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OVIPOSITION PREFERENCES OF *CULEX PIPIENS* AND *CULEX RESTUANS* FOR INFUSION-BAITED TRAPS

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ABSTRACT. The number of egg rafts oviposited by *Culex pipiens* and *Culex restuans* in infusion-baited ovitraps varied with the type of infusion substrate, infusion age, method of infusion preparation, and calendar date. In one or more tests, more *Culex* egg rafts were collected from water infused with Kentucky bluegrass sod, mixed grass clippings, straw, or a commercial rabbit chow than from a water control. Infusions prepared with oak leaves, maple leaves, and sod soil were ineffective as *Culex* oviposition attractants; however, alfalfa infusions ranged from marginally effective to unattractive. Rabbit chow infusions required one or more reinfusions before becoming active oviposition lures when prepared in unconditioned (unused) ovitraps. Infusions prepared in conditioned ovitraps (those that had previously contained the same type of infusion) had shorter delay periods. Effective oviposition lures for *Culex* species included continuous infusions, such as sod and sod grass, and those in which the substrate was infused in the water for only a brief period (24-48 h), such as infusions of rabbit chow and mixed grass clippings. The percentage of egg rafts from *Cx. restuans* was usually greater in sod and grass infusions than in rabbit chow infusions, whereas *Cx. pipiens* showed a slight preference for rabbit chow infusions over sod and grass infusions. The temporal shift in relative oviposition activity of the 2 *Culex* species (cross-over) varied from year to year and between sites in one year.

INTRODUCTION

Oviposition site selection by a mosquito consists of a series of search, examination, and acceptance behaviors that are initiated, maintained, and modified by the interaction of positive and negative inputs from the environment and the internal state of the mosquito (Klowden 1990). The primary exogenous factors that govern the discrimination between potential oviposition sites are the visual, tactile, and chemosensory characteristics of the resource (Bentley and Day 1989). Species that prefer to oviposit in eutrophic areas, like *Culex pipiens* Linn., *Culex quinquefasciatus* Say, and *Culex restuans* Theobald, are probably selecting these sites, at least in part, because of the production of volatile microbial by-products (Ikeshoji et al. 1975, Benzon and Apperson 1988, Millar et al. 1992). For example, *Cx. pipiens* and *Cx. restuans* are attracted to aqueous infusions of cow manure, laboratory chow, various grasses, and hay; *Cx. quinquefasciatus,* to infusions of hay, sod, tree leaves, wood creosote, and horse, steer, and chicken manures; *Culex tarsalis* Coquillett, to infusions of grass and sod; and *Culex salinarius* Coquillett and *Cx. pipiens pallens* Coquillett, to infusions of ricestraw (Kramer and Mulla 1979, Prasad and Daniel 1988, Bentley and Day 1989, Brust 1990, Reisen and Meyer 1990, Steinly and Novak 1990, and references therein). Ovitraps and gravid traps, baited with these infusions, provide relatively rapid, inexpensive, and selective tools for monitoring oviposition activity (Reiter 1986). Infusion-baited traps can also be relatively species- specific depending on the type of infusion, the physical characteristics of the trap, and trap placement (Bentley and Day 1989).

In Illinois, *Cx. pipiens* and *Cx. restuans* are believed to be the major vectors of St. Louis encephalitis (SLE) virus (Mitchell et al. 1980). The seasonal shift in relative abundance of these 2 species (crossover) is considered an important event in the surveillance of SLE (Steinly and Novak 1990) because the early season *Cx. restuans* probably amplifies the virus among the local bird populations and the later season *Cx. pipiens* is the putative vector of the SLE virus to birds and other mammals, including man. Infusion-baited ovitraps are important tools for studying the population dynamics of these *Culex* species because adults are difficult to separate visually and may not be equally attracted to other types of traps (Madder et al. 1980). Unfortunately, temporal changes in infusion quality can complicate the interpretation of oviposition trends (Brust 1990).

The objectives of this study were 1) to compare the oviposition rates of local *Culex* species in ovitraps baited with several types of infusion materials, 2) to demonstrate the potential influence of infusion age, preparation methods, and ovitrap condition (new or used) on the attraction of ovipositing females, and 3) to compare the daily percentage of *Cx. pipiens* and *Cx. restuans,* in our study site with an independent study conducted in a nearby area. One of our main goals was to identify oviposition lures that exhibit relatively long periods of activity yet require a minimum amount of additives, handling, and aging before becoming active.

MATERIALS AND METHODS

Rationale for infusions and preparation methods: Rabbit chow infusion has been used in Illinois for several years to monitor the oviposition activity of *Culex* species (Steinly and Novak 1990, 1993; Steinly et al. 1993) and was chosen as the initial standard with which other organic substrates would be compared in this study. The first group of treatments in our initial study in 1991 (rabbit chow, mixed grass clippings, alfalfa, oak leaves, and maple leaves) were chosen based on their availability and similarity to organic matter used to prepare oviposition lures for *Aedes* and *Culex* species (Reiter 1986, Prasad and Daniel 1988, Bentley and Day 1989, Reisen and Meyer 1990, Steinly and Novak 1990). All of the infusions were prepared by steeping a relatively large amount of substrate (150 g) in 6 liters of water for a short period of time (24–48 h), a modification of the standard rabbit chow protocol used by Steinly and Novak (1990). In order to compare these infusions when the relative oviposition activity of *Cx. pipiens* and *Cx. restuans* differed, 2 complete sets of infusions were tested twice in adjacent woodlots in 1991. Between the first and 2nd tests in both woodlots, all of the infusions were discarded. The ovitraps were rinsed with water, and new infusions of the same material were prepared in the ovitraps, as described above.

In 1992, a continuous lawn sod infusion, one in which the sod substrate remained submerged in the ovitrap throughout a test, was included in our study. Sod infusions, prepared in this manner, have been used as oviposition lures for *Cx. pipiens, Cx. restuans* and *Cx. quinquefasciatus* (Maw and Bracken 1971, Madder et al. 1980, Brust 1990, Millar et al. 1992). The other treatments in this 2nd series of infusions included mixed grass clippings, rabbit chow, alfalfa, and wheat straw. These substrates were used to prepare infusions by the rabbit chow protocol as in 1991. The infusions were not discarded between tests in 1992, as had been done in 1991, for the following reasons: 1) we were attempting to follow the rabbit chow protocol in our laboratory in which infusions are maintained with periodic reinfusion for 5–6 months, and 2) we wanted to determine whether reinfusing aged media affected the relative activity of the infusions. Although the infusions were not discarded between tests, fresh organic matter was added to each aged infusion media 24 h before the onset of a new test, except for the sod infusion. After the first test, the used sod material was replaced with a section of fresh sod. This process was repeated with this group of infusions for each subsequent test.

In 1991, infusions were prepared in conditioned (used) ovitraps, that is, the ovitraps had previously contained the same type of infusion for about 3–4 wk in a pilot study conducted at a different site. In the first test of 1992, the infusions were prepared in unconditioned (new) ovitraps. In order to determine whether differences in *Culex* oviposition between the 2 series of tests were due to ovitrap condition or some other source of variation, the next set of infusions included aged rabbit chow and sod in conditioned ovitraps and fresh infusions of rabbit chow and sod in unconditioned ovitraps. This allowed us to verify the effect of ovitrap condition and, potentially, to estimate how long it would take fresh infusions in unconditioned ovitraps to reach the level of activity of the aged infusions in conditioned ovitraps. Two additional treatments in this group included fresh, continuous infusions of sod grass and sod soil in unconditioned ovitraps. These treatments were included in order to show whether either or both of the sod components could make an effective oviposition lure. The sod in the fresh and aged treatments, as well as the sod grass and sod soil, was not replaced between tests. We wanted to estimate how long the sod and grass infusions remained active with the same substrate and how their relative activity changed with infusion age. The rabbit chow infusions were reinfused at the onset of each test because it had been determined previously that they were active for about 2–3 wk without reinfusion (Steinly and Novak 1990).

The final series of tests in 1992 compared the most effective infusions from the preceding tests in order to document their relative activity. The infusions were prepared in conditioned ovitraps and were reinfused with fresh organic substrate at the onset of each subsequent test.

A detailed description of the ovitraps, substrates, and preparation methods for each group of treatments is provided below.

Preference tests and infusion preparation: All ovitraps were white, 19-liter plastic buckets with 6, 7.5-cm-diam holes evenly spaced around the bucket about 15 cm from the top (Steinly and Novak 1990). The ovitraps were classified as either conditioned (previously containing the same type of infusion) or unconditioned (new and unused). Egg rafts were collected daily from infusion-baited ovitraps in 2 woodlots (about 0.4 ha each) on the South Farms at the University of Illinois in 1991 and 1992. The predominant tree species in these woodlots were silver maple (*Acer saccharinum* Linn.), sycamore (*Plantanus* *occidentalis* Linn.), and various oaks (a mixture of *Quercus* species). Infusion treatments were placed either along the perimeter or 10 m inside the woodlots. Ovitraps in each test were rotated daily to eliminate positional effects. Collection of egg rafts began the day after infusions were started. Larvae from individual rafts were reared in separate Petri dishes at room temperature until identified to species at the 3rd or 4th instar.

The daily number of egg rafts oviposited by *Cx. pipiens* and *Cx. restuans* was recorded for 4 separate groups of infusions. The first group of treatments included 1) roadside grass, a mixture of dried *Bromus, Festuca,* and *Poa* species collected from the edge of a rural road, 2) prepared rabbit chow (Rabbit Chow Brand Feed, Purina Mills Co., St. Louis, MO), 3) alfalfa, *Medicago sativa* Linn., from a single bale, 4) locally collected pin oak leaves, *Quercus palustris* Muench, 5) a mixture of silver and sugar maple leaves, *A. saccharinum* and *Acer saccharum* Marshall, and 6) tap water. Each substrate (150 g) was wrapped in cheesecloth and allowed to infuse for 48 h in 6 liters of water before removal. The tap water treatment (control) had cheesecloth added to the water for 48 h. A complete set of infusions was prepared in conditioned ovitraps on August 1 in one of the woodlots and August 4, 1991 in the other woodlot. On August 21, 1991, the infusions in both sets were discarded, the buckets rinsed with tap water, and fresh infusions prepared in the same manner as before. The ovitraps in both woodlots were arranged in a line about 10–15 m inside the woodlot with 10 m between adjacent treatments.

The 2nd group of treatments included 1) tap water, 2) Kentucky bluegrass sod, *Poa pratensis* Linn., 3) rabbit chow, 4) alfalfa, 5) partially dried grass clippings from a yardwaste site, and 6) wheat straw, *Triticum aestivum* Linn., from a single bale. These infusions were compared in 4 different tests between August 2 and September 21, 1992. In the first test (August 2–13), 3 replicates of each treatment were prepared in unconditioned ovitraps. The ovitraps were randomly positioned in 3 rows about 10 m apart inside one of the woodlots. Each substrate (150 g) was wrapped in cheesecloth and added to 6 liters of water for 48 h, except for the sod and water treatments. For sod-baited ovitraps, a 25 × 25-cm section of Kentucky bluegrass sod was wedged into the bottom of the ovitrap and covered with 6 liters of water. The sod remained in the ovitrap during a test. On August 14, 2 of the 3 replicates were removed and the remaining set of ovitraps (a single replicate of each treatment) was relocated to the northern periphery of the woodlot where the comparison of these treatments was continued. Fresh rabbit chow, alfalfa, straw, and grass clippings (150 g of each) were added to the aged liquid media for 24 h (hence each infusion was reinfused). In the sod ovitrap, the old sod was removed and a fresh 25 × 25-cm section of sod was submerged in the bucket. The aqueous portion of each infusion was not discarded between tests, as it had been in 1991; however, the water level in each ovitrap was adjusted to 6 liters. This procedure was repeated for all treatments on 2 other occasions at the same site (August 27, and September 15, 1992). The ovitraps were unconditioned only for the first test because they were reused for each subsequent comparison. One of the discontinued sets of rabbit chow and sod treatments from the first set of infusions in 1992 was included in a new comparison of infusions described below. The 2nd set of replicates was held for chemical and microbial analyses (R. L. Lampman, unpublished data).

The 3rd series of tests included aged infusions of sod and rabbit chow in conditioned ovitraps from one of the discontinued sets of infusions discussed above, as well as fresh infusions of sod and rabbit chow in unconditioned ovitraps and sod grass and sod soil in unconditioned ovitraps. The aged infusions were 14–16 days old. The fresh infusions of sod and rabbit chow were prepared in the manner previously described. The sod grass infusion was started with about 15 g of freshly cut grass from a 25 × 25-cm section of sod. The closely trimmed sod soil was used to start a separate infusion. The first test of this series of treatments was started on August 14, 1992. The rabbit chow treatments (aged and fresh) were reinfused with 150 g of material for 24 h on August 27 and September 9, 1992 for 2 additional tests. The sod, sod grass, and sod soil substrates were not changed in any of the comparisons of these infusions, although the water level was adjusted to 6 liters at the onset of each test. The infusions and ovitraps were classified as aged and fresh and conditioned and unconditioned based on their initial state for the sake of continuity. After the first test, the difference between infusion age and ovitrap condition is less distinct. In all 3 tests, the infusions were placed in a single row at the southern periphery of a woodlot with 10 m between ovitraps.

The final series of infusions were prepared in conditioned ovitraps on August 30, 1992. The treatments included a 25 × 25-cm section of Kentucky bluegrass sod, 150 g of grass clippings from Kentucky bluegrass sod, and 150 g of rabbit chow in 6 liters of water. All infusions, including the sod, were reinfused with fresh organic matter on September 17, 1992. The liquid portion of each infusion was reused. Four rep-

Table 1. Total number of *Culex* egg rafts and percentage of *Cx. restuans* collected from ovitraps baited with different infusion treatments in 2 woodlots in 1991.

Treatment	Woodlot 1		Woodlot 2	
	Aug. 2–19	Aug. 22–Sept. 2	Aug. 4–21	Aug. 22–Sept. 2
Roadside grass	112a[1] (49%)	46a (7%)	77a (47%)	99a (1%)
Rabbit chow	124a (38%)	29ab (3%)	52a (31%)	107a (3%)
Alfalfa	18b (23%)	24b (8%)	6b (0%)	25b (8%)
Oak leaves	8c (37%)	5c (0%)	2c (0%)	0c (0%)
Maple leaves	0c (0%)	10c (20%)	0c (0%)	0c (0%)
Tap water	1c (0%)	0c (0%)	0c (0%)	0c (0%)

[1] The daily number of egg rafts was transformed by log(x + 1) and analyzed by one-way ANOVA for each test with days as replicates. Total numbers of egg rafts with the same letter in the same column represent means that were not significantly different at the 5% level of significance by Fisher's least significant difference test (StatView; Abacus Concepts 1992).

licates of each treatment were randomly positioned about 10 m inside one woodlot with 10 m between adjacent treatments.

Data analysis: The data from each test were transformed by logarithm (x + 1) and subjected to analysis of variance with days as replicates (StatView; Abacus Concepts 1992). The mean number of *Culex* egg rafts per treatment was separated by Fisher's least significant difference (LSD) test with separations made at the 5% level of significance. The data are presented as total number of *Culex* egg rafts per treatment per period. Replicated data were pooled for analyses if there were no significant differences due to date of test by ANOVA. For the graphic presentation of egg raft deposition by each species, the daily percentage of *Cx. restuans* and *Cx. pipiens* was determined from all treatments for each calendar date.

RESULTS

Over 99% of the egg rafts (n = 4,023) from all treatments in 1991 and 1992 were from *Cx. pipiens* and *Cx. restuans,* with *Culex territans* Walker and *Cx. salinarius* accounting for less than 1%. The total numbers of egg rafts per day for all species were pooled for statistical analyses of oviposition preference.

Culex females exhibited considerable selectivity in their choice of oviposition traps. In 1991, there was a significant difference in the number of egg rafts between treatments (tap water, grass clippings, rabbit chow, alfalfa, oak leaf, and maple leaf) at both woodlots and both tests (in one woodlot, df = 5,102, F = 27.2, $P < 0.001$ for the first test and df = 5,66, F = 11.5, $P < 0.001$ for the 2nd test; in the 2nd woodlot, df = 5,102, F = 10.6, $P < 0.001$ for the first test and df = 5,66, F = 29.5, $P < 0.001$ for the 2nd test). Ovitraps baited with infusions made from roadside grass and rabbit chow had more egg rafts than did those baited with either oak or maple leaf infusions or tap water (Table 1). The number of egg rafts collected from the alfalfa infusion was greater than those collected from oak and maple leaf infusions, as well as tap water, but substantially less than those collected from the grass and rabbit chow infusions in all tests except one.

The total numbers of egg rafts (and percentages of total) in each infusion from both woodlots in 1991 were 334 egg rafts in roadside grass (45%), 312 in rabbit chow (42%), 73 in alfalfa (10%), 15 in oak leaf (2%), 10 in maple leaf (1%), and one in tap water. The first egg rafts were collected from the grass and rabbit chow infusions within 1–3 days, indicating a short delay period for both infusions under these conditions.

Initially, the percentage of egg rafts from *Cx. pipiens* was higher than that from *Cx. restuans*; however, the ovipositing populations of the 2 species were almost equally abundant in the 2 woodlots from August 7 to 20 (Fig. 1). During this cross-over period (most of first test in Table 1), the grass-baited ovitraps had a higher percentage of *Cx. restuans* than did the rabbit chow-baited ovitraps and, conversely, the rabbit chow-baited traps had a higher percentage of *Cx. pipiens* than did the grass-baited traps.

Ovitrap condition and infusion age dramatically affected the number of egg rafts collected from each treatment (Table 2). The ANOVA revealed no significant difference between number of egg rafts and date in the first test in 1992, therefore the data from each infusion type were pooled. The number of egg rafts was significantly different between treatments in all tests (in the first test, df = 5,210, F = 90.7, $P < 0.001$; 2nd test, df = 5,66, F = 15.1, $P < 0.001$; 3rd test, df = 5,102, F = 10.6, $P < 0.001$; 4th test, df = 5,30, F = 17.6, $P < 0.001$). However, the relative activity of the infusions changed from test to test (Table 2). Sod was the only

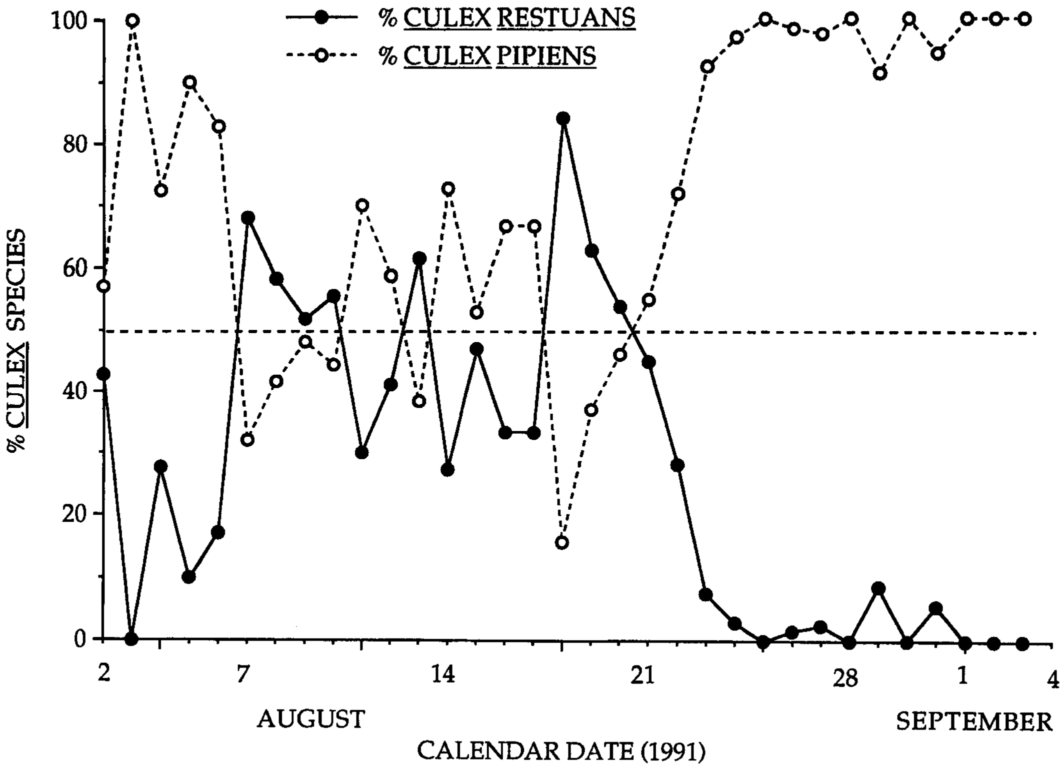

Fig. 1. Daily percentage of *Culex restuans* and *Culex pipiens* determined from all egg rafts collected in 1991, Urbana, IL.

treatment significantly different from tap water in the first test, starting with unconditioned ovitraps and fresh infusions. The alfalfa infusion was not significantly different from tap water in any of the tests. Three of the treatments (rabbit chow, straw, and grass clippings) exhibited an increase in relative activity after one or more reinfusions. All of the infusions, except the mixed grass infusion, were inactive in the final test when *Culex* oviposition activity was low. The grass infusion exhibited the greatest overall increase, accounting for 3% of the total number of egg rafts in the first test (August 2–13), 32% of the total egg rafts in the 2nd test (August 15–

Table 2. Total number of *Culex* egg rafts and percentage of *Cx. restuans* collected from ovitraps baited with different infusion treatments in a woodlot in 1992.

Treatment	Tests			
	Aug. 2–12[1]	Aug. 15–26	Aug. 28–Sept. 14	Sept. 16–21
KY bluegrass sod	360a[2] (94%)	87a (75%)	68a (57%)	6a (83%)
Rabbit chow	14b (100%)	22bc (82%)	38b (32%)	3a (33%)
Straw	2b (100%)	4c (50%)	59ab (28%)	0a
Grass clippings	11b (91%)	59ab (70%)	191c (73%)	19b (47%)
Alfalfa	0b	4c (50%)	12bd (50%)	0a
Tap water	0b	0c	0d	0a

[1] The infusions were initially prepared in unconditioned ovitraps with 3 replicates of each treatment. In each subsequent test, there was only one replicate of each treatment. Before the onset of each test after the first, the infusions were reinfused with organic matter for 24 h in the same ovitraps.

[2] The daily number of egg rafts was transformed by log(x + 1) and analyzed by one-way ANOVA for each test with days as replicates. The replicates were pooled for the first test. Total numbers of egg rafts with the same letter in the same column represent means that are not significantly different at the 5% level of significance by Fisher's least significant difference test (StatView; Abacus Concepts 1992).

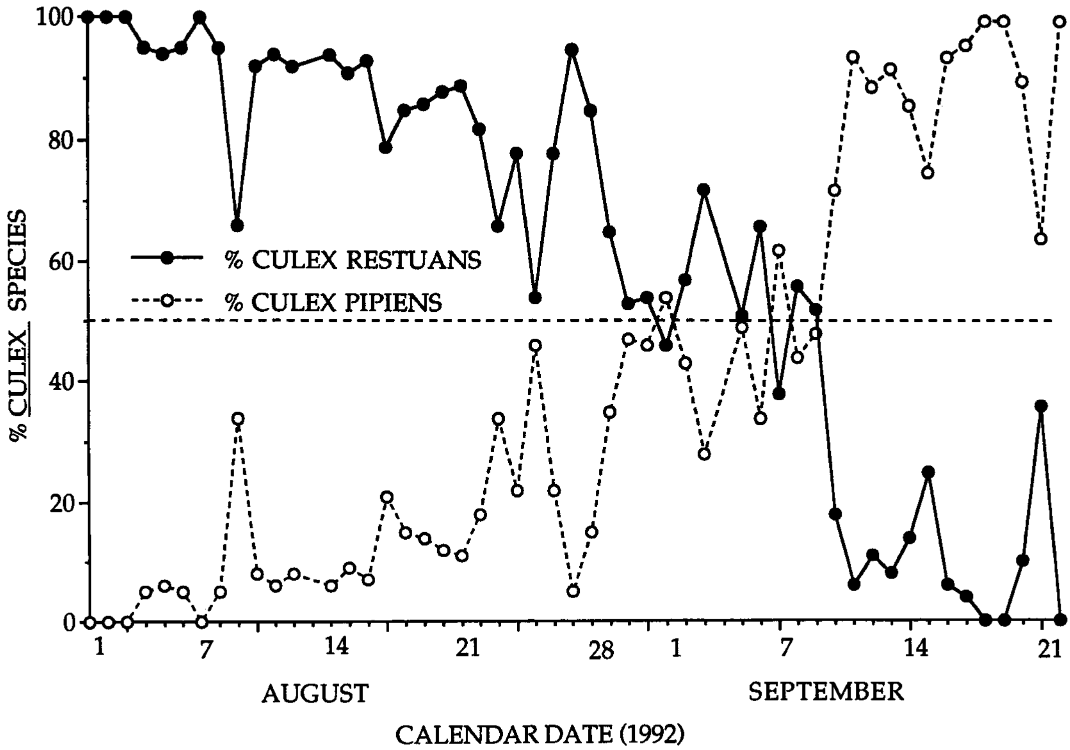

Fig. 2. Daily percentage of *Culex restuans* and *Culex pipiens* determined from all egg rafts collected in 1992, Urbana, IL.

26), 52% in the 3rd test (August 28–September 14), and 68% in the final test (September 16–21). In contrast, the sod treatment was most active in the first and 2nd tests (93 and 49%, respectively, of the total number of eggs rafts) and intermediate to inactive in the 3rd and 4th teste (19% and 21%, respectively). The total numbers of egg rafts per treatment from all tests (and the percentages of total) were 521 (54%) egg rafts in sod, 77 (8%) egg rafts in rabbit chow, 65 (7%) egg rafts in straw, 280 (30%) egg rafts in mixed grass clippings, 16 (2%) egg rafts in alfalfa, and 0 egg rafts in tap water.

The percentage of days a treatment was positive for one or more egg rafts also appeared to vary with ovitrap condition (new or used ovitraps). The conditioned ovitraps with fresh infusions in 1991 (Table 1) had egg rafts on 87% of the total trap days for grass clippings and 72% of the total trap days for rabbit chow. Unconditioned ovitraps with fresh infusions in 1992 (the first test in Table 2) were positive for egg rafts 8% of the total trap days for rabbit chow, 11% for grass, and 94% for sod. Sod was the only substrate that appeared to have a short delay period in unconditioned and conditioned ovitraps.

During the 1992 preference tests, the oviposition activity of *Cx. restuans* and *Cx. pipiens* was almost equal from August 28 to September 7 (Fig. 2). This is a shift in cross-over by about 20–30 days from 1991 (Fig. 1). As previously observed, ovitraps baited with infusions of grass clippings and sod had a higher percentage of *Cx. restuans* than did the rabbit chow infusion during this period in 1992 (the August 28–September 14 test in Table 2).

The next test included aged infusions in conditioned ovitraps and fresh infusions in unconditioned ovitraps, as well as fresh sod grass and sod soil infusions in unconditioned ovitraps (Table 3). There were significant differences between treatments for each test (in the first test, df = 5,66, F = 27.2, $P < 0.001$; 2nd test, df = 5,66, F = 12.8, $P < 0.001$; 3rd test, df = 5,30, F = 19.4, $P < 0.001$). In the first test, more egg rafts were oviposited in conditioned ovitraps with either aged rabbit chow infusion or aged sod infusion than in unconditioned ovitraps with sod, rabbit chow, sod grass, or sod soil. The aged rabbit chow and aged sod were not significantly different; however, in the unconditioned ovitraps, fresh sod had more *Culex* egg rafts than did all other fresh infusions. This supports

Table 3. Total number of *Culex* egg rafts and percentage of *Cx. restuans* collected from ovitraps baited with different treatments at a woodlot in 1992.

	Tests		
Treatments[1]	Aug. 15–26	Aug. 28–Sept. 8	Sept. 10–15
Aged sod in used ovitrap	265a[2] (91%)	36a (75%)	20a (55%)
Fresh sod in new ovitrap	166b (85%)	101b (74%)	8ab (25%)
Aged rabbit chow in used ovitrap	329a (82%)	52ab (57%)	13a (30%)
Fresh rabbit chow in new ovitrap	12c (67%)	11c (73%)	0b
Fresh sod grass in new ovitrap	28c (21%)	79b (53%)	14a (23%)
Fresh sod soil in new ovitrap	1c (100%)	0c	0b

[1] Aged rabbit chow and sod infusions in used (conditioned) ovitraps were 14–16 days old at the start of the first test. Rabbit chow infusions were reinfused for 24 h the day before each new test. Sod, sod grass, and sod peat soil were left in the ovitraps for the duration of each test.

[2] The daily number of egg rafts was transformed by log(x + 1) and analyzed by one-way ANOVA for each test with days as replicates. Total numbers of egg rafts with the same letter in the same column represent means that were not significantly different at the 5% level by Fisher's protected least significant difference test (StatView; Abacus Concepts 1992).

the earlier observation that sod infusions have short delay periods before attracting gravid females. In all subsequent tests with this group of infusions, the designation of aged and fresh and conditioned and unconditioned becomes less distinct because of the reuse of ovitraps and infusion media. Nevertheless, the comparison of infusion activity in subsequent tests provides insight into temporal changes in infusion quality. For example, the aged sod (started in a conditioned ovitrap) was less attractive than was the fresh sod (started in an unconditioned ovitrap) in the 2nd test, although the 2 sod infusions were equally preferred in the final period when oviposition activity was low. The aged rabbit chow infusion had more egg rafts than did the fresh rabbit chow infusions in all tests, even after reinfusion. In fact, the fresh rabbit chow infusion never became a significant oviposition lure, although rabbit chow infusions, prepared in conditioned ovitraps, were active at a later date (see below). Furthermore, the active, aged rabbit chow infusion in the first test (August 15–26 in Table 3) had been inactive for 12–14 days before this test (it was from one of the discontinued sets in Table 2 that was run from August 2 to 13). The infusions from sod grass and sod peat soil showed that the grass component of sod was the important substrate for the infusion (Table 3). Sod grass was inactive in the first test, but by the 2nd and 3rd tests it was among the most active infusions in this series. In contrast, only one egg raft was collected from the ovitrap with sod soil after all 3 tests. Ovitraps with the complete sod had egg rafts deposited in them earlier than did those with only the sod grass clippings.

The total numbers of egg rafts per treatment (and percentages of total) for all tests with this series of infusions were 321 (28%) for aged sod started in a conditioned ovitrap, 275 (24%) for fresh sod started in an unconditioned ovitrap, 394 (35%) for aged rabbit chow started in a conditioned ovitrap, 23(2%) for fresh rabbit chow started in an unconditioned ovitrap, 121 (11%) for sod grass started in an unconditioned ovitrap, and one egg raft for sod soil started in an unconditioned ovitrap. Higher percentages of egg rafts from *Cx. restuans* were oviposited in the aged sod than the fresh sod in all 3 tests. The fresh sod infusion had a higher percentage of *Cx. restuans* than did rabbit chow in the first and 2nd tests (Table 3). In the 3rd test, the number of egg rafts recovered from all infusions was low.

The last series of infusions included sod, rabbit chow, and sod grass prepared in conditioned ovitraps. In the first test (August 31–September 17, 1992), the complete sod had 133 egg rafts (59% *Cx. restuans*); rabbit chow, 22 egg rafts (9% *Cx. restuans*); and sod grass, 48 egg rafts (2% *Cx. restuans*). After reinfusion, sod-baited ovitraps had a total of 52 egg rafts (2% *Cx. restuans*); rabbit chow, 117 egg rafts (no *Cx. restuans*); and sod grass, 75 egg rafts (1% *Cx. restuans*) from September 17 to 22, 1992. The sod treatment attracted more gravid *Cx. restuans* than did the sod grass and rabbit chow in the first test. The rabbit chow infusion in this series, although prepared in a conditioned ovitrap, was not active until after reinfusion (the 2nd test). The egg rafts from all treatments were primarily from *Cx. pipiens* in the 2nd test.

DISCUSSION

Oviposition in infusion-baited traps varied with type of infusion substrate, infusion age, ovitrap condition (new or unused), infusion preparation method, and calendar date. *Culex pipiens* and *Cx. restuans* oviposited in water infused with Kentucky bluegrass sod, grass clippings from Kentucky bluegrass sod, mixed grass clippings, wheat straw, and a commercial rabbit chow in one or more tests. Infusions of oak leaves, maple leaves, and sod soil were ineffective as oviposition attractants for *Culex* when prepared as in this study. The sod, grass, and rabbit chow infusions attracted both *Culex* species; however, during crossover, there were usually more egg rafts from *Cx. restuans* in sod and grass infusions than in rabbit chow infusions and, conversely, more egg rafts from *Cx. pipiens* in rabbit chow infusions than in sod and grass infusions. The active oviposition lures included those that allowed the substrate to remain in the ovitrap throughout a test (sod and sod grass) and those in which the substrate was infused in the water for only 24–48 h before the onset of a test (rabbit chow and mixed grass clippings). Additives, such as lactalbumen, brewer's yeast, decanoic acid, ethyl alcohol, and ammonium nitrate, were not required to produce effective oviposition substrates, as was the case in several other studies (Maw and Bracken 1971, Reiter et al. 1986, Millar et al. 1992).

In 1992, the attraction of *Culex* spp. to rabbit chow infusions was quite variable. Rabbit chow infusions often required a long aging period with several reinfusions before becoming active, especially when prepared in unconditioned (unused) ovitraps. Previous studies with rabbit chow infusions seldom encountered this problem, probably because conditioned ovitraps were often used and the infusions were usually prepared early in the season and reinfused several times throughout the monitoring period (Steinly and Novak 1990, 1993). The number of egg rafts oviposited in the rabbit chow infusion was associated with a readily detectable change in the visual and olfactory characteristics of the infusion. Inactive rabbit chow infusions were usually opaque and highly odiferous. After a variable aging period, the rabbit chow infusion transformed to a dark brown, transparent, and less-pungent mixture. The early sewage-like odor from these infusions may have been caused by the production of short chain carboxylic acids, like butyric and iso-butyric acids (R. L. Lampman, unpublished data). In conditioned ovitraps, the conversion of the rabbit chow infusion occurred within days (Table 1), whereas it took several weeks and one or more reinfusions when prepared in unconditioned ovitraps (Tables 2 and 3). In one series of tests, the rabbit chow infusion was inactive even after 3 reinfusions. All of the ovitraps containing attractive infusions (e.g., sod, rabbit chow, and various grass infusions) also had a dark red to reddish brown deposit on the inner lining of the plastic bucket after they became active.

Infusion baits for mosquitoes are inherently unstable just as natural oviposition sites are. They undergo temporal changes in bacterial content, chemistry, clarity, and color (Bentley and Day 1989). These changes are affected, at least in part, by seasonal and daily fluctuations in temperature (Brust 1990). Changes in the concentration of volatiles released by infusions may also alter the mosquito's perception of the infusion from unattractive or repellent to attractive or *vice versa* (Knight and Corbett 1991). Infusion quality may be modified further by the introduction of contaminants from the environment, as well as from ovipositing mosquitoes and developing larvae (Prasad and Daniel 1988). To minimize variability in mosquito attraction to the ovitraps, infusions are usually replaced (Reiter et al. 1986) or reinfused on a periodic basis (Steinly and Novak 1990). Few studies have investigated how variability in infusion quality affects mosquito response (Bentley and Day 1989). In this study, sod infusions were the most consistent lures for gravid *Cx. pipiens* and *Cx. restuans.* Females usually oviposited in sod infusions within 1–3 days after preparation in either conditioned or unconditioned ovitraps. Continuous infusions of sod maintained a high rate of egg deposition from *Culex* species, ranging from 3 to 5 wk. In Canada, similar sod infusions were unattractive to *Cx. restuans* after 1–3 wk and to *Cx. tarsalis* after 3 wk depending on seasonal changes in temperature (Brust 1990). Grass clippings from sod, yard waste, and roadside cuttings were also used to prepare active infusions. The sod soil infusion was unattractive in all tests. For most attractive infusions, there was a shorter delay in conditioned ovitraps, probably caused by the retention of the distinctive infusion odor by the plastic, the contamination of the new infusion media with bacteria, or both.

Recent studies suggest variation in infusion quality may be minimized by standardizing either the chemistry or microbial content of lures for *Culex* species. For example, Millar et al. (1992) found that a Bermuda grass infusion, supplemented with yeast and lactalbumen, generated several phenolic and nitrogen-containing compounds. Skatole, one of the odor components, was highly preferred over water for oviposition by *Cx. quinquefasciatus* in laboratory tests. Ikeshoji et al. (1975) showed that the oviposition of *Culex* species was affected by many of the phenolic compounds from wood creosote. We identified several short-chain carboxylic acids, cresols, skatole, indole, and a few aromatic compounds from sod, rabbit chow, and mixed grass infusions, and *Cx. pipiens* preferred skatole-treated water to water controls in caged laboratory experiments (R. L. Lampman and R. J. Novak, unpublished data). Beehler et al. (1994) found that gravid traps baited with skatole were effective for collecting gravid *Cx. quinquefasciatus*. This suggests that synthetic baits may be able to replace crude infusions; however, we have not been able to duplicate these results with skatole or indole for *Cx. pipiens* or *Cx. restuans* in field tests. As an alternative to chemically defined baits, Maw and Bracken (1971) recommended that aged infusions be kept frozen in order to introduce the necessary bacteria to fresh infusions. Bacterial species associated with attractive infusions are generally common environmental contaminants, such as *Pseudomonas aeruginosa, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli,* and *Acinetobacter calcoaceticus* (Rockett 1987, Benzon and Apperson 1988, Prasad and Daniel 1988, Bentley and Day 1989).

The relative oviposition activities of *Cx. pipiens* and *Cx. restuans* in areas within a 8–10-km radius of our study site were independently recorded in Urbana and Champaign, IL, from May to October 1991 (Steinly et al. 1993). Rabbit chow infusions, reinfused approximately every 10 days, were used to monitor the daily oviposition of *Culex* spp. at 7 peridomestic and domestic locations. The number of egg rafts from *Cx. pipiens* became more abundant than those from *Cx. restuans* by the end of the week of July 10, 1991; however, in our test sites, almost equal numbers of both species were present about 4 wk later (August 7–20, 1991). The initial percentage of *Cx. pipiens* collected from all ovitraps in our study was higher than that of *Cx. restuans* (Fig. 1), which suggests our collections may have been during a 2nd crossover of the 2 species, because *Cx. restuans* is almost always the more prevalent early species (Steinly and Novak 1993). The woodlots in 1991 were probably localized areas with an extended period of overlap between *Cx. restuans* and *Cx. pipiens*. Factors that affect female recruitment to a particular habitat (e.g., the number and characteristics of oviposition sites and blood hosts; see Reisen and Meyer 1990) may have differed between our rural study area and the urban locales used by Steinly and Novak (1993). Alternatively, differences in the experimental protocols of the 2 studies (i.e., using one infusion as compared with several types of infusions) may have yielded different estimates of relative species abundance. In 1992, the crossover of the 2 *Culex* species occurred between August 28 and September 7 in our test area (Fig. 2), which coincided with the city-wide crossover data from rabbit chow infusions (Steinly et al. 1993).

Oviposition activity, as measured by infusion-baited ovitraps, may be affected by a combination of interacting factors, including temporal changes in *Culex* population dynamics, weather conditions, infusion composition, and mosquito preference. Despite this variability, many simple infusions of organic matter adequately mimic natural oviposition sites without the addition of yeast or lactalbumen and without long aging periods. A continuous infusion of lawn sod exhibited the least amount of variability in this study and had a relatively short delay period before becoming active in both conditioned and new ovitraps. Continuous and periodic infusions of grass clippings were also effective oviposition lures, especially if started in conditioned ovitraps. The rabbit chow infusion is a simple infusion to prepare and can rival the other infusions in number of *Culex* egg rafts. Unfortunately, the activity of the rabbit chow infusion is quite variable. Infusion-baited ovitraps are important tools for collecting egg rafts and gravid females to estimate relative oviposition activity and to collect vector species for viral analyses.

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