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FIELD EVALUATION OF CDC GRAVID TRAP ATTRACTANTS TO PRIMARY WEST NILE VIRUS VECTORS, *CULEX* MOSQUITOES IN NEW YORK STATE

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ABSTRACT. A field study was conducted to evaluate two CDC gravid trap attractants available for the West Nile virus surveillance program in New York State (NYS). According to potential attractiveness, a common lawn sod in NYS, Kentucky bluegrass (*Poa pratensis*) infusion and a rabbit chow infusion were compared for attractiveness to primary West Nile virus vectors, *Culex* mosquitoes. Attractiveness of each infusion was measured by the number of adult mosquitoes caught in CDC gravid traps and the number of egg rafts laid in ovitraps. Both gravid trap and ovitrap studies demonstrated that lawn sod infusion with a 7-day incubation period had better attractiveness to *Culex restuans*/*Culex pipiens* than rabbit chow infusion with the same incubation period. Attractiveness of lawn sod infusions was increased as they became aged within a week's period. Lawn sod infusion also attracted more *Ochlerotatus japonicus,* a potentially important West Nile virus vector in New York.

KEY WORDS West Nile virus vector, *Culex* mosquitoes, CDC gravid trap, attractants, New York State

INTRODUCTION

In 1999, the first outbreak of West Nile virus in the Western Hemisphere occurred in New York City. The West Nile causes life-threatening disease not only in humans and horses but also in birds. Emergence of the disease in New York City has prompted an intensive and extensive surveillance program in New York State (NYS) (White et al. 2001). This large-scale West Nile surveillance program includes weekly mosquito collections by county health departments.

West Nile virus is maintained in natural cycles involving wild avian populations as amplification hosts and mosquitoes as vectors. *Culex* mosquitoes (*Culex restuans* Theobald, *Cx. salinarius* Coquillett, *Culex pipiens* L.) are primary vectors of West Nile in the northeastern USA (Andreadis et al. 2001, Nasci et al. 2001, White et al. 2001).

The gravid traps (Reiter 1983, 1986) baited with infusions have been demonstrated to collect *Culex* mosquitoes efficiently for both Saint Louis encephalitis virus and West Nile surveillance (Reiter et al. 1986, Andreadis et al. 2001). The gravid traps have proven to collect more Saint Louis encephalitis- (Reiter et al. 1986) and West Nile-infected mosquitoes (Andreadis et al. 2001) than CDC light traps. The gravid trap mosquito collections are predominantly *Culex* mosquitoes that have fed on host blood, while the light trap collections are host-seeking mosquitoes that have not necessarily fed on hosts before.

Mosquito collections in CDC gravid traps depend on the addition of oviposition substrate that attracts mosquitoes (Bentley and Day 1989). Several different organic infusions have been used as *Culex* mosquito attractants in gravid traps: grass-hay (Reiter 1983, Reiter et al. 1986, Meyer 1991), alfalfa (Reisen et al. 1999), bulrush (Reisen et al. 1999), and Kentucky bluegrass (Andreadis et al. 2001). However, little information is available as to superiority of an infusion to the others. Besides, as most studies were done in Tennessee (Reiter 1983, Reiter et al. 1986) and California (Meyer 1991, Reisen et al. 1999), same infusions may not be available in NYS.

An ovitrap study in Illinois (Lampan and Novak 1996) showed that a commercial rabbit chow or Kentucky bluegrass infusions were better *Culex* oviposition substrate than other infusions such as oak, maple, sod soil, and alfalfa. Both of these oviposition substrates are widely available in NYS and could be used as mosquito attractants in CDC gravid traps in NYS. However, ovitrap collections rely on contact chemical cues as well as olfactory and physical cues of oviposition substrates upon which only gravid trap collections are dependent. It would be appropriate to evaluate attractiveness of the potential attractants in gravid traps.

The study was conducted to provide information for gravid mosquito trapping in NYS West Nile surveillance. In this study, we evaluated potential *Culex* attractants in NYS: infusions of Kentucky bluegrass (*Poa pratensis*) lawn sod and a commercial rabbit chow in CDC gravid traps and ovitraps in 2001.

MATERIALS AND METHODS

Study sites and period

A CDC gravid trap and an ovitrap study were conducted at a landfill and sewage treatment plant site in Monticello, NY (41°38.587′N, 74°40.230′W,

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elevation 430.4 m) and an ovitrap study was conducted at a residential site in Montgomery, NY (41°31.336′N, 74°14.333′W, elevation 109.7 m). The landfill and sewage treatment in Monticello, NY, is approximately 20.3 hectares (50 acres) surrounded by marshes and forests in three sides and a residential area in west side. The studies were conducted from June 30 through September 27, 2001.

Infusion preparations for gravid and ovitraps

Rabbit chow and lawn sod infusion preparations followed previously published protocols (Lampan and Novak 1996, Lee and Rowley 2000). Both lawn sod and rabbit chow infusions were made at the same time in 20-liter white buckets by flooding either a 15- × 45-cm section Kentucky bluegrass lawn sod or 200 g of a commercial rabbit chow (BlueSeal®) with 8 liters of tap water. As the studies were intended to provide information helpful for NYS counties' weekly mosquito collections, attractiveness of infusions with incubation time of up to 7 days was tested in ovitraps. For gravid trap attractiveness tests, 4-day-old infusions made at the same time and incubated in 24 ± 1°C were used.

Trap placement and operation

Two different sites were chosen at the landfill and the sewage treatment plant, Monticello, NY. A pair of CDC gravid traps (Model 1712; John W. Hock Co., Gainesville, FL) with green oviposition pans (34 × 24.5 × 17 cm) was placed in each site. The commercial CDC gravid trap follows original descriptions of the trap developed by Reiter (1983) except size of the pan. Briefly, the CDC gravid traps consist of a collecting bag, an 8-cm-diameter inlet tube with a motor, two cross bars, and a pan. The mosquitoes are attracted to an oviposition substrate in the pan. The trap uses 4 D-size batteries to create an upward current of air within the confines of the pan. The mosquitoes attracted to the substrate are blown into the collecting bag during their oviposition behavior.

In our study, 4 CDC gravid traps were operated 13 nights (1 night/wk) between June 30 and September 27. Each pair of gravid traps was baited with 3.5 liters of an undiluted 4-day-old infusion made at the same time, one with lawn sod infusion and the other with rabbit chow infusion. The traps in each pair were placed 5 m apart in protected areas of both locations. The trap placements in each site were randomized each week. The traps were started at least 2 h before sunset and collected the following morning. Captured mosquitoes were brought to the laboratory for counting and sorting.

Ovitrap studies were conducted at two different sites: one at the sewage treatment plant in Monticello, NY, and the other at a residential area of Montgomery, NY. Two ovitraps, 1 each infused with lawn sod and rabbit chow, were placed at each site. Two ovitraps at each site were placed 10 m apart under the eaves of buildings and the trap placements each week were determined by randomization. New infusions were made in the field every 7 days to replace the old ones. To measure effectiveness of each infusion, numbers of egg rafts were counted and removed daily from the 2nd day through the 7th day of trapping throughout the study period. Due to difficulty in distinguishing *Cx. restuans* from *Cx. pipiens* of gravid trap collections and to estimate *Culex* species attracted to each infusion, egg rafts were collected for species identification on the 4th day of trapping each week at the time when adult mosquitoes were collected by CDC gravid traps. A total of 24 egg rafts were randomly chosen and brought from each ovitrap to the laboratory in 24-well tissue culture plates if there were more than 24 egg rafts in an ovitrap. In the laboratory, individual egg rafts were transferred to 0.47-liter ice cream cartons and reared with tropical fish food, Tetramine®, to the 4th instar. Larvae from each egg raft were identified to species (Means 1987).

Table 1. Mosquito collections in CDC gravid traps baited with 4-day-old Kentucky bluegrass (*Poa pratensis* L.) lawn sod infusion and with the same age of rabbit chow infusion. The gravid traps were operated in Monticello, NY, 13 nights between June 30 and September 27, 2001.

Species	Lawn sod infusion	Rabbit chow infusion
Culex restuans/pipiens	447	25
Ochlerotatus japonicus	104	54
Ochlerotatus triseriatus	23	3
Others	8	7
Total	582	89

Data analysis

Analysis of variance was used for statistical analysis (SAS Institute Inc. 1988) to compare attractancy of infusions in gravid and ovitraps and to determine incubation time effects of oviposition substrates on the attractiveness to *Culex* mosquitoes.

RESULTS

Comparison of attractancy of infusions in gravid traps

A total of 582 mosquitoes were collected in gravid traps with lawn sod infusion and 89 collected with rabbit chow infusion (Table 1). In lawn sod infusion-baited traps, 447 (76.8%) *Cu. restuans/pipiens,* 104 (17.9%) *Ochlerotatus japonicus* (Theobald), 23 (4%) *Ochlerotatus triseriatus* (Say), and 8 (1.3%) mosquitoes in other species were cap-

Table 2. Number of adult *Culex* mosquitoes/trap/night collected in CDC gravid trap baited with 4-day-old Kentucky bluegrass (*Poa pratensis* L.) lawn sod infusion and with the same age of rabbit chow infusion. The gravid traps were operated in Monticello, NY, 13 nights between June 30 and September 27, 2001.

Week (collection date)	Lawn sod infusion	Rabbit chow infusion
1 (7/03/01)	22.5	0.0
2 (7/10/01)	25.5	0.0
3 (7/17/01)	24.0	3.0
4 (7/24/01)	9.5	2.5
5 (7/31/01)	14.5	0.0
6 (8/9/01)[1]	55.0	2.5
7 (8/14/01)	27.0	0.0
8 (8/21/01)	23.5	2.0
9 (8/28/01)	1.5	0.0
10 (9/04/01)	14.0	0.5
11 (9/11/01)	5.0	0.0
12 (9/18/01)	0.5	0.0
13 (9/25/01)	1.0	2.0
Total	223.5	12.5
Average	17.19[2]	0.96

[1] Due to unfavorable weather for gravid mosquito trapping on 8/6/01, trapping was conducted with 6-day-old infusions on 8/8/01.
[2] $P < 0.001$ (F = 23.22, df = 1,50).

tured. The mosquitoes in other species were 2 *Coquillettidia purturbans* (Walker), 2 *Anopheles punctipennis* (Say), 1 *Ochlerotatus canadensis* (Theobald), 1 *Uranotaenia sapphirina* (Osten-Sacken), and 2 *Aedes/Ochlerotatus* spp.

Whereas, in gravid traps with rabbit chow infusion *Oc. japonicus* and *Cx. restuans/pipiens* constituted 54 (60.7%) and 25 (28.1%) of the collections, respectively. The rest of the collections were 3 (3.4%) *Oc. triseriatus*, 2 (2.2%) *Oc. canadensis*, and 5 (5.6%) *Aedes/Ochlerotatus* spp.

An average of 17.19 adult *Culex* mosquitoes per trap night were collected in a lawn sod-baited trap and 0.96 in a rabbit chow-baited trap ($P < 0.001$, F = 23.22, df = 1,50) (Table 2). The highest *Culex* collection was 55 per trap night in a lawn sod-baited trap (6th wk of the study, mid-August) and 3 in a rabbit chow-baited trap (3rd wk of the study, mid-July). The lawn sod infusion-baited trap caught more *Culex* mosquitoes than the rabbit chow infusion-baited trap throughout the study period.

Comparison of attractiveness of infusions in ovitraps

Similar results to those in the gravid trap study were observed in the ovitrap studies. In the Monticello site, more *Culex* egg rafts were deposited on lawn sod infusion, 43.6 per night, than on rabbit chow infusion, 15.0 per night ($P < 0.001$, F = 34.06, df = 1,154). Daily mean number of egg rafts laid on lawn sod infusion was higher (9.13) than that on rabbit chow infusion (3.40) in the Montgomery site ($P < 0.001$, F = 28.89, df = 1,154) (Table 3). Unlike the results of the gravid trap study, daily average number of egg rafts on lawn sod infusion was not always higher than that on rabbit chow infusion. In the 9th wk of the study (August 25–30), similar numbers of egg rafts were laid on grass infusion and rabbit chow infusion in both study sites. In the 10th wk (September 1–9), rabbit chow infusion had more *Culex* egg rafts (11.5) than lawn sod infusion (10.5) in the Monticello site (Table 3). In the weeks when rabbit chow infusion had more or equal numbers of egg rafts,

Table 3. Daily mean number of egg rafts (estimated % *Culex restuans*/% *Culex pipiens*) collected in ovitraps infused with Kentucky bluegrass (*Poa pratensis* L.) lawn sod and rabbit chow. This study was conducted in Monticello and Montgomery, NY, from June 30 through September 27 for 13 weeks in 2001.

	Monticello, NY		Montgomery, NY	
Week	Lawn sod infusion	Rabbit chow infusion	Lawn sod infusion	Rabbit chow infusion
1 (6/30–7/05)	108.3 (100/0)	7.8 (100/0)	8.5 (89/11)	0.0
2 (7/07–7/12)	75.2 (100/0)	5.2 (100/0)	9.2 (75/25)	0.5 (100/0)
3 (7/14–7/19)	64.8 (100/0)	16.2 (100/0)	11.8 (17/83)	1.0 (100/0)
4 (7/21–7/26)	74.2 (100/0)	21.5 (100/0)	17.7 (50/50)	5.7 (71/29)
5 (7/28–8/02)	60.2 (100/0)	34.0 (100/0)	6.5 (55/45)	3.5 (75/25)
6 (8/04–8/09)	38.0 (100/0)	13.8 (100/0)	20.0 (75/25)	5.3 (63/37)
7 (8/11–8/16)	58.0 (100/0)	39.2 (100/0)	7.7 (50/50)	4.8 (100/0)
8 (8/18–8/23)	33.3 (95/0)[1]	17.8 (100/0)	9.2 (64/36)	4.8 (100/0)
9 (8/25–8/30)	20.0 (100/0)	19.5 (100/0)	5.7 (100/0)	5.7 (100/0)
10 (9/01–9/06)	10.5 (94/6)	11.5 (100/0)	6.0 (50/50)	4.3 (25/75)
11 (9/08–9/13)	16.0 (56/44)	7.0 (20/80)	8.0 (60/40)	4.0 (50/50)
12 (9/15–9/20)	6.8 (80/20)	0.7 (100/0)	5.3 (67/33)	2.5 (100/0)
13 (9/22–9/27)	1.5 (83/17)	0.5 (100/0)	2.8 (33/67)	1.0 (0/100)
Total	566.8 (98.1/1.6)	194.7 (97.1/2.9)	118.4 (60.5/39.5)	43.1 (75.1/24.9)
Average	43.60[2]	14.98	9.13[3]	3.40

[1] *Culex territans* constituted 5% of egg rafts.
[2] $P < 0.001$ (F = 34.06, df = 1,154).
[3] $P < 0.001$ (F = 28.89, df = 1,154).

Table 4. Effects of incubation time (2–7 days) of oviposition substrates made of Kentucky bluegrass (*Poa pratensis* L.) lawn sod and rabbit chow infusion on mean number of *Culex* egg rafts in Monticello, NY, in 2001.

	Incubation time (day)		
Weeks	2–3	4–5	6–7
Lawn sod infusion[1]			
1–4 (6/30–7/26)	55.5	94.5	91.8
5–8 (7/28–8/23)	22.3	42.0	77.9
9–12 (8/25–9/20)	17.3	11.0	11.8
Average	31.70	49.17	60.5
Rabbit chow infusion[2]			
1–4 (6/30–7/26)	5.8	13.0	19.3
5–8 (7/28–8/23)	12.9	27.5	38.3
9–12 (8/25–9/20)	12.0	11.4	5.6
Average	10.23	17.3	21.07

[1] ($P < 0.05$, df = 2,70, F = 3.46).
[2] ($P = 0.09$, df = 2,70, F = 2.49).

egg rafts were predominantly laid by *Cx. restuans* in both study sites. In the Monticello site, *Cx. restuans* constituted 98.1% of egg rafts in lawn sod infusion and 97.1% in rabbit chow infusion during the study. The overall percentage of *Cx. restuans* in the Montgomery site was 60.5 in lawn sod infusion and 75.1 in rabbit chow infusion (Table 3). In Monticello, *Culex territans* Walker laid egg rafts on lawn sod infusion in the 8th wk (August 18–23) of the study. *Culex salinarius* egg rafts were not detected in either study site.

Incubation time effects of infusions on attractiveness

The number of egg rafts laid on lawn sod infusion increased in a range (2–7 days) tested as infusion became aged ($P < 0.05$, F = 3.46, df = 2,70) (Table 4). In rabbit chow infusion, older infusion (6–7 days old) tended to have more *Culex* egg rafts than 2–3- or 4–5-day-old infusion (P = 0.09, F = 2.49, df = 2,70) (Table 4). The differences between the mean number of egg rafts laid on 2–3-day-old infusion and that on 4–5-day-old infusion were 17.47 in lawn sod and 7.07 in rabbit chow infusion, while the differences between 4–5- and 6–7-day-old infusion were 11.33 in lawn sod infusion and 3.77 in rabbit chow infusion. However, in 9–12 wk of the study, both infusions showed that 4–7-day-old infusions had fewer egg rafts than 2–3-day-old ones.

DISCUSSION

Gravid traps and ovitraps use infusions that elicit oviposition behavior of mosquitoes. Mosquitoes use chemical and physical cues of oviposition substrates to select the sites for their ovipositions (Bentley and Day 1989). Our gravid trap study showed that a 4-day-old common lawn sod, Kentucky bluegrass, infusion was more attractive to *Cx. restuans/pipiens* than a rabbit chow infusion with the same age during the entire study period. Lawn sod infusion seems to produce more attractive volatile chemicals (attractants) for *Culex* mosquitoes than rabbit chow infusion. In addition to attractants of the lawn sod infusion, the color might enhance its attractiveness to *Cx. restuans/pipiens.* Beehler et al. (1993) demonstrated that *Cx. quinquefasciatus* Say preferred to oviposit on darker substrates. In our study, lawn sod infusion was always dark and transparent; however, rabbit chow infusion was yellow and opaque and then changed its color to darkish yellow over the 1-wk incubation time. As Bentley and Day (1989) noted that both chemical and physical natures of oviposition substrates change over time, it would be of interest to see how attractiveness of both infusions in gravid traps are changing beyond the incubation time we tested.

Lawn sod infusion also attracted more *Oc. japonicus* than rabbit chow infusion (Table 1). *Ochlerotatus japonicus* is a newly introduced mosquito in the USA (Peyton et al. 1999) and is a potentially important West Nile virus vector (Turell et al. 2001, White et al. 2001). Oviposition preference of *Oc. japonicus* is little known in the USA. Our results suggest that lawn sod infusion could be used as a potential oviposition attractant for *Oc. japonicus.*

Results of the ovitrap study in 2 sites supported that of the gravid trap study (Table 3). Lawn sod infusion had more egg rafts than rabbit chow infusion. However, both infusions had similar numbers of egg rafts in 1 or 2 wk of 13-wk studies (Table 3). Lampman and Novak (1996) indicated that more egg rafts on rabbit chow infusion was a reflection of abundance of *Cx. pipiens. Culex pipiens* was reported to have slightly higher preference to oviposit on rabbit chow infusion than grass or sod infusion (Lampman and Novak 1996). Unlike Lampman and Novak (1996), our study in both sites showed that the estimated *Culex* species composition that was attracted to rabbit chow and lawn sod infusions were 100% *Cx. restuans* when both infusions had similar numbers of egg rafts (August 25–30) (Table 3). In our study, increased numbers of *Culex* egg rafts on rabbit chow infusion compared with those on lawn sod infusion do not seem to be attributable to abundance of *Cx. pipiens* but more likely are due to a change in the chemical and/or physical nature of the infusion. The chemical and/or physical nature of an infusion is changed over time by environmental factors, especially temperature (Bentley and Day 1989). It is likely that either lawn sod or rabbit chow infusion did not provide *Culex* mosquitoes with distinctive advantages on which to oviposit. It is not known whether both infusions produced a similar degree of attractiveness or some environmental factors hampered mosquitoes finding a better oviposition substrate. However, a recent study (McCall and Eaton 2001) showed that gravid *Cx. quinquefasciatus* was attracted to the odors that they had experienced either in development or pupal eclosion, suggesting a higher portion of gravid *Culex* mosquitoes of our study sites in 9th and 10th wk (August 25–September 6) might be previously exposed to similar attractive cues produced by rabbit chow infusion while they were in the development stage.

Selection of an oviposition site is a species-dependent process (Kramer and Mulla 1979, Bentley and Day 1989), which indicates that the effect of various infusions may be species specific; an infusion that attracts one species may be inactive or repellent to others. In NYS, *Cx. restuans, Cx. pipiens,* and *Cx. salinarius* are primary West Nile vectors (White et al. 2001). All three species of primary West Nile vectors in New York readily oviposited on Kentucky bluegrass in other areas of the states (Corsaro and Munstermann 1984, Lee and Rolwey 2000), although *Cx. salinarius* was not collected in our studies. In addition to our gravid and ovitrap studies, no adult *Cx. salinarius* was collected in CDC light traps at the same sites in 2001 (Lee and Kokas, unpublished data), indicating that *Cx. salinarius* may not be breeding or be a rare species of mosquitoes at the study sites. Means (1987) and Nasci et al. (2001) reported that *Cx. salinarius* was mainly collected in New York City, Lower Hudson Valley, and Long Island, NY.

Incubation time to make effective attractants for CDC gravid traps would be an important factor to consider in an intensive and extensive surveillance program like the one in NYS. A study in California showed that 7-day-old Bermuda grass infusion was more effective to attract *Cx. tarsalis* than infusions of any other ages, whereas *Cx. quinquefasciatus* showed oviposition preferences to 2–4-wk-old infusion (Isoe et al. 1995). Other studies demonstrated that 10-day-old infusion with Purina Laboratory Chow repelled gravid *Cx. tarsalis* and *Cx. quinquefasciatus* (Kramer and Mulla 1979, Hwang et al. 1980). As an infusion changes over time (Bentley and Day 1989) and it is hard to standardize a process to make effective infusion, Millar et al. (1992) isolated several biologically active semiochemcials from Bermuda grass infusion that had been known to attract and stimulate *Cx. quinquefasciatus* to oviposition. A field test with the semiochemicals identified by Millar et al. (1992) found that 3-methylindole was mainly responsible for the attraction of *Cx. quinquefasciatus* (Beehler et al. 1994). Blackwell et al. (1993) reported that concentration ranges of 3-methylindole for the attraction and the repulsion of *Cx. quinquefasciatus* oviposition. However, no information is available as to either optimum incubation times or biologically active semiochemicals of the infusions we tested for *Cx. pipiens* and/or *Cx. restuans* attraction. Our study showed that Lawn sod infusion had about 3 times more egg rafts than rabbit chow infusion of equal incubation time (7 days) tested (Table 4). Over a week's time, more *Cx. restuans/pipiens* oviposited progressively on older lawn sod infusion. Unlike overall trends, mosquitoes laid egg rafts on aged infusions (4–7 days old) less than on fresh infusions in 9–12 wk of the study (Table 4). The unexpected result in the specific period of the study might be due partly to big fluctuations of daily oviposition activities with low population density of mosquitoes as temperatures became lower. In our study, we tested attractivenss of infusions with up to 7 days of incubation; it would be of interest to see how long the infusions need to be incubated to produce the best attractivenss to the primary West Nile vectors in NYS.

In summary, Kentucky bluegrass lawn sod is widely available in NYS and its infusion, within a range of incubation period (7 days) we tested, provides better attractiveness to primary West Nile vectors, *Cx. restuans/pipiens* than rabbit chow infusion with the same incubation period. Within a 7-day incubation period, attractiveness of lawn sod infusion increases as it becomes older. Lawn sod infusion appeared to have more attractiveness to *Oc. japonicus,* a potential West Nile virus vector in New York than rabbit chow infusion.

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