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EXPANDED POLYSTYRENE (EPS) FLOATS FOR SURVEILLANCE OF *OCHLEROTATUS JAPONICUS*

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ABSTRACT. Blocks of expanded polystyrene (EPS) were placed in a variety of habitats to investigate their potential as an egg-collection device for container-dwelling *Aedes* and *Ochlerotatus* species. Eggs from *Ochlerotatus japonicus, Oc. triseriatus, Oc. hendersoni,* and *Aedes albopictus* were collected with EPS floats. The float provides an inexpensive, low-maintenance alternative to the Centers for Disease Control ovitrap for sampling container-dwelling mosquito species that are important vectors of disease. Eggs collected on the floats have many potential applications, including use in routine population surveillance; detection of *Oc. japonicus, Ae. albopictus,* and other container-dwelling species in new areas; species distribution studies; natural transovarial transmission studies; ovipositional studies; collection of local field populations for insecticide resistance assays; assessment of adulticiding efficacy; and establishment of new laboratory colonies.

KEY WORDS *Ochlerotatus japonicus,* surveillance, eggs, expanded polystyrene (EPS) float, ovitrap

INTRODUCTION

Ochlerotatus japonicus japonicus (Theobald) is an Asian mosquito that was 1st discovered in the United States in 1998 (Peyton et al. 1999). In the 5 years since its discovery, the mosquito has spread to 14 states (Connecticut [Andreadis et al. 2001], Delaware [Chris Lesser, personal communication], Maine [Foss and Dearborn 2001], Maryland [Sardelis and Turell 2001], Massachusetts [Ellen Bidlack, personal communication], New Hampshire [Sarah McGregor, personal communication], New Jersey [Peyton et al. 1999], New York [Peyton et al. 1999], Ohio [Robert Restifo, personal communication], Pennsylvania [Sardelis and Turell 2001], Rhode Island [Alan Gettman, personal communication], Vermont [Graham and Turmel 2001], Virginia [Harrison et al. 2002; David Gaines, personal communication], and West Virginia [Jim Amrine, personal communication]) in the eastern USA and Quebec, Canada (R. Savignac, personal communication), and has been discovered on the Pacific Coast near the port city of Seattle, WA (Washington State Department of Health 2002).

Laboratory studies have demonstrated that *Oc. japonicus* is an efficient vector of La Crosse virus (Sardelis et al. 2002b), eastern equine encephalitis virus (Sardelis et al. 2002a), Japanese encephalitis virus (Takashima and Rosen 1989), and West Nile virus (WN) (Sardelis and Turell 2001). West Nile virus–positive *Oc. japonicus* have been collected from New Hampshire (Centers for Disease Control [CDC], unpublished data), New Jersey (W. J. Crans, unpublished data), New York (White et al. 2001), Ohio (CDC, unpublished data), Pennsylvania (Michael Hutchinson, PA Department of Environmental Protection, West Nile Virus Program, personal communication), and Massachusetts (Werner 2001). The vector potential of this mosquito for WN and other viruses requires that we find effective surveillance methods for it. But since its arrival in the United States in the late 1990s, few traditional methods of mosquito surveillance have been effective in detecting *Oc. japonicus*. Infusion-baited gravid traps (Reiter 1983) have been the most efficient means of collecting adults of this species for virus surveillance (Andreadis et al. 2001, Scott et al. 2001, Falco et al. 2002), but in low numbers relative to other species and do not provide information about its abundance. Larval surveillance is the most reliable method for detecting populations of *Oc. japonicus*; however, this may be time-consuming and laborious.

Egg collections are a sampling method that few mosquito control agencies include as part of their standard surveillance program. Most egg surveillance programs use the traditional CDC ovitrap as described by Fay and Eliason (1966), which consists of a 1-pint glass jar with a painted black exterior surface, containing water and a wooden tongue depressor with a strip of brown blotter paper as the ovipositional surface inside the open jar. The blotter paper is collected and replaced with fresh paper weekly, and the water in the ovitrap is replaced or topped off as needed.

Ovitraps have many applications, and have been used to detect new populations of introduced species such as *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) (Richardson et al. 1995, Bellini et al. 1996), for species distribution studies (Hanson et al. 1988, Yap 1995), and for ecological and ovipositional studies (Loor and DeFoliart 1969, Clark and Craig 1985, Aziz and Hayes 1987, Ballard et al. 1987, Kitron et al. 1989, Beehler and DeFoliart 1990, Trexler et al. 1997). Over the years, ovitraps have collected many species of container-dwelling mosquitoes, including *Ochlerotatus atropalpus* (Coq.), *Oc. triseriatus* (Say), *Ae. albopictus, Aedes mediovittatus* (Coq.), *Orthopodomyia signifera* (Coq.) (Pratt and Kidwell 1969), *Oc. hendersoni* (Cockerell) (Clark and Craig 1985, Ballard et al.

Fig. 1. (A) Locality, date, and other information is written directly onto the float. Two sides of the same expanded polystyrene (EPS) float are shown in (B) and (C). More eggs were deposited in the crevices and contours produced by breaking the EPS panels (B) than on the smooth surfaces produced by cutting the foam panels (C).

1987), and *Ae. aegypti* (Reuben et al. 1977, Reiter et al. 1991).

Because *Oc. japonicus* is a container-dwelling mosquito, it is a natural candidate for surveillance with ovitraps. *Ochlerotatus japonicus* was 1st detected in Ohio by using ovitraps (Berry 1999), but only 6 specimens were collected. In Connecticut, Andreadis et al. (2001) reported that ovitraps were not effective in detecting *Oc. japonicus,* even though many ovitraps were set in areas where gravid and light traps collected adult *Oc. japonicus.*

During routine larval collections of *Oc. japonicus* from automated livestock watering devices, the senior author observed large numbers of mosquito eggs on the polystyrene floats used to regulate the water level. These eggs were collected and returned to the laboratory, where they were hatched and the larvae were identified as *Oc. japonicus.* This observation provided the inspiration to investigate whether *Oc. japonicus* would lay its eggs on similar substrates placed in other containers, and if a floating polystyrene block could be used as an alternative to the traditional CDC ovitrap.

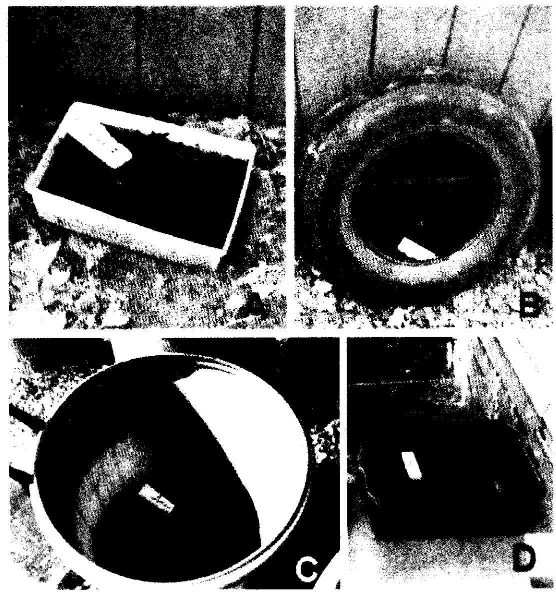
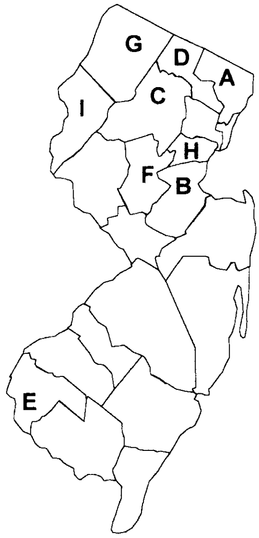


Fig. 2. Typical habitats suitable for use of expanded polystyrene floats. (A) Plastic tray; (B) discarded tire; (C) trash can; and (D) gravid trap.

MATERIALS AND METHODS

Sheets of Shelterfoam, a commercially available expanded polystyrene (EPS) panel insulation (Shelter Enterprises, Inc., Cohoes, NY) used for insulating homes, were purchased from Home Depot (Bound Brook, NJ). Shelterfoam is sold in packages of 6 panels, with each panel measuring 0.75 $\times$ 13.625 $\times$ 48 in. (1.9 $\times$ 34.6 $\times$ 121.9 cm). The EPS panels were broken into 12-cm-wide strips, and the strips were further broken into 5-cm-long pieces to produce a float measuring 12 $\times$ 5 $\times$ 2 cm (Fig. 1A). Sixty similarly sized EPS floats were produced from each panel. Initial attempts using a utility knife to cut the EPS panels into the small size needed for surveillance were abandoned because this was a slow process that produced smooth edges on the float. We found that breaking the EPS panels on the edge of a table or workbench was a faster process and produced textured surfaces. A single worker can produce several hundred floats in less than an hour.

Most EPS floats for this study were set between March 2001 and November 2002 in existing artificial containers in Hutcheson Memorial Forest, Franklin Township, NJ (Fig. 2). Since 1998, routine larval surveillance conducted at this site revealed a typical mix of container-dwelling mosquito species for the region, including *Oc. triseriatus, Culex pipiens* L. and *Cx. restuans* Theobald. *Ochlerotatus japonicus* was 1st detected at this site on August 15, 2000. Other species collected as larvae from containers at the study site included *Anopheles punctipennis* (Say), *An. quadrimaculatus* Say, *Cu-*



Key	County	Containers	Habitat	No. of EPS Floats	Species collected	Mean number of larvae per float (range)
A	Bergen	Various plastic containers, tires	Forested	7	*Oc. japonicus* *Oc. triseriatus* *Oc. hendersoni*	81.0 (6-253) 9.0 (0-41) 3.7 (0-22)
B	Middlesex	Tires	Industrial/ Urban	5	*Oc. japonicus* *Oc. triseriatus*	24.0 (0-69) 54.0 (0-152)
C	Morris	Tires	Forested	17	*Oc. japonicus* *Oc. triseriatus* *Oc. hendersoni*	180.7 (19-573) 25.5 (0-84) 2.6 (0-16)
D	Passaic	Plastic barrels	Rural/ Forested	4	*Oc. japonicus* *Oc. triseriatus* *Oc. hendersoni*	206.8 (63-416) 36.3 (2-86) 0.8 (0-3)
E	Salem	Tires	Industrial	6	*Ae. albopictus* *Oc. triseriatus*	60.7 (18-110) 12.7 (0-32)
F	Somerset	Tires, various plastic containers, tarps, woodland pools	Rural/ Forested	100	*Oc. japonicus* *Oc. triseriatus*	175.4 (0-1245) 26.8 (0-311)
G	Sussex	Tires, various plastic containers	Rural/ Forested	18	*Oc. japonicus* *Oc. triseriatus* *Oc. hendersoni*	157.8 (0-503) 51.7 (0-201) 2.3 (0-23)
H	Union	Tires	Industrial/ Urban	4	*Oc. japonicus* *Oc. triseriatus*	19.8 (0-79) 13.3 (0-22)
I	Warren	Cement catch basins, various plastic containers	Suburban/ Rural	41	*Oc. japonicus* *Oc. triseriatus*	25.7 (0-233) 4.1 (0-55)

Fig. 3. Expanded polystyrene float locations with container types, habitats, and species collected.

lex salinarius Coq., and *Toxorhynchites rutilus septentrionalis* (Dyar and Knab). No *Oc. atropalpus*, *Oc. hendersoni*, *Ae. albopictus*, or *Ae. aegypti* have been collected from this site.

From May through October 2002, additional EPS floats were set in Bergen, Middlesex, Morris, Passaic, Salem, Sussex, Union, and Warren counties (Fig. 3) for 7–52 days to determine if the floats would collect other container-dwelling species not found at the primary study site. Finally, the longevity and durability of the EPS floats were assessed by leaving additional floats out in a variety of habitats for extended periods up to 350 days.

The majority of EPS floats were set in artificial containers, except in Warren County, where previous larval sampling had revealed a significant population of *Oc. japonicus* in cement catch basins, so 20 of the 41 floats in this county were set in the catch basins. The employees of the Warren County Mosquito Extermination Commission (WCMEC) used lengths of monofilament line to tether EPS floats in catch basins to facilitate retrieval and prevent their loss during rain events (Christine Musa, WCMEC Director, personal communication).

Floats were collected and placed directly into individual sandwich-sized resealable plastic bags and transported to the laboratory. Floats were stored in these bags for 7–14 days at room temperature (20–24°C) and 16:8 h light : dark. The water adhering to the floats provided high relative humidity inside the sealed plastic bags, and allowed eggs to complete embryonation without desiccation. Individual EPS floats were submerged in pans containing 1.0 liter of 24-h aged tap water with 10 mg of powdered rat chow added. Floats remained submerged for 7 days, and larvae were identified and the number of each species was recorded.

RESULTS

The EPS floats collected eggs from *Oc. japonicus*, *Oc. hendersoni*, *Oc. triseriatus*, and *Ae. albopictus* (Fig. 3). Eggs were generally deposited at or slightly above the waterline on the floats. Eggs above the waterline rarely hatched before immersion in the laboratory. Where *Oc. japonicus* was present, the species made up the majority of eggs deposited on the EPS floats, with smaller numbers (generally less than 20%) of *Oc. triseriatus*. *Ochlerotatus hendersoni* was collected in low numbers from 7 locations, all in wooded areas of the rural northwestern region of the state (Fig. 3). When present, *Ae. albopictus* readily deposited eggs on the floats (Fig. 3). Small numbers of eggs of *An. punctipennis* occasionally were collected as well, but these reflected incidental adhesion to the floats, not direct oviposition.

More eggs were deposited on the textured sides produced by breaking than on the smooth sides produced by cutting the Shelterfoam panels. This may be related to greater surface area on the broken sides relative to the cut sides; however, field observations showed that the mosquitoes tended to deposit their eggs in the many crevices created along contour lines of the cells (Fig. 1B) rather than on the smooth surfaces (Fig. 1C). So the textured surfaces produced by breaking the EPS sheets appeared to increase their attractiveness to ovipositing mosquitoes.

The floats collected *Oc. japonicus* eggs from a wide variety of larval habitats. The majority of collections were made from natural and artificial containers, including discarded vehicle tires, hubcaps, cement catch basins, tarps, birdbaths, tree holes, plastic milk jugs, plastic trash cans, and a variety of plastic buckets, pans, and planters (Fig. 2).

Interestingly, eggs of *Oc. japonicus* also were collected from several floats placed in woodland pools. Repeated larval collections during the spring, summer, and fall of 2002 failed to collect any larval *Oc. japonicus* from these woodland pools, although many typical woodland pool species were taken, including *Aedes vexans* (Meigen), *Oc. canadensis* (Theobald), *Ochlerotatus grossbecki* (Dyar and Knab), *Cx. restuans*, *Psorophora ferox* (von Humboldt), and *Ps. columbiae* (Dyar and Knab). No eggs of other species were collected on the EPS floats in woodland pools.

After mid-October only eggs of *Oc. japonicus* were collected on the floats. *Ochlerotatus japonicus* continued to deposit eggs through early December in 2001, and through mid-November in 2002. Concurrent larval and adult surveillance also revealed that *Oc. japonicus* was the most abundant species from April though mid-October, and the only mosquito species present from mid-October though December.

The durability and longevity of the floats was examined by allowing additional floats to remain outdoors in a variety of containers from July 14, 2001, to April 30, 2002 (286 days). The floats were very durable, and withstood exposure to sunlight, a wide range of temperatures (−12 to 37°C), and repeated drying without any apparent deterioration. These long-term floats collected several thousand eggs each, with as many as 4 layers of eggs deposited on top of each other. After several weeks, these EPS floats were noticeably heavier because they had absorbed water. None became sufficiently waterlogged to sink; however, some of the eggs deposited closest to the waterline were submerged and hatched.

During the study, only 3 of the 202 floats were lost: 2 were damaged by squirrels gnawing on the floats, and 1 was blown from a dry container in windy conditions. However, note that the surface tension of the water on the float is generally sufficient to prevent the float from being blown away in windy conditions.

DISCUSSION

The EPS float has several advantages over other egg-collection methods. It is small, lightweight, easily transported, and easy to use. The white color of the EPS floats contrasts with the dark eggs, and permits rapid assessment in the field for the presence or absence of eggs. The EPS floats are very durable and do not degrade, even when exposed to direct sunlight, drying and reflooding, and freezing temperatures for several months. Their durability contributes to their low maintenance as a passive sampling method; EPS floats may be set out early in the season, and checked as frequently as is convenient. They are very inexpensive to produce: 1 package of 6 EPS panels costs about U.S. $5.00 at most hardware stores and about sixty 12 × 5-cm floats can be produced from each sheet. The final production cost per float is approximately U.S. $0.03 (1.2¢ for materials and 1.9¢ for labor). Although the floats used in this study were all of a standardized size, floats may be broken into smaller sizes to accommodate smaller containers. Monofilament may be used to tether EPS floats in hard-to-reach locations, such as deep tree holes, or where intermittent flooding may carry away the float, such as in catch basins. This may provide useful and cost-efficient mosquito surveillance in the early and late seasons when conventional trapping may be infrequent. The EPS floats may be placed in existing containers, which tend to hold more water for longer periods of time than the CDC ovitraps, and thus do not require frequent, regular inspections to maintain the water level. Information such as locality data, date, and agency contact information may be written directly on the float.

Although identification of the eggs is possible, this is a labor-intensive process and comparative photos and keys to the eggs of the North American container-dwelling *Aedes* and *Ochlerotatus* species are not readily available. The larvae are identified quickly and easily, and many of the diagnostic characters for container-dwelling species are present in the early instars.

The eggs collected on the floats may be used for many different purposes, including routine population surveillance; detection of *Oc. japonicus*, *Ae. albopictus*, and other container-dwelling species in new areas; species distribution studies; natural transovarial transmission studies; ovipositional studies; collection of local field populations for insecticide resistance assays; assessment of adulticiding efficacy; and establishment of new laboratory colonies.

The collection of eggs of *Oc. japonicus* from floats set in woodland pools raises an interesting question: are ovipositing *Oc. japonicus* attracted to the EPS floats no matter what the habitat, or do *Oc. japonicus* deposit their eggs in a broader range of habitats than previously reported? The failure to collect larval *Oc. japonicus* from these woodland pools during subsequent samples suggests that *Oc. japonicus* may not be a successful competitor in these habitats, or that the species only deposited eggs on the floats and nowhere else in the pools.

Eggs were deposited on floats set in containers that had been treated with mosquito control products including Altosid® and VectoLex®. Most of these eggs hatched, but none of the larvae survived to the pupal stage, suggesting that some residual larvicide remained on these EPS floats. The apparent lack of adult repellency to these larvicidal products may permit the EPS floats to be used for monitoring posttreatment populations.

The EPS float provides a simple, inexpensive, and reliable method for sampling *Oc. japonicus*, *Oc. triseriatus, Ae. albopictus*, and other container-dwelling mosquito species that are important vectors of disease.

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