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Research Paper

Efficacy of Resmethrin Aerosols Applied from the Road for Suppressing *Culex* Vectors of West Nile Virus

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ABSTRACT

We determined whether aerosol applications of resmethrin, delivered from the road, suppress the reproductive activity of *Culex pipiens pipiens* and *Cx. restuans* mosquitoes in suburban sites located near Boston. Oviposition implies a prior blood-feeding event and hence a potential West Nile virus (WNV) transmission-related event. Droplet size, rate of delivery and meteorological conditions were monitored. The target populations proved to be fully susceptible to the insecticide that was used. The roads in the test sites generally gave adequate opportunity for insecticidal coverage. We found that the aerosol plume may have failed to contact the target mosquitoes and conclude that such insecticidal aerosols, delivered from the road, may not effectively reduce the force of transmission of WNV in our test sites. Key Words: Mosquito control—Adulticide—ULV—Resmethrin—*Culex pipiens pipiens*—*Culex restuans*—Arbovirus—West Nile virus. Vector-Borne Zoonotic Dis. 6, 117–127.

INTRODUCTION

The remarkable expansion of the range of West Nile virus (WNV) since its appearance in the United States in 1999 continues to challenge public health and mosquito abatement agencies (Roehrig et al. 2002). The various *Culex* mosquitoes that are regarded as the principal vectors of this pathogen are widely distributed in North America (Taylor et al. 1953, Hurlbut et al. 1956). Source reduction efforts frequently are directed against the aquatic stages of these vector mosquitoes. Catch basins, for example, are treated routinely with microbial or hormonomimetic insecticides (McCarry 1996, Siegel and Novak 1999). Interventions recommended by federal, state and local health authorities generally focus on the application of adulticides delivered from aircraft and road vehicles (Mount et al. 1996). Such "ultra-low volume" (ULV) applications of aerosols (Mount 1996) are widely used to protect residents of North America against the mosquito-borne encephalitides.

Large-scale applications of insecticidal aerosols became practical shortly after World War II, with the advent of potent insecticides and when the devices that had been used for generating naval smokescreens were "beaten into plowshares." The resulting thermal aerosol generators dispensed dilute insecticidal formulations with reasonable efficacy, and this methodology

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rapidly became widespread (Mount 1998). The technology permitting ULV applications of insecticide was developed during the 1950s (Knapp and Roberts 1965). Although the apparatus for dispensing such aerosols originally had been mounted on aircraft, such devices soon were adapted for use on ground-based vehicles, thereby becoming the "worldwide standard ground aerosol method of mosquito adulticiding . . . because of the inherent advantages over [high volume] aerosols" (Mount 1998). Although numerous field trials have demonstrated that insecticidal aerosols are lethal to caged mosquitoes (Mount 1998), few have monitored their impact on mosquitoes in nature. One such study demonstrated an 80% reduction of *Culex* species on the night after treatment, but concluded that a single application was probably inadequate for meaningful reduction of human risk of arboviral infection (Reiter et al. 1990). The general epidemiological impact of vehicle-mounted ULV insecticidal applications, therefore, remains to be determined.

It may be that risk of human infection by WNV can be reduced by aerosol formulations of insecticide applied from the road. Accordingly, we determined the impact of ULV applications of resmethrin on *Culex* mosquitoes in suburban sites in the vicinity of Boston. We used ovitraps to record the abundance of egg rafts deposited by *Culex pipiens pipiens* and *Cx. restuans* mosquitoes in the treated sites before and after aerosols were dispersed, as well as in sites in which no such aerosols were applied.

METHODS

Monitoring the Culex *spp. population*

The abundance of *Culex* mosquitoes was assessed by counting egg rafts deposited each night in arrays of ovitraps placed where aerosols were to be dispersed (Reiter 1986). Each ovitrap was a standard, black plastic tote box (54.0 × 40.0 × 12.7cm; Tablecraft Products Company, Inc, Gurnee, IL). The attractant was prepared by steeping 500 g of hay in 120 L of water for 7 days. Four liters of this infusion were placed in each pan several hours before sunset and renewed daily. Pans were spaced about 50 m apart. Egg rafts were counted each morning and a sample of 24 rafts transported to the laboratory and allowed to hatch. The resulting larvae were identified to species by examining the egg tooth and the surrounding cranial surface (Reiter 1986). Oviposition activity was monitored for a minimum of two days

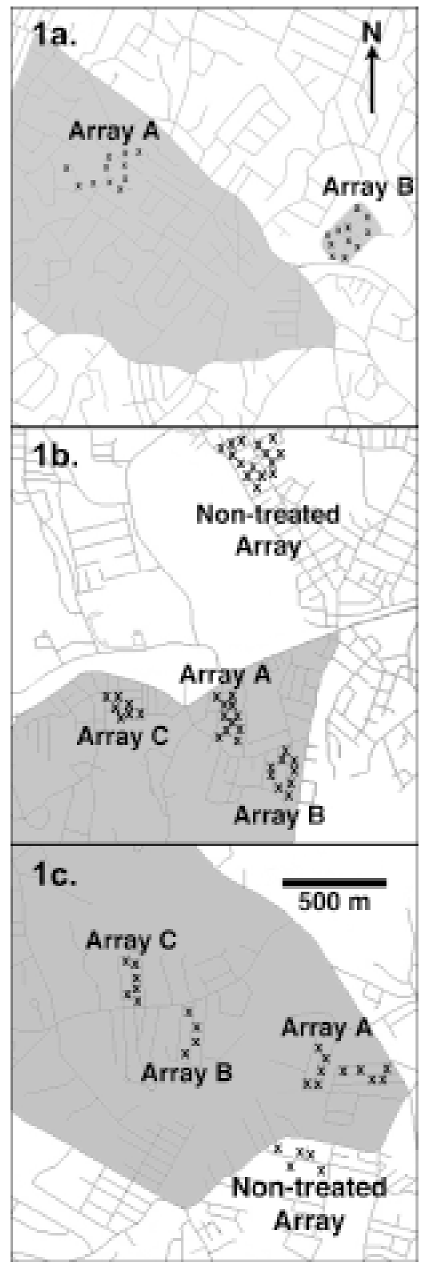

FIG. 1. The distribution of streets in the towns subjected to insecticidal treatment, the location of ovitraps (marked X), and the extent of the aerosol applications (shaded). (a) Burlington, MA. (b) Framingham, MA. (c) Tewksbury, MA.

prior to treatment and for up to two weeks thereafter.

Study sites

Six trials were completed in 2001–2002. Two were conducted in Burlington, eastern Massachusetts. The treatment area (Fig. 1a) included two arrays of ovitraps. Although many streets in the region are spaced irregularly, those where the ovitraps were set were spaced less than 30 m apart. Most of the houses were large, single-family homes, spaced well apart and set back from the road, each covering about 0.1 ha. Most were surrounded by lawns and gardens, including trees and shrubs of various sizes. Some house-lots included areas of dense secondary-growth forest.

Three trials were conducted in the nearby community of Framingham. The treatment area (Fig. 1b) included three arrays of ovitraps, all set along streets with similar layout and appearance. Although many houses were free standing, some were attached, multi-family units. Houses were spaced about 10m apart, and had a somewhat less affluent appearance.

A third array of ovitraps was set in the nearby community of Tewksbury (Fig. 1c). The street layout, lot-size and landscaping characteristics resembled that of Burlington. For comparison, we monitored arrays of ovitraps in non-treated areas in Framingham, Tewksbury (Fig. 1b,c), and Cambridge. Non-treated sites in Framingham and Tewksbury were similar to those that were treated.

Insecticide application

Resmethrin aerosols (Scourge® 18% A.I.+54% PBO; Bayer Environmental Science, Montvale, NJ) were generated from an 18-hp Grizzly® Cold Aerosol PDS ULV generator (Clarke Mosquito Control, Roselle, IL). Droplet size was determined with an AIMS hot wire portable droplet counter (Model DC-III; KLD Laboratories, Inc., Huntington Station, NY). The sprayer was adjusted to generate a surface mean diameter of 16–18 μm and to release the aerosol 45 degrees above the horizon. Flow rate was adjusted to 89 mL/min for trials 1–3 and 106 mL/min for trials 4–6. Windspeed ranged from 1km/h to 8 km/h, and air temperature was above 18°C when the application commenced. Insecticide was applied at three of four U.S.Environmental Protection Agency (EPA)–approved treatment rates (1.12, 1.96, and 7.85 g/ha). Spraying commenced 0.5 h after astral sunset and ended within about 2 h. Wind direction, speed, ambient temperature and relative humidity were recorded immediately

TABLE 1. RATE OF RESMETHRIN TREATMENT, LOCATION AND DATE OF EACH TRIAL

					Dates			
			Traps		Observations			
Trial	*Resmethrin, g/ha*	*Town*	*Array*	*No.*	*Begun*	*Ended*	*Aerosol applied*	*Year*
1	1.12	Burlington	A	14	15 Jul	26 Jul	18 Jul	2001
"	"	"	B	12	"	"	"	"
2	"	"	A	14	30 Jul	16 Aug	2 Aug	"
"	"	"	B	12	"	"	"	"
3	1.96	Framingham	A	11	5 Aug	3 Sep	13 Aug	"
"	"	"	B	9	"	"	"	"
4	7.85	Tewksbury	A	10	4 Sep	13 Sep	6 Sep	"
"	"	"	B	4	"	"	"	"
"	"	"	C	7	"	"	"	"
"	"	"	Untreated	5	"	"	None	"
5	"	Framingham	A	11	8 Jul	25 Jul	16 Jul	2002
"	"	"	B	9	"	"	"	"
"	"	"	C	10	"	"	"	"
6	"	"	A	8	5 Aug	12 Aug	6 Aug	"
"	"	"	B	7	"	"	"	"
"	"	"	Untreated	15	"	"	None	"

prior to each application. Local weather data were compared to data recorded by an automated meteorological station operated by the National Weather Service in the nearby town of Bedford, MA.

Insecticide susceptibility

Larval *Cx. p. pipiens* and *Cx. restuans* obtained from egg rafts collected at the Cambridge and Burlington, MA, sites were sent to CDC Malaria Branch where they were reared to adulthood and tested by bioassay and enzyme analysis. Adults were held for 1 h in bottles containing residues of 20 μg of resmethrin, 43 μg of deltamethrin, or 25 μg of cypermethrin. All mosquitoes died after 30 min of exposure, indicating full pyrethroid susceptibility.

Statistical analysis

Multi-variate regression analysis was performed using a statistical software program (MINITAB, release 13.31, MINITAB Inc., State College, PA). Variables compared in this analysis included (1) whether or not insecticide had been applied, (2) days elapsed since such an application (limited to four days) and (3) mini-

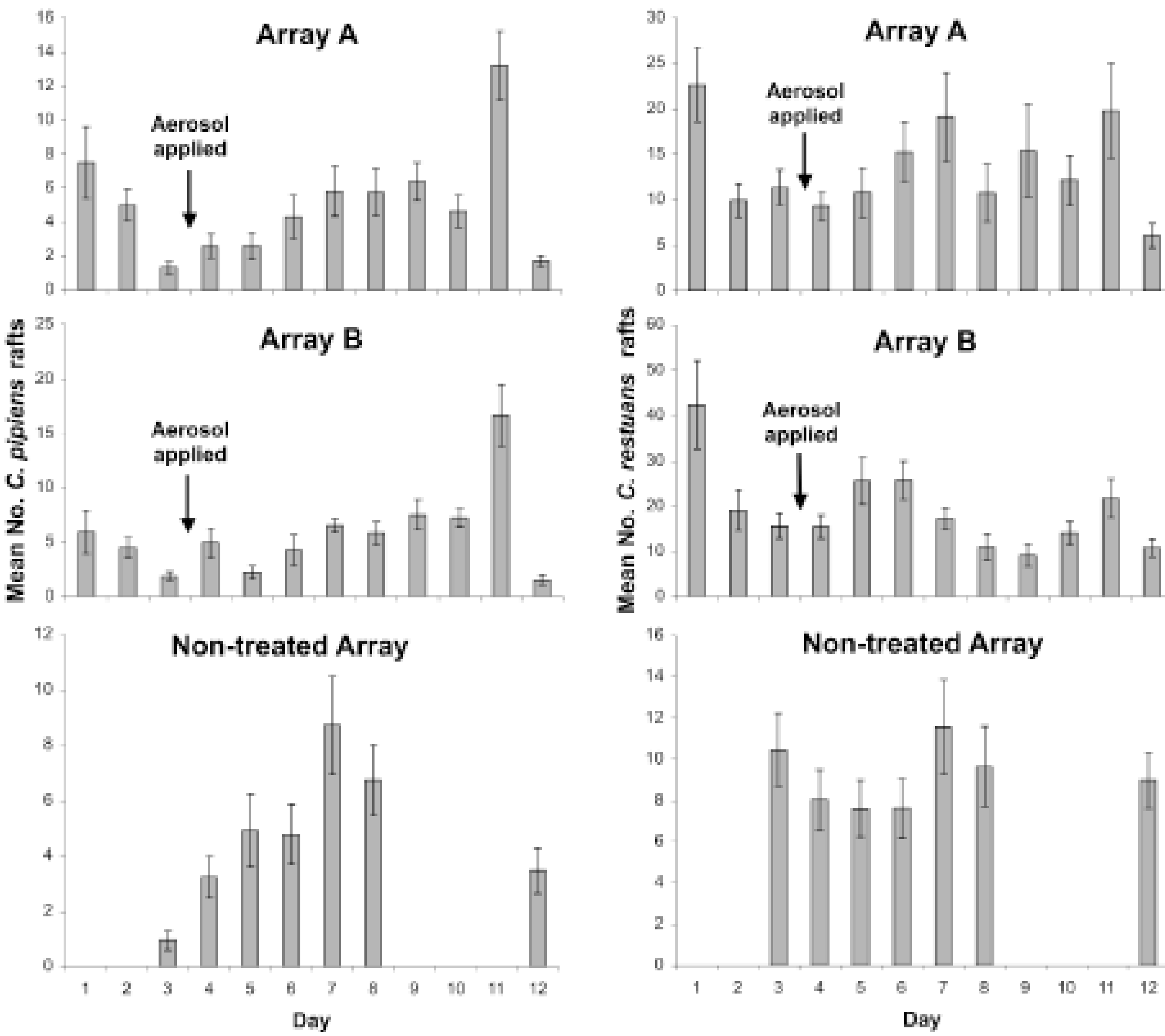

FIG. 2. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 1 following an aerosol application of resmethrin at 1.12 g/ha in Burlington, MA, during 15–26 July 2001. Non-treatment observations were recorded at sites in Cambridge, MA, and Jamaica Plain, MA. No collections made on days 1, 2, 9, and 11.

mum ambient temperature on each of these days. A *p* value of <0.05 was considered to be significant.

RESULTS

The first trial was conducted in Burlington during mid-July of 2001 (Table 1). Insecticide was applied at the minimum specified rate of 1.12 g/ha. This evaluation was based on an array of traps set near the center of a larger treated site (Array A) and another that covered the entire treated area (Array B). Insecticide was applied on the fourth evening after observations commenced. During the 12-day period of observation, the number of egg rafts deposited in each array fluctuated synchronously (Fig. 2), with *Cx. restuans* depositing more eggs than *Cx. p. pipiens*. About as many eggs were deposited by *Cx. p. pipiens* ($p < 0.073$) and *Cx. restuans* ($p < 0.400$) before the insecticidal application as after, more or less in parallel with that in the non-treated sites.

Trial 2 was conducted in the same sites two weeks later using the same application rate, and continued for 18 days (Table 1). Insecticide was applied on the fourth evening. Oviposition frequency was relatively constant in both arrays of traps, with the exception of a depression during the second night after treatment (Fig. 3). Rain fell heavily throughout that night,

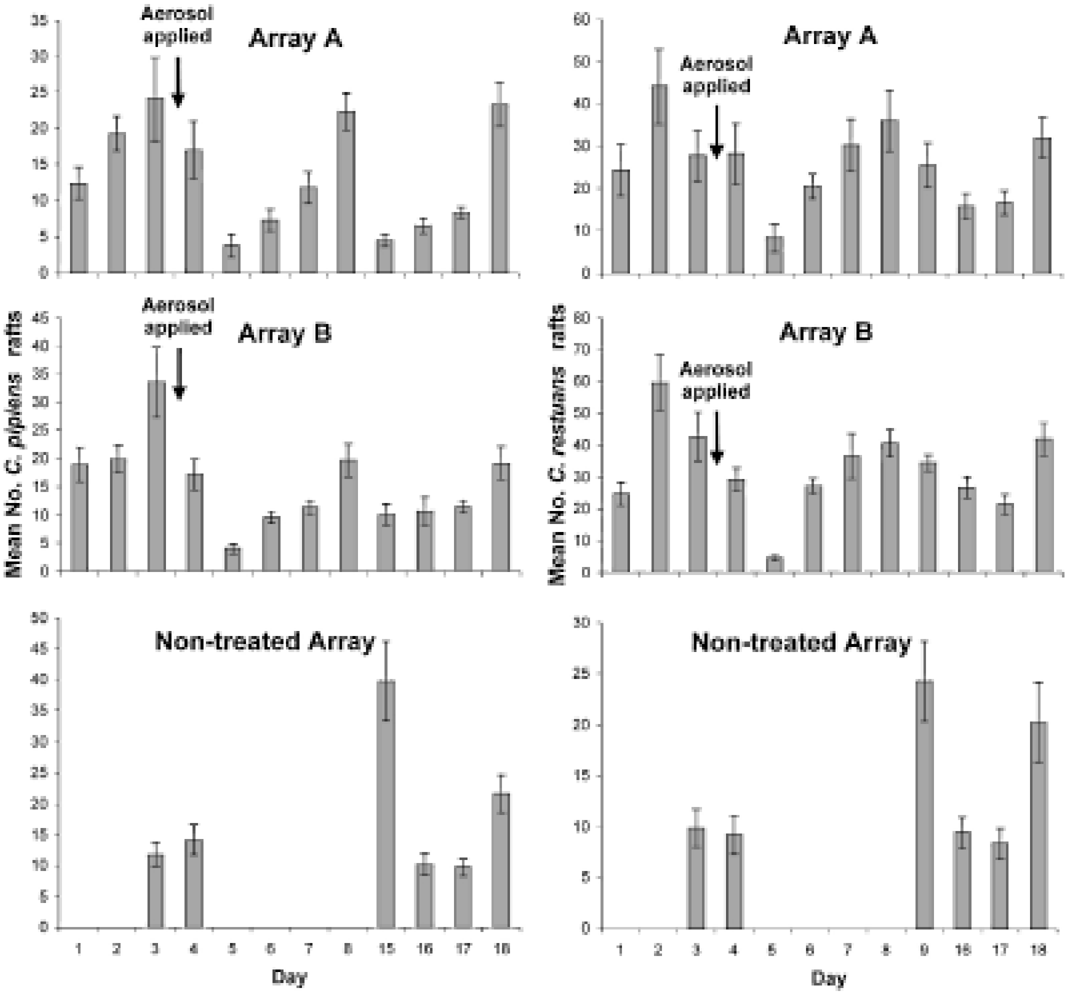

FIG. 3. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 2 following an aerosol application of resmethrin at 1.12 g/ha in Burlington, MA, during 30 July to 16 August 2002. Non-treatment observations were recorded at sites in Cambridge, MA, and Jamaica Plain, MA. No collections made on days 1, 2, and 5–15.

and observations in the non-treatment sites were interrupted during this critical period. Somewhat more *Cx. p. pipiens* eggs were deposited in treated sites before the intervention than after ($p < 0.001$). No such effect was observed on *Cx. restuans* oviposition activity ($p < 0.266$).

In Trial 3, conducted in Framingham during August of 2001, the application rate was increased to 1.96 g/ha (Table 1) with treatment on the seventh evening after observations commenced. Oviposition activity rose after the treatment in parallel with that in the non-treatment sites (Fig. 4). Only a minimal effect by the aerosol application on *Cx. p. pipiens* was evident ($p < 0.046$). A significant, yet transient reduction in oviposition by *Cx. restuans* was noted ($p < 0.003$).

Trial 4 was conducted in Tewksbury early in September of 2001. Insecticide was applied during the third evening of the trial at the maximum rate specified on the label, which was four times greater than in Trial 3 (Table 1). For comparison, a nearby non-treated site was monitored, and three arrays of traps were set within the treated neighborhoods. This aerosol application, too, appeared not to have affected the reproductive activity of *Cx. p. pipiens* ($p < 0.593$) or *Cx. restuans* ($p < 0.879$; Fig. 5).

Trial 5 was carried out in Framingham during mid July of 2002. Insecticide was again applied at the maximum allowable rate, nine days after the first observation. Somewhat fewer egg rafts of *Cx. p. pipiens* ($p < 0.013$) and of *Cx. restuans* ($p < 0.034$) were deposited after the treatment than at other times (Fig. 6).

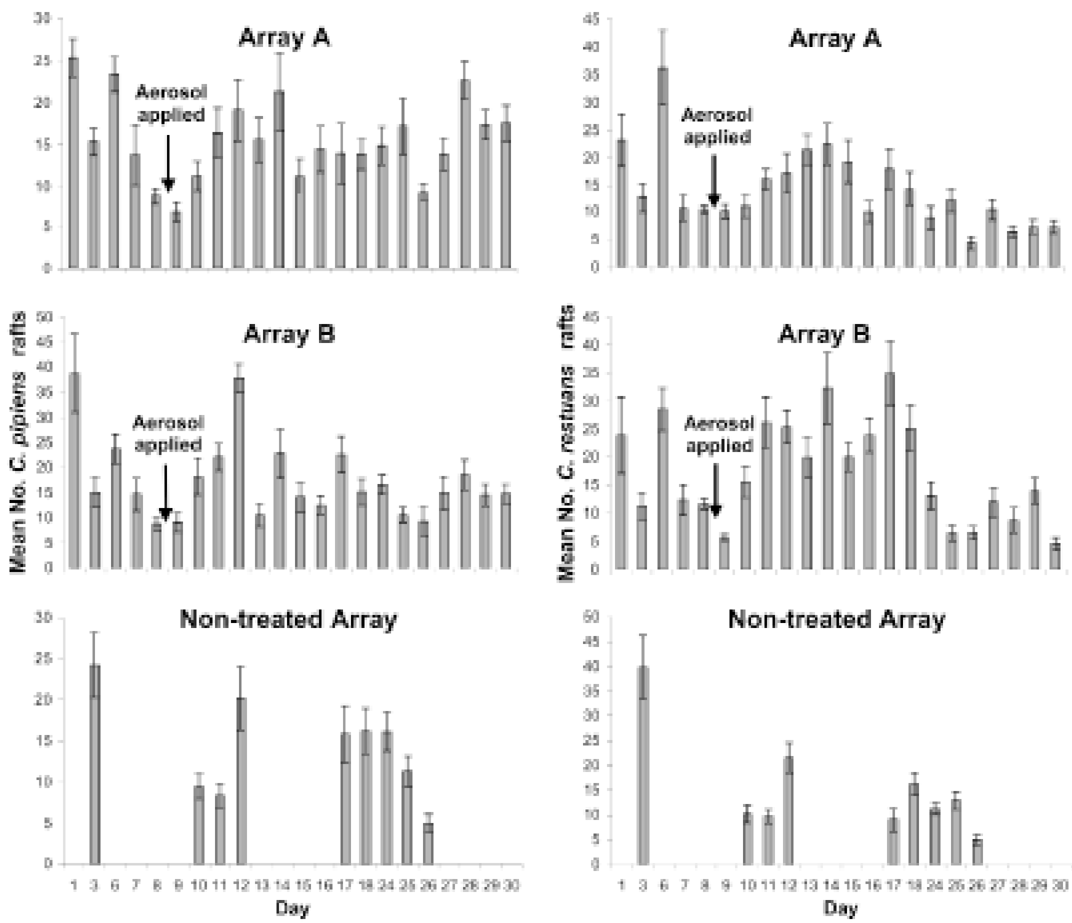

FIG. 4. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 3 following an aerosol application of resmethrin at 1.96 g/ha in Framingham, MA, during 5 August to 3 September 2002. Non-treatment observations were recorded at sites Cambridge, MA, and Jamaica Plain, Ma. No collections made on days 1, 2, 6–9, 13–16, and 27–30.

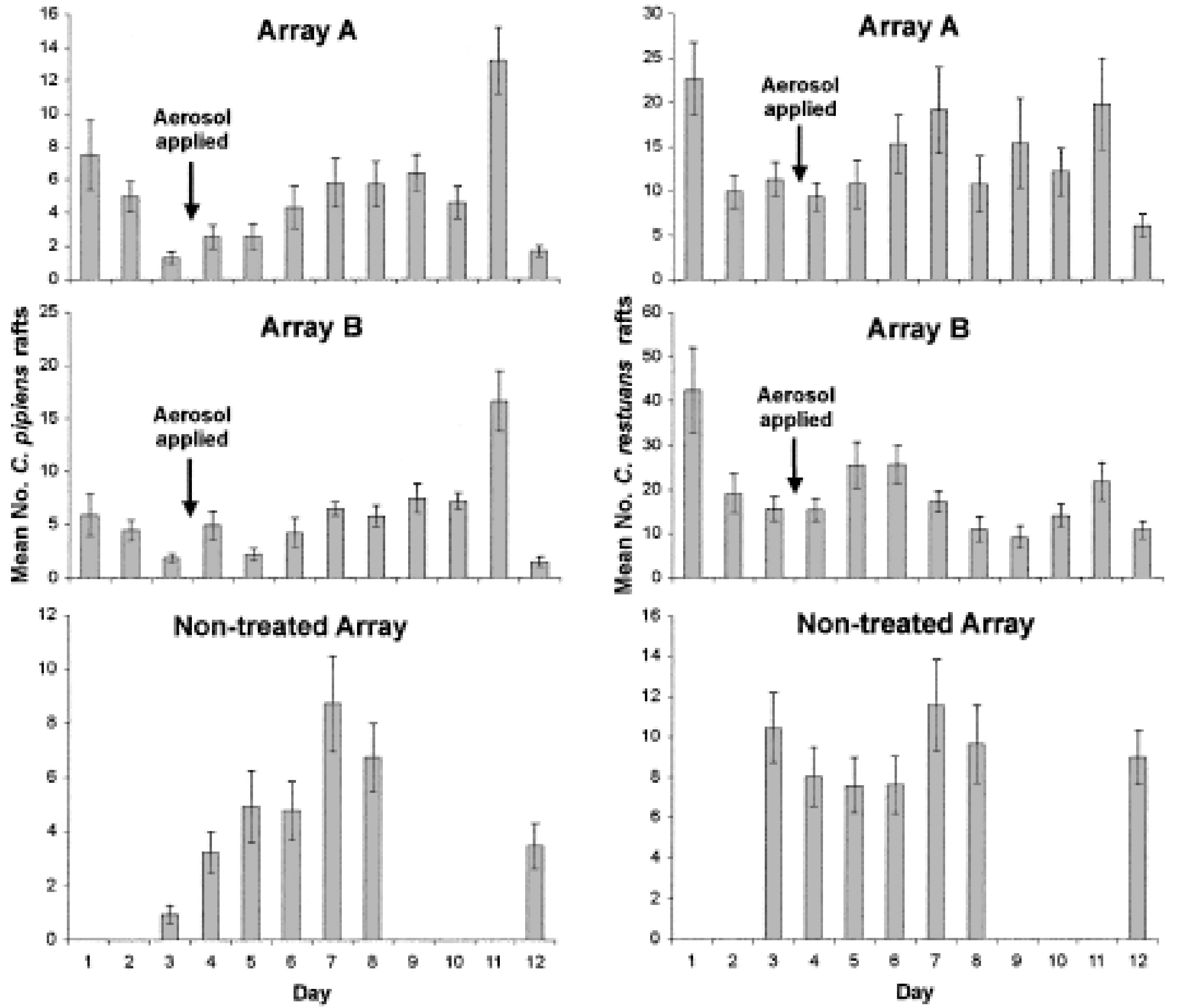

FIG. 5. Deposition of egg rafts in three arrays of ovitraps (designated A, B, and C) by *Cx. p. pipiens* and *Cx. restuans* in trial 4 following an aerosol application of resmethrin at 7.85 g/ha in Tewksbury, MA, during 4–13 September 2002. Non-treatment observations were recorded in Tewksburg, MA. No collections made on days 1 and 5–7.

In Trial 6, conducted in Framingham early in August of 2002, treatment was on the second evening, and was again at the maximum permitted rate. No reduction in the number of egg rafts of *Cx. p. pipiens* ($p < 0.295$) or of *Cx. restuans* ($p < 0.346$) was noted (Fig. 7).

DISCUSSION

The emergence of WNV in North America calls for interventions to protect the public health. Source reduction by sanitary and larvicidal measures receives much attention, but the preventive efficacy of such measures has not been demonstrated. Although many communities rely on adulticidal aerosols, this approach also awaits critical evaluation. Spraying from the road may reduce the density of "bridge vectors" that can carry infection from the avian cycle to human hosts; but our results suggest that this is not the case for the two kinds of mosquitoes that are considered important in enzootic transmission (Kilpatrick 2005). Moreover, a model of short-term reductions of adult mosquitoes on transmission suggests that the impact and sustainability required to reduce the force of transmission may be unattainable in practice (Newton and Reiter 1992).

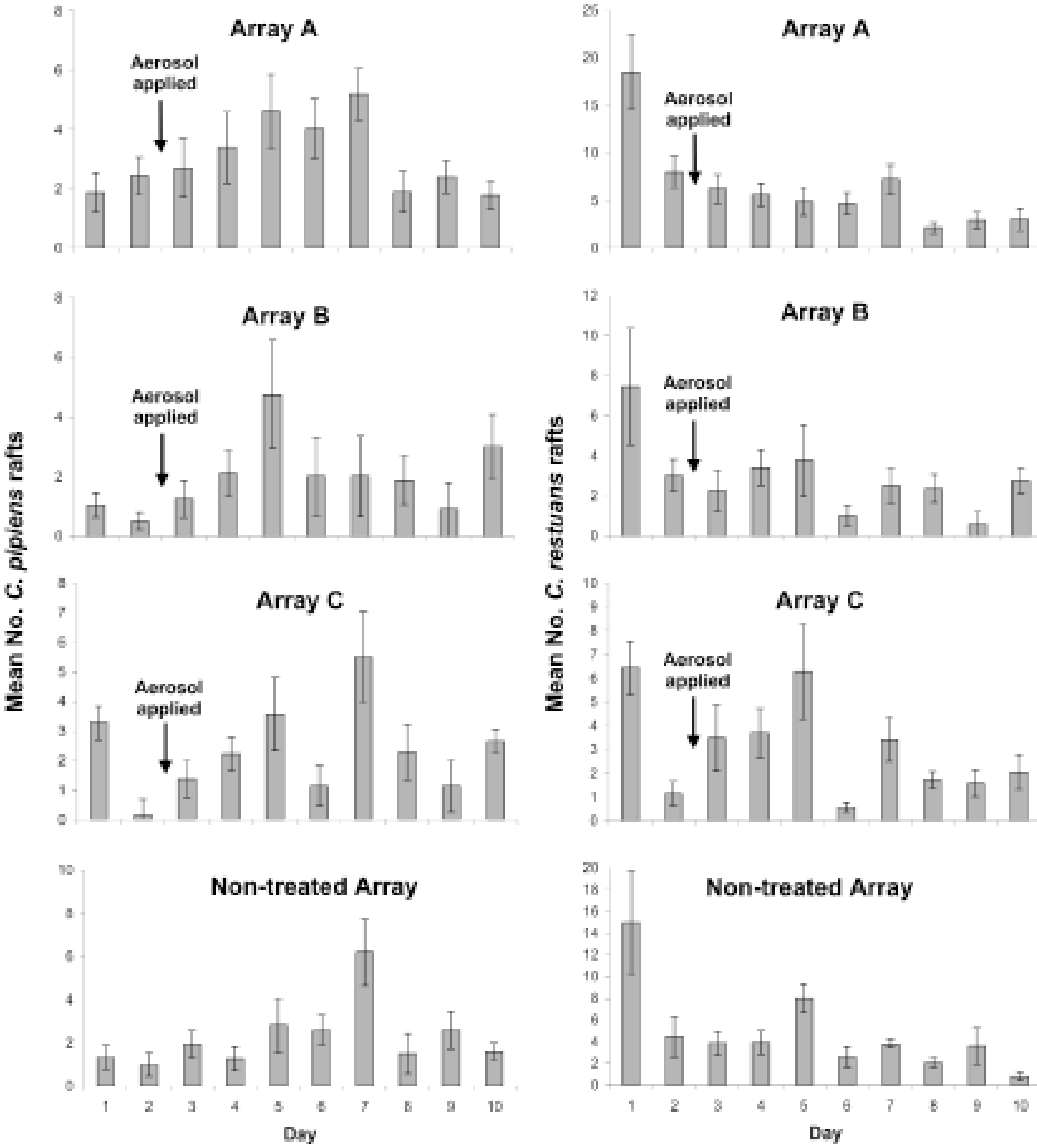

FIG. 6. Deposition of egg rafts in three arrays of ovitraps (designated A, B, and C) by *Cx. p. pipiens* and *Cx. restuans* in trial 5 following an aerosol application of resmethrin at 7.85 g/ha in Framingham, MA, during 8–25 July 2002. Non-treatment observations were recorded at sites in Cambridge, MA. No collections made on days 1, 2, 4–7, 12–14, and 16.

Factors that may affect the impact of an insecticidal aerosol applied from road vehicles include insecticidal insusceptibility, inadequate application rate, inadequate or excessive wind speed, inappropriate air temperature, lack of a suitable temperature inversion, physical obstacles to drift, an irregular network of roads, and the timing of treatments in relation to flight activity by the target species (Mount 1998).

In our study, droplet size and flow rate met EPA specifications. Insecticide was applied when ambient temperatures were 15–24°C. Wind conditions ranged from 1 to 8 km/h. Careful attention was paid to vehicle speed. Treatments were made under a variety of conditions. Application rates covered the full range specified by the U.S. EPA, and the target mosquitoes were fully susceptible to the-

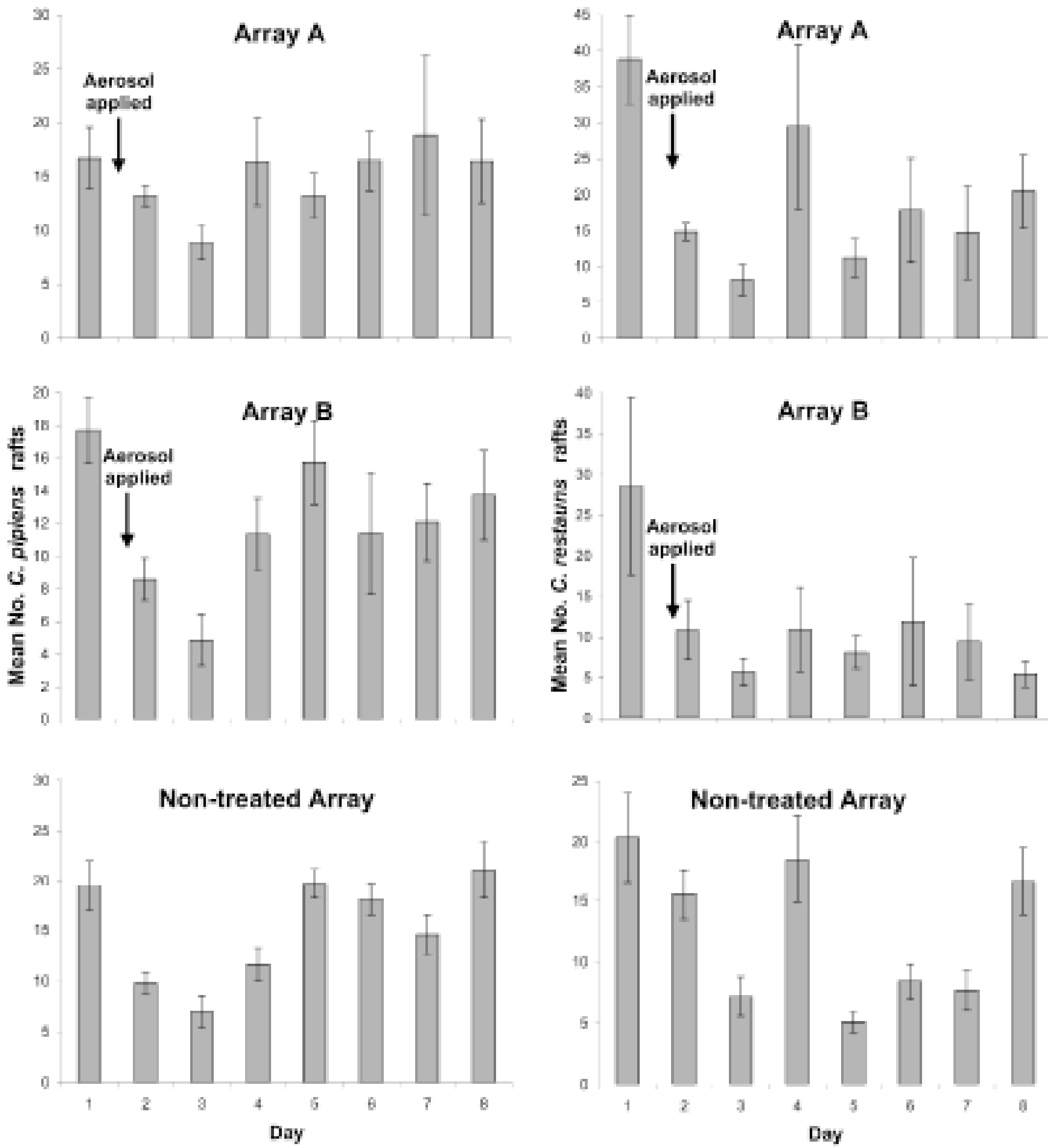

FIG. 7. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 6 following an aerosol application of resmethrin at 7.85 g/ha in Framingham, MA, during 5–12 August 2002. No collections made on days 4–7.

insecticide. In summary, we tested the method under a variety of circumstances, and made every attempt to optimize conditions, yet none of our treatments had any demonstrable impact.

The effective swath width for ULV treatments is about 90 m. The streets in our test areas were all less than 90 m apart and were selected to give maximum opportunity for optimum coverage. As in many parts of New England, however, a large proportion of nearby streets are spaced irregularly, thus limiting area-wide uniformity of aerosol coverage.

Culex p. pipiens, is said to fly up to 0.91 km in a single night (Schreiber et al. 1988). Spray evaluations may therefore be confounded by immigration if the monitoring devices are located too close to the periphery of the treatment area. Because the dimensions of residential areas in our studies are characteristic of the region, our trapping arrays were clustered near the center of the treatments, several were nearer than 0.9 km to the non-treated periphery.

Buildings, trees, dense brush and other obstacles affect drift (Mount et al. 1966) and create eddies that may result in variable rates of exposure of target mosquitoes (Reiter et al. 1990, Reiter and Gubler 1997). In addition, our studies (Lepore et al. 2004) confirmed other published evidence (Main et al. 1966, Anderson et al. 2004) that *Cx. p. pipiens* and *Cx. restuans* quest in the tree canopy, rather than near the ground; the temperature inversions required for optimum drift may reduce effective delivery of aerosol to such sites.

The timing of insecticidal applications is crucial because aerosols mainly are effective against mosquitoes when they are in flight. We observed that questing and oviposition by *Cx. p. pipiens* and *Cx. restuans* peaked about two hours after sunset, and declined steadily thereafter until sunrise (T.J. Lepore unpublished data). Our applications were targeted for the two hours of maximum activity, and may have missed a major portion of mosquitoes that were active later in the night.

In a previous study, one of us (P.R.) reported an 80% reduction of populations of *Cx. p. pipiens* and *Cx. restuans* in Memphis, TN. The insecticide, methods of evaluation and the target mosquito species were essentially the same. A relative lack of obstacles to drift may account for this difference: in Memphis, plots were two to five times larger than in New England, houses were separated by extensive lawns and open spaces, with little shrubbery or other ground vegetation. In addition, the regular grid plan of streets was optimal for even coverage. By contrast, in our treatment areas, houses were closer together, and vegetation was abundant, often dense.

We find that ULV applications of resmethrin had little or no impact on the *Culex* vectors of WNV, even at maximum permitted rates of application. A model simulating the major outcomes of such treatments indicates that they are unlikely to reduce the force of transmission of such an arbovirus (Newton and Reiter 1992). We conclude that insecticidal aerosols dispersed from the road may not effectively reduce the force of transmission of WNV.

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