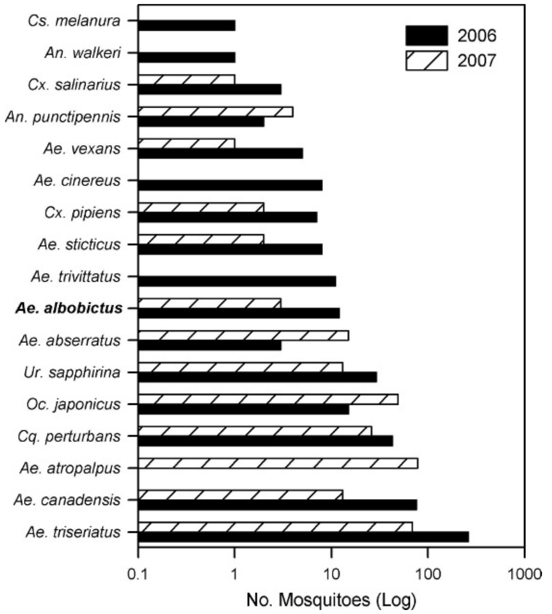

Fig. 3. Comparative number (log scale) of *Aedes albopictus* and 16 other mosquito species collected in a CO_2-baited Mosquito Magnet® Liberty trap placed within the tire recycling plant from June through October 2006 and 2007. Species are ranked in order of overall abundance.

week of September 8, but thereafter, adults were continually collected until the cessation of trapping on October 16. Twelve female *Ae. albopictus* were identified with on-site trapping from a total of 484 mosquitoes (2.5%) collected during the season, representing 16 species, more than one-half of which (53.9%) were *Aedes triseriatus* (Coq.) (Fig. 3). Other species previously shown to utilize used tire casings for larval development in this region (Andreadis 1988, Andreadis et al. 2001) included *Anopheles punctipennis* (Say), *Culex pipiens* L., *Culiseta melanura* (Coq.), *Culex salinarius* Coq., and *Aedes japonicus* (Theobald).

Host-seeking female *Ae. albopictus* attempting to alight on human subjects in the woodlands surrounding the tire plant were first detected on August 21, 1 wk after the first bait stations were begun, and thereafter, on each subsequent weekly visit through October 2 (Fig. 2). On each occasion, host-seeking females were generally encountered within 5 to 10 min upon entering the woodlands. No attempt was made to quantify the frequency of attack, but 4 to 10 females were routinely aspirated from human subjects over a typical 30-min exposure period. Other mosquitoes attempting to bite human subjects included *Ae. triseriatus*.

Eggs collected in ovitraps initially placed in the woodlands surrounding the tire plant from August 14 to 21 only produced larvae of *Ae. triseriatus* (98.5%) and *Ae. japonicus* (1.5%) upon hatch ($n = 413$). The first larvae of *Ae. albopictus* recovered from field-collected eggs were obtained from ovitraps placed in the field the week of August 21 to 28 (Fig. 3) ($n = 4$ of 123; flooded September 18). From that point on, eggs of *Ae. albopictus* continued to be collected in the ovitraps until the cessation of trapping on October 2. Although the number of larvae hatching from field-collected eggs was not quantified, the overwhelming majority of mosquitoes hatching were *Ae. triseriatus*, and number of larvae of *Ae. albopictus* recovered represented less than 1% of the total hatch ($n =$ approximately 2,000 larvae). Ovitraps placed on the ground were consistently more effective for collecting eggs of all 3 species than those attached to trees at a height of 2 m. Adult *Ae. albopictus* reared from egg collections made the week of September 18 were used to successfully establish a free mating colony that is currently maintained in a 0.9-m^2 cage using guinea pigs as a source of blood.

Evaluation of colonization in 2007: No larvae of *Ae. albopictus* were recovered from eggs collected in any of the 8 ovitraps that were placed in the woodlands surrounding the tire plant perimeter or in the 6 traps placed 1.0–1.6 km away, nor were any host-seeking females detected by human subjects for the entire sampling period, July to October (Fig. 2). Mosquito species collected in the ovitraps included *Ae. japonicus* and *Ae. triseriatus* only; the number and comparative proportion of larvae of each species that hatched from eggs are shown in Fig. 4. Significantly more *Ae. triseriatus* were recovered at both locations (overall = 86.1%, $n = 5{,}045$, chi-square = 203.3, 1 df, $P < 0.001$) and the relative proportion of each species did not differ by site ($P = 1.0$, Fisher Exact Test).

One adult female *Ae. albopictus* was collected in the CO_2-baited Mosquito Magnet Liberty trap placed within the confines of the tire recycling plant during the week of July 28 to August 3, and an additional 2 females were collected during the week of October 1 to 5 (Fig. 2). This was from a total of 276 mosquitoes collected during the season representing 17 species composed mostly of *Aedes atropalpus* (Felt & Young) (28.3%), *Ae. triseriatus* (25.0%), and *Ae. japonicus* (17.8%) (Fig. 3).

Temperature data: Average monthly temperatures from July through October of 2006 were slightly above or near normal, whereas temperatures recorded from November (7.8°C) to January 2007 (−0.9°C) were notably milder, ranging from 2.3°C to 3.6°C above normal (Fig. 5). The coldest temperatures recorded were for the month of February (−5.8°C), which was 4.0°C below normal. Temperatures from March through July 2007 were near normal whereas September and October were markedly warmer.

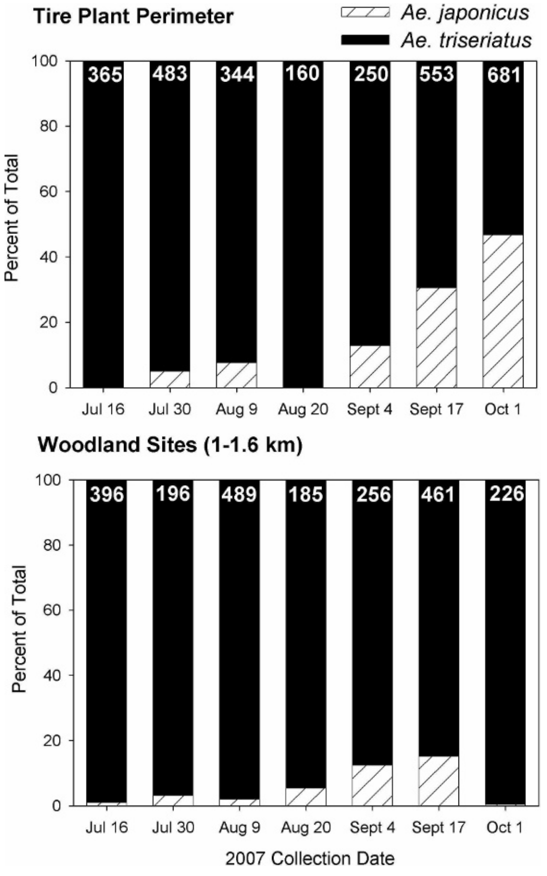

Fig. 4. Comparative abundance of *Aedes japonicus* and *Aedes triseriatus* larvae hatching from eggs collected in ovitraps placed along the perimeter of the tire plant and at a distance of 1.0–1.6 km in 2007. Numbers on bars denote sample size.

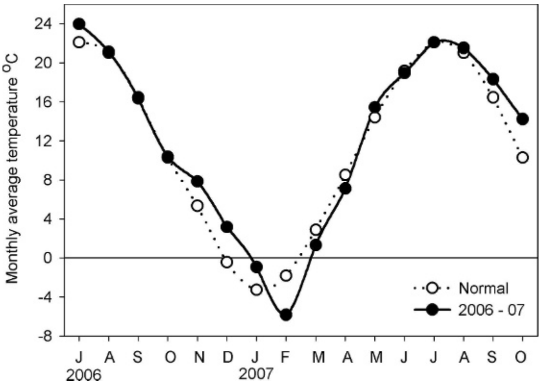

Fig. 5. Normal and actual monthly average temperatures recorded from June 2006 to October 2007 from the central region of Connecticut (Division 2).

DISCUSSION

With this report the introduction and seasonal establishment of *Ae. albopictus* in New England is documented for the first time. However, the inability to recover viable eggs or larvae in ovitraps, or to detect biting adults the following year clearly indicates that the species did not survive winter conditions in northern Connecticut to enable successful colonization in the surrounding woodlands. Although it was not possible to identify the exact geographic source of the introduction, it is logical to infer that *Ae. albopictus* was introduced via transport of infested used tires originating from either northern New Jersey or metropolitan New York City where populations are established and from which tire shipments are regularly received.

The ability of *Ae. albopictus* to invade this climatic region of the USA during the midsummer but its apparent failure to successfully overwinter is in full agreement with estimations of the potential overwintering and summer expansion ranges for *Ae. albopictus* in North America based on the 0°C and −5°C daily mean January temperature isotherms (Nawrocki and Hawley 1987). Average daily temperatures recorded in January and February of 2007 were both below the limiting 0°C isotherm. Results are also consistent with the absence of *Ae. albopictus* in statewide mosquito surveillance conducted in Connecticut since 1999, wherein a single female had been previously trapped (August 26, 2003, Fairfield County) (Andreadis et al. 2005), and prior surveys of commercial waste tire disposal sites in the state where no larvae or adults were detected (Andreadis 1988, Andreadis et al 2001). Connecticut would thus appear to be at the northern overwintering limit for *Ae. albopictus* as nuisance populations have been recognized in all the major boroughs of neighboring New York City (Kulasekera et al. 2001) where average daily temperatures in January are just above 0°C (0.14°C).

The failure of *Ae. albopictus* to overwinter and establish itself in the forested woodlands surrounding the tire plant following several weeks of breeding and oviposition during the summer and early fall was conceivably due to winter egg mortality. Overwintering survivorship of eggs of temperate US strains of *Ae. albopictus* is a function of absolute minimum temperature and the duration of exposure to lethal temperatures (Hawley et al. 1989; Hanson et al. 1993; Hanson and Craig 1994, 1995). The lower limit of survival of temperate strains appears to be an absolute minimum temperature of approximately −12°C as this is the temperature that causes mortality in diapause, cold-acclimated eggs in the laboratory (Hanson and Craig 1994). However, high survivorship in the field (78%) has been noted in eggs exposed to temperatures of −12°C for brief periods (<16 consecutive hours) (Hanson and Craig 1995) suggesting that the number of hours exposed to temperatures below this threshold is also an important factor affecting the survivorship of *Ae. albopictus* eggs during the winter (Hanson 1995, Hanson and Craig 1995). In the present study, minimum daily temperatures below −12°C were recorded on 3 days during the month of January and 12 days (5 consecutive) in February with an extreme low of −16°C consistent with this hypothesis. It is noteworthy that average daily temperatures in February were 4°C below the norm, raising the possibility that populations could perhaps survive in a normal or warmer than normal winter.

Another barrier to the establishment of *Ae. albopictus* in the woodlands surrounding the tire plant may have included interspecific competition from *Ae. triseriatus* and *Ae. japonicus* in natural larval habitats. This competition would have been further exacerbated by the comparatively small founding population of *Ae. albopictus* eggs as detected in ovitraps the preceding fall. Teng and Apperson (2000) have suggested that competition from *Ae. triseriatus* may suppress the rate of growth of *Ae. albopictus* populations early in the spring due to the combined detrimental effects of overwinter egg mortality and higher threshold temperatures required for larval development. It is noteworthy that eggs of another invasive species, *Ae. japonicus*, which was similarly thought to have been introduced into the region via used tires in the mid-1990s and is now firmly established throughout the state (Andreadis et al. 2001), were regularly collected in ovitraps placed in the woodlands, albeit at lower densities than *Ae. triseriatus* (Fig. 4).

The inability to colonize natural development sites in the forested woodland could also have been a contributing factor. Limited invasion of wooded habitats by *Ae. albopictus* and a complete absence of larvae in natural tree holes was noted in central Illinois following successful overwintering and establishment of the species in an urban/suburban setting (Swanson et al. 2000). Indeed, Lounibos et al. (2001) have further demonstrated that in central Florida, where *Ae. albopictus* is firmly established, *Ae. triseriatus* consistently occupies more tree holes than *Ae. albopictus*, which occurs infrequently in undisturbed woodland habitats. Barker et al. (2003) also reported that ovitraps from open, unforested sites in southwestern Virginia had a higher proportion and greater intensity of *Ae. albopictus* eggs than those from densely forested areas and forest border locations, whereas *Ae. triseriatus* showed no clear preference.

The continual transport of used tires into recycling facilities in New England from locales where *Ae. albopictus* is firmly established will most certainly result in periodic reintroduction of this invasive mosquito as documented in 2006 and 2007. Based on findings reported here, however, there appears to be an inability of *Ae. albopictus* to overwinter whether because of egg mortality, interspecific competition, or other ecological barriers that may preclude permanent establishment in the region. Continued monitoring of such facilities for potential reinvasion is warranted, especially in urban/suburban environs where global warming and milder winter temperatures may provide more suitable conditions in the future for permanent establishment in the region.

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