COMPARATIVE STUDY OF GRAVID-TRAP INFUSIONS FOR CAPTURING

BLOOD-FED MOSQUITOES (DIPTERA: CULICIDAE) OF THE GENERA

AEDES, *OCHLEROTATUS*, AND *CULEX*

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COMPARATIVE STUDY OF GRAVID-TRAP INFUSIONS FOR CAPTURING BLOOD-FED MOSQUITOES (DIPTERA: CULICIDAE) OF THE GENERA *AEDES*, *OCHLEROTATUS*, AND *CULEX*

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Nathan Daniel Burkett, son of Donald Levi Burkett and Brenda (Lowery) Burkett, was born April 28, 1976, in Creola, Alabama. He graduated from LeFlore High School in 1994. He attended Alabama State University in Montgomery, Alabama, for one year, and then entered Springhill College in Mobile Alabama where he studied for two years before entering the University of South Alabama, also in Mobile. In 2001 he graduated with a Bachelor of Science degree in Biology. In 2002 he entered the Master of Science program in the Department of Entomology and Plant Pathology at Auburn University.

THESIS ABSTRACT

COMPARATIVE STUDY OF GRAVID-TRAP INFUSIONS FOR CAPTURING BLOOD-FED MOSQUITOES (DIPTERA: CULICIDAE) OF THE GENERA *AEDES*, *OCHLEROTATUS*, AND *CULEX*

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Field experiments were conducted in east-central Alabama to compare the attractiveness of selected gravid-trap infusions to ovipositing female mosquitoes of 3 medically important genera: *Aedes, Ochlerotatus* and *Culex*.

Evaluations of infusions to collect medically important *Culex* females were performed in 2003 and 2004. Infusions were produced from Bermuda hay and 3 species of emergent aquatic plants: soft rush, *Juncus effuses*; a common sedge, *Rhynchospora corniculata;* and broad-leaf cattail, *Typha latifolia,* which are typical of *Culex* larval habitats. Experimental sites included a hardwood bottomland site bordering a marsh in Tuskegee National Forest, Macon County, AL and a soils bioremediation site

in Lee County, AL. Carbon dioxide-baited miniature light traps were operated concurrent with gravid traps to monitor activity of various mosquito species at both sites.

Gravid traps with hay infusion collected the greatest mean *Culex quinquefasciatus* and *Culex restuans* females in 2003 and in 2004. Traps with sedge infusion collected the second greatest mean of females for both species, followed by infusions of cattail and rush. The results indicate that hay infusion is highly attractive to *Culex* spp., such as *Cx. quinquefasciatus* and *Cx. restuans,* and is the infusion of choice for collecting medically important *Culex* spp. in gravid traps.

In 2004 field experiments were conducted to compare the attractiveness of infusions made from commercially available garden products to an oak-leaf infusion standard to container-breeding *Aedes* and *Ochlerotatus* females.

Three products selected from preliminary experiments (composted horse manure, dyed (red) hardwood mulch, and pine straw) were compared to an oak-leaf infusion under field conditions at 2 sites in Lee County, AL: an Automobile Salvage Yard, Phenix city and a suburban forested lot, Dean Road, Auburn.

Container-breeding mosquitoes collected from gravid-traps included females of *Ae. albopictus* and *Oc. triseriatus.* Neither of these species demonstrated a preference for any of the infusions evaluated in experiments at either field site.

In general, females of *Culex* spp. demonstrated selectivity when choosing an oviposition site while *Aedes* and *Ochlerotatus* females did not. Factors associated with oviposition behavior of the latter 2 genera most likely account for their lack of preference for any single infusion type.

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I. INTRODUCTION

Mosquitoes are responsible for vectoring a number of disease agents around the world. In North America 3 genera of particular importance are *Aedes, Ochlerotatus,* and *Culex*. Several of the *Aedes* and *Ochlerotatus* species of medical significance for humans are mosquitoes that breed in tree-hole and water-filled containers. These insects often reproduce in water-filled tires and other artificial containers. In the eastern U.S. they include the yellow fever mosquito (*Aedes aegypti*), Asian tiger mosquito (*Aedes albopictus*), eastern tree-hole mosquito (*Ochlerotatus triseriatus*), and *Ochlerotatus japonicus*.

Aedes aegypti, an introduced species from the Old World, is known to be the primary vector of yellow fever and is also a competent vector of dengue. This mosquito breeds primarily in artificial containers in North America, but is occasionally found in tree-holes. In its native African jungles this insect develops in water-filled rot holes of trees far from human habitation (Carpenter and LaCasse 1974). In the U. S. *Ae. aegypti* is found in the southeastern states (Darsie and Ward 1981). This mosquito is being displaced in North America by *Ae. albopictus,* another introduced species (Sprenger and Wuithiranyagool 1989).

Aedes albopictus, an Asian species first detected in the continental U.S. in 1985 (Francy et al. 1990), has spread throughout the eastern half of the U. S. (Moore and

Mitchell 1997, Moore 1999). This mosquito is a competent vector of Dengue virus (Mitchell et al. 1987), Eastern Equine Encephalitis virus (Scott et al. 1990) and other arboviruses not normally encountered by humans in the U.S. (Francy et al 1990). It breeds in a variety of natural and artificial containers such as bamboo stumps, rot holes of trees, and discarded automobile tires.

Ochlerotatus triseriatus, the primary vector of La Crosse Encephalitis in the upper Midwest (DeFoliart et al. 1986), is widely distributed across the eastern half of the U.S. and southern Canada (Darsie and Ward 1981). This common (Szumlas et al. 1996) species breeds primarily in water-filled cavities of deciduous trees, but also in artificial containers such as automobile tires (Joy et al. 2003), barrels and watering troughs (Carpenter and LaCasse 1974). Shaded habitats are preferred (Joy and Hildreth-Whitehair 2000, Joy and Clay 2002).

Ochlerotatus japonicus, a native of Japan and Korea (Tanaka et al. 1979), was first detected in the Americas in New York in 1998 (Peyton et al. 1999). This mosquito has recently extended its southern range into Maryland (Sardelis and Turell 2001) and is reaching lower latitudes each year (Harrison et al. 2002). In July of 2005 it was reported from 2 counties in northern Alabama (TVA, unpublished data). In its native range *Oc. japonicus* is the primary vector of Japanese Encephalitis virus. Concern has been expressed about the possibility of this species serving as a vector of other mosquito-borne viruses in the Western Hemisphere. *Oc. japonicus* has been shown to be an efficient laboratory vector of West Nile Virus (Sardelis and Turell 2001) and Eastern Equine Encephalitis virus (Sardelis et al. 2002). This species usually breeds in artificial containers and occasionally in tree-holes and rock pools (Scott et al. 2001).

The 4 species of *Culex* principally responsible for disease transmission in the eastern U.S. are the southern house mosquito (*Culex quinquefasciatus*), unbanded salt-marsh mosquito (*Culex salinarius*), *Culex restuans*, and *Culex nigripalpus*.

Culex quinquefasciatus is considered to be the primary bridge vector (i.e., transmits disease agents from wild reservoir hosts to humans and domestic animals) of West Nile virus (WNV) in the U.S. (Turell et al. 2001). This mosquito is also known to be the principal urban vector of Bancroftian filariasis in the tropics (Carpenter et al. 1946) as well as a secondary vector of Saint Louis encephalitis in the United States (Reiter et al. 1986). Relative to other *Culex* spp., *Cx. quinquefasciatus* is primarily an urban pest and often breeds in foul or polluted waters in a variety of natural and artificial habitats (Carpenter and LaCasse 1974). Due to its medical importance much effort has been spent on the surveillance of this species.

Culex restuans, *Cx. nigripalpus*, and *Cx. salinarius* are all primarily rural species that breed in permanent or semi-permanent fresh-water marshes, grassy pools and ditches (Carpenter and LaCasse 1974). These 3 species feed on avian and mammalian blood and are important as bridge vectors of encephalitis viruses.

Culex salinarius is known to be a vector of both West Nile and St. Louis encephalitis viruses. This mosquito is widely distributed across the eastern half of the U. S. and southeastern portions of Canada. Larvae are most commonly encountered grassy pools, ditches and ponds containing fresh or polluted water (Carpenter and LaCasse 1974).

Culex nigripalpus is a vector of the viruses causing St. Louis encephalitis, eastern equine encephalitis and West Nile encephalitis (Rutledge et al. 2003). Females prefer to lay eggs in freshly flooded roadside ditches and agricultural furrows (Day and Curtis 1994). The distribution of this species is limited to coastal states of the southeastern U.S. and western portions of Kentucky and Tennessee (Darsie and Ward 1981). Carpenter and LaCasse (1974) report this rural species to be less prone to bite man than *Culex salinarius.*

Culex restuans is a vector of Eastern Equine Encephalitis and West Nile viruses (Nasci et al. 2001). This pestiferous biter of man (Moore 1999) is distributed widely across much of the eastern U.S. and Southern Canada. In western states the distribution of *Cx. restuans* is limited and patchy (Darsie and Ward 1981, Carpenter and LaCasse 1974).

Gravid Traps

A number of methods for trapping mosquitoes have been used in mosquito research and surveillance programs. Electric nets, counterflow geometry traps, CO_2 traps, oviposition traps, light traps (baited and unbaited), resting boxes and CDC gravid traps are some of the different types that have been employed in this endeavor (Vaidyanathan and Edman 1997). Mosquito-borne pathogens are normally acquired from hosts and transmitted by blood-feeding female mosquitoes. Because these individuals are the most likely to have become infected with viruses, a trap targeted towards the capture of blood-fed females is particularly helpful in monitoring mosquito-borne viruses. Of the traps mentioned above, only the CDC gravid trap is specifically designed to attract and catch blood-fed female mosquitoes. The other traps also attract males and newly emerged or non-gravid females. Since the chance of these individuals having picked up pathogenic agents is minimal, eliminating them from catches leads to more efficient monitoring for mosquito-borne diseases.

The CDC gravid trap consists of a dark rectangular basin that holds a water-based gravid-mosquito attractant. Suspended over the basin is a battery-powered fan mounted inside a hollow pipe. Gravid mosquitoes attempting to oviposit in the liquid medium are drawn up the pipe and trapped in a collection net at the top. Paul Reiter (1983), who designed the trap, describes the preovipositional behavior in which females "examine" oviposition waters in "a rapid series of brief landings at many points." During this preovipositional behavior mosquitoes are drawn into the trap as they pass under the hollow pipe.

Each species of mosquito has specific larval habitat requirements. Understanding the cues that female mosquitoes utilize in locating breeding sites can be helpful in developing oviposition attractants for use in trapping particular mosquito species. Laboratory studies and field data have shown that gravid female mosquitoes exhibit significant preference when selecting waters for oviposition (Lampman 1996, Weber and Horner 1993). Research has also shown that the volatile small molecules released by oviposition waters play a primary role in the attraction of gravid females searching for oviposition sites (Millar et al. 1992). Decomposition of organic matter is the most likely source of the volatile chemicals that have been found to lure gravid female mosquitoes (Ikeshoji et al. 1975, Benzon and Apperson 1988).

Ovipositional Attractants

Fermented mixtures of various plants have been used successfully to attract *Culex* mosquitoes for over 2 decades. Reiter's (1983) original medium was made by adding 1lb of Bermuda hay and 1oz each of dried brewer's yeast and lactalbumen powder to 30 gallons of tap water. This mixture is left to incubate (ferment) for 5 days in a closed container before being used.

Infusions made of a variety of materials have proven to be attractive to gravid *Culex* mosquitoes. Infusions of Bermuda grass (Millar et al. 1992), steer manure (Reisen and Meyer 1990), Kentucky bluegrass (Lampman et al. 1996), and bulrush (Du et al. 1999) all have been shown be more attractive to ovipositing *Culex* mosquitoes than water-only controls in gravid traps. Of the plants used in infusions noted above, only bulrush is a dominant emergent plant in larval habitats of some medically important *Culex* species, namely *Culex tarslis* (a western U. S. species) and *Cx. quinquefasciatus* (Du et al. 1999). Berkelhamer and Bradley (1989) found that larvae of certain *Culex* spp. were associated with decaying mats of cattails (Typhaceae) and bulrush (Cyperaceae) *Schoenoplectus californicus*.

Later, researchers demonstrated the ability of infusions made from decaying *S. californicus* to stimulate oviposition in *Cx. tarsalis* (Walton and Workman 1998). Bentley and Day (1989) showed that container-breeding mosquitoes use both chemical and physical cues when selecting oviposition sites. Ahmadi et al. (1983) suggested that decayed organic matter in natural breeding water may act as an attractant for ovipositing *Aedes sierrensis*, a tree-hole breeding mosquito of the western U.S.

Results of this work point toward the presence of specific chemical cues for gravid mosquitoes created by decomposition of the dominant vegetation in those areas.

Through fractionation by liquid chromatography Millar et al. (1992) identified several small molecules (phenol, 4-methylpnenol, 4-ethylpnenol, indole and 3-methylindole) from Bermuda grass infusions that acted as strong ovipositional stimulants for *Cx. quinquefasciatus.*

While progress has been made in finding effective attractants for some *Culex* mosquitoes, there has been little success in discovering ovipositional attractants for gravid *Aedes* or *Ochlerotatus* females (Trexler et al. 1998). Lampman and Novak (1996) reported large catches (mean = 105.25/trap/night) of *Aedes albopictus* in gravid traps baited with infusion made from fermented Kentucky bluegrass sod (*Poa pratensis*) at a waste tire site in Illinois, but only 5% of females were gravid. In addition, Trexler et al. (1998) indicated that oak-leaf infusions enhanced oviposition of *Aedes albopictus* and *Ochlerotatus triseriatus* in a field study in North Carolina.

II. FIELD COMPARISON OF GRAVID-TRAP INFUSIONS FOR COLLECTING *CULEX* FEMALES

Objective: To compare the attractiveness of infusions made from emergent aquatic plants to a Bermuda-hay infusion standard for attraction of ovipositing *Culex quinquefasciatus* and other *Culex* species in Alabama.

Study Sites

Field evaluations were conducted at 2 sites. The first site was located in a hardwood bottomland in the Uphapee Creek floodplain adjacent to national forest road 937 in Tuskegee National Forest, Macon County, AL. Dominant tree species included water oak; *Quercus nigra*, slash pine; *Pinus elliotti*, sweetgum; *Liquidambar styraciflua,* swamp tupelo; *Nyssa biflora*, and southern magnolia; *Magnolia grandiflora.*

The second site, a soils bioremediation plot located near the intersection of Samford Ave. and Wire Rd. on the campus of Auburn University, was characterized by a gently sloping red-clay hill with a small (8 x 3m) artificial pool situated at its top. Grasses dominated the site, which was mowed regularly. The open grassy area, where the traps were located, was surrounded by mixed hardwood and coniferous forest. Vegetation included water oak; *Q. nigra*, slash pine; *P. elliotti*, sweetgum; *L. styraciflua,* Chinese privet*; Ligustrum sinense,* and kudzu; *Pueraria montana.*

Materials and Methods

Evaluation of gravid-trap infusions for collection of ovipositing *Culex* females was conducted in summer of 2003, and a follow-up study was conducted in spring of 2004. Research was conducted at 2 sites in 2003. At a hardwood bottomland location in Tuskegee National Forest in Macon County, AL experiments were conducted over a 6-week period and replicated 7 times. At the Soils Bioremediation site in Lee County, AL, experiments were conducted over a 10-week period and replicated 12 times. In 2004 the follow-up study was conducted at a single location, the Soils Bioremediation site in Lee County, AL, and replicated 4 times over a 3-week period.

Experimental attractants were produced from 3 common species of emergent aquatic plants for comparison to a Bermuda-hay-infusion standard that has been used to collect blood-fed females of *Culex quinquefasciatus*. Entire plants of *Typha latifolia* (broadleaf cattail), *Juncus effusus* (soft rush), and *Rhynchospora corniculata,* (a common sedge), were harvested prior to the study from a wetland area at Tuskegee National Forest in Macon Co., AL. Plant matter was washed and then allowed to dry in outdoor conditions. A bale of Bermuda hay, *Cynodon dactylon,* was purchased for making hay infusion. Dry materials from these 4 plant species were then used to prepare 4 separate infusions following the protocol of Reiter (1983).

Infusions were prepared in 15-gallon batches, this being half the size of that originally described by Reiter (1983). Dried plant material (250g), brewer's yeast (5g) and lactalbumen (5g) were added to 15 gallons of tap water and stirred with a wooden paddle. The mixture was allowed to incubate at outdoor temperatures for 5 days in closed plastic containers before use in traps. Infusions utilized in gravid traps were discarded after a single use. Fresh infusion was added to gravid traps for each trapping session. Trapping sessions were initiated in late afternoon and collections were retrieved approximately 24 hours later.

A gravid trap with 1 infusion type served as single replicate. At both sites each infusion type was replicated 6 times for each trapping occasion. A randomized complete block design was utilized. Each block consisted of 4 gravid traps, each with a different infusion. At both sites gravid traps were placed along a single 125m transect. Treatment positions were randomized within blocks for each trapping period. At the Tuskegee National Forest site the transect was oriented parallel to a dirt road in the vicinity. At the Soils Bioremediation site traps were placed on open ground adjacent to a fencerow concealed by dense vegetation consisting of *L. sinense* and *P. montana*

Carbon dioxide-baited CDC miniature light traps were operated at each field location during 3 trapping sessions to provide an index for assessing the activity of mosquito species of interest to this study.

Female mosquitoes collected by gravid traps were returned to the laboratory for identification to species using Darsie and Ward's (1981) key. Age and type of infusion were recorded, together with species identifications of the mosquitoes collected.

Collection data were analyzed by ANOVA for variation among mean numbers of female mosquitoes collected by gravid traps with each of the 4 treatments (infusions). Pair-wise comparisons of infusions were achieved via Tukey-Kramer adjusted statistical test at a 95% confidence level.

Results

2003 Field Evaluations

At the Tuskegee National Forest site only 78 mosquitoes were trapped in a total of 7 trap nights, for an average of 0.5 mosquitoes per trap per night. A complete list of mosquito species collected by gravid traps at this site is provided in Table 1 along with the total numbers of females collected.

Culex spp. comprised 63% (n=26) of total collections. Seventeen of the *Culex* specimens (65% of the total *Culex* collections) were not identifiable to species due their worn condition or damage from the trapping process. The color and arrangement of scales on the thorax and abdomen are the most common characters used for species level identification within the genus *Culex*.

The following *Culex* spp. were collected: *Culex quinquefasciatus* 44% (4/9); *Cx. nigripalpus:* 11% (1/9); *Cx. salinarius*: 11% (1/9); and *Cx. territans:* 33% (3/9). *Culex restuans* was not recovered from gravid traps at this site. Table 2 presents the total numbers of females captured by gravid traps with 4 infusion types for each species of *Culex* collected at the Tuskegee National Forest site.

Culex quinquefasciatus: 50% (n = 3) of total *Cx. quinquefasciatus* collections were from gravid traps with hay infusion, 33% (n=2) with cattail infusion, and 17% (n=1) with sedge infusion. No *Cx. quinquefasciatus* females were collected from traps with rush infusion. The variation among infusions was not significant (overall p = 0. 6834).

Culex nigripalpus: 100% (n=1) of *Cx. nigripalpus* collections were from gravid traps with rush infusion. The variation among infusions was not significant (overall p = 0. 5328).

Table 1. Total female mosquitoes collected by gravid traps at a hardwood bottomland site in Tuskegee National Forest, Macon Co., AL, 2003. Figures represent actual specimens collected during seven 24-hour trapping periods.

Mosquito species	Females collected
Aedes albopictus	11
Aedes atlanticus	2
Ochlerotatus triseriatus	17
Aedes/Ochlerotatus spp.	18
Coquillettidia perturbans	1
Culex erraticus	0
Culex nigripalpus	1
Culex quinquefasciatus	4
Culex restuans	0
Culex salinarius	1
Culex territans	3
Culex spp.	17
Orthopodomyia signifera	1
Psorophora horrida	1
Psorophora ferox	1
Total	78

Table 2. Total *Culex* females collected by gravid traps with 4 experimental infusions at a hardwood bottomland in Tuskegee National Forest, Macon Co., AL, 2003. Figures represent combined numbers of actual specimens collected during seven 24-hour trapping periods.

Species	Cattail	Hay	Rush	Sedge	Total
Culex nigripalpus	0	0	1	0	1
Culex quinquefasciatus	2	1	0	1	4
Culex salinarius	1	0	0	0	1
Culex territans	2	1	0	0	3
Culex spp.	3	3	3	8	17

Culex salinarius: 100% (n=1) of *Cx. salinarius* collections were from gravid traps with cattail infusion. The variation among infusions was not significant (overall p = 0. 5328).

Culex territans: 67% (n=2) of *Cx. territans* collections were from gravid traps with cattail infusion and 33% (n=1) with hay infusion. The variation among infusions was not significant (overall p = 0.2958).

Light traps operated at the Tuskegee National Forest site collected 2 *Culex* species. *Culex territans* was collected on 1 of 3 (7/29/2003) trapping sessions and *Cx .erraticus* on 2 of 3 (7/29/2003 and 8/22/2003) trapping sessions. A list of mosquito species collected by light traps at this site and their respective totals is provided in Table 3.

At the Soils Bioremediation site 4838 mosquitoes were trapped during a total of 12 trapping occasions for an average of 16.8 mosquitoes per trap per night. A list of mosquito species collected by gravid traps, together with the total numbers of females collected at this site are provided in Table 4.

Culex spp. comprised 84% (4078/4838) of total collections. Thirty-nine percent of the total *Culex* collections (1571/4078) were not identifiable to species due to damaged specimens.

The following *Culex* spp. were collected in gravid traps at the Soils Bioremediation site: *Culex quinquefasciatus:* 98% (n=2449), *Cx. restuans*: 2% (n=46), *Cx. nigripalpus*: < 1% (n=2), and *Cx. territans:* < 1% (n=4). *Culex salinarius* was not collected by gravid traps at this site. Table 5 presents the total numbers of females captured by gravid traps with 4 infusion types for each species of *Culex* collected.

Table 3. Total female mosquitoes collected by carbon-dioxide-baited light traps during 3 trapping occasions at a hardwood bottomland in Tuskegee National Forest, Macon Co., AL, 2003. Figures represent the number of specimens collected during a 24-hour trapping period.

Mosquito species	7/29/20003	8/8/2003	8/22/2003
Aedes atlanticus	20	0	0
Aedes infirmatus	10	0	0
Aedes trivittatus	3	0	0
Aedes vexans	2	0	0
Aedes/Ochlerotatus spp.	1	49	16
Anopheles spp.	0	6	0
Coquillettidia perturbans	0	0	2
Culiseta melanura	1	0	0
Culex erraticus	5	0	12
Culex nigripalpus	0	0	0
Culex quinquefasciatus	0	0	0
Culex restuans	0	0	0
Culex salinarius	0	0	0
Culex territans	1	0	0
Psorophora ferox	4	0	3
Psorophora howardii	0	0	1
Psorophora spp.	0	3	0
Totals	47	58	34

Table 4. Total female mosquitoes collected by gravid traps at the Soils Bioremediation site, Auburn, AL 2003. Figures represent actual specimens collected during twelve 24-hour trapping periods.

Mosquito species	Females collected
Aedes albopictus	732
Aedes vexans	13
Ochlerotatus triseriatus	5
Aedes/Ochlerotatus spp.	4
Culex erraticus	0
Culex nigripalpus	2
Culex quinquefasciatus	2449
Culex restuans	46
Culex salinarius	0
Culex territans	4
Culex spp.	1577
Orthopodomyia signifera	5
Psorophora spp.	1
Total	4838

Table 5. Total *Culex* females collected by gravid traps with 4 experimental attractants at the Soils Bioremediation site, Lee Co., AL, 2003. Figures represent combined numbers of actual specimens collected during twelve 24-hour trapping periods.

Species	Cattail	Hay	Rush	Sedge	Totals
Culex nigripalpus	2	0	0	0	2
Culex quinquefasciatus	170	1532	146	584	2432
Culex restuans	3	38	1	4	46
Culex territans	2	1	0	1	4
Culex spp.	69	836	78	588	1571

Culex quinquefasciatus: 63% (1532/2449) of *Cx. quinquefasciatus* females were collected by gravid traps with hay infusion, 24% (584/2449) with sedge infusion, 7% (170/2449) with cattail infusion and 6% (146/2449) with rush infusion (Figure 1). The variation among infusions was significant (overall $p < 0.0001$). The mean number of females collected by gravid traps with hay infusion was significantly greater than that of traps with infusions of cattail ($p < 0.0001$), rush ($p < 0.0001$) and sedge ($p < 0.0001$). The mean number of females collected by traps with sedge infusion was significantly greater than that of traps with cattail ($p = 0.0002$) and rush ($p = 0.0006$) infusions. No other pair-wise comparisons of infusions showed significant difference.

Culex restuans: 83% (38/46) of *Cx. restuans* females collected by gravid traps at the Soils Bioremediation site were from traps with hay infusion, 9% (4/46) with sedge infusion, 7% (3/46) with cattail infusion, and 2% (1/46) with rush infusion (Figure 2). The variation among infusions was significant (overall $p < 0.0001$) when compared by the mean number of females collected by gravid traps with each infusion type. The mean number of females collected by traps with hay infusion was significantly greater than that of traps with infusions of cattail ($p < 0.0001$), rush ($p < 0.0001$), and sedge ($p = 0.0001$). No other pair-wise comparisons of infusions showed significant difference.

Culex nigripalpus: 100% (2/2) of *Cx. nigripalpus* females collected by gravid traps at the Soils Bioremediation site were from traps with cattail infusion. The variation among infusions was not significant (overall $p < 0.1171$).

Light traps operated concurrent with gravid traps at the Soils Bioremediation site yielded 53 intact *Culex* specimens and approximately 200 mosquito thoraxes. The dismembered bodies (presumably of *Culex* spp.) resulted from ant predation inside the

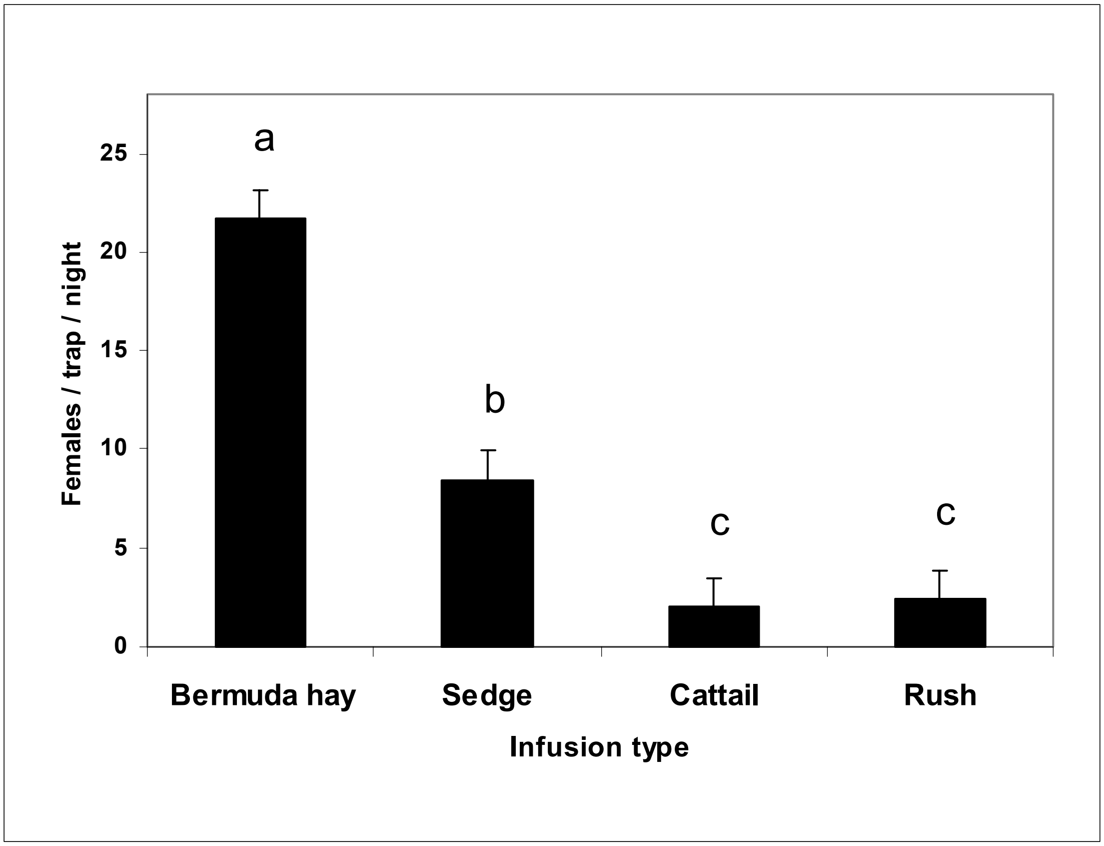

Figure 1. Mean number of *Culex quinquefasciatus* females collected by gravid traps with 4 different experimental infusions during 12 trapping occasions, Soils Bioremediation site, Lee Co., Alabama, 2003.

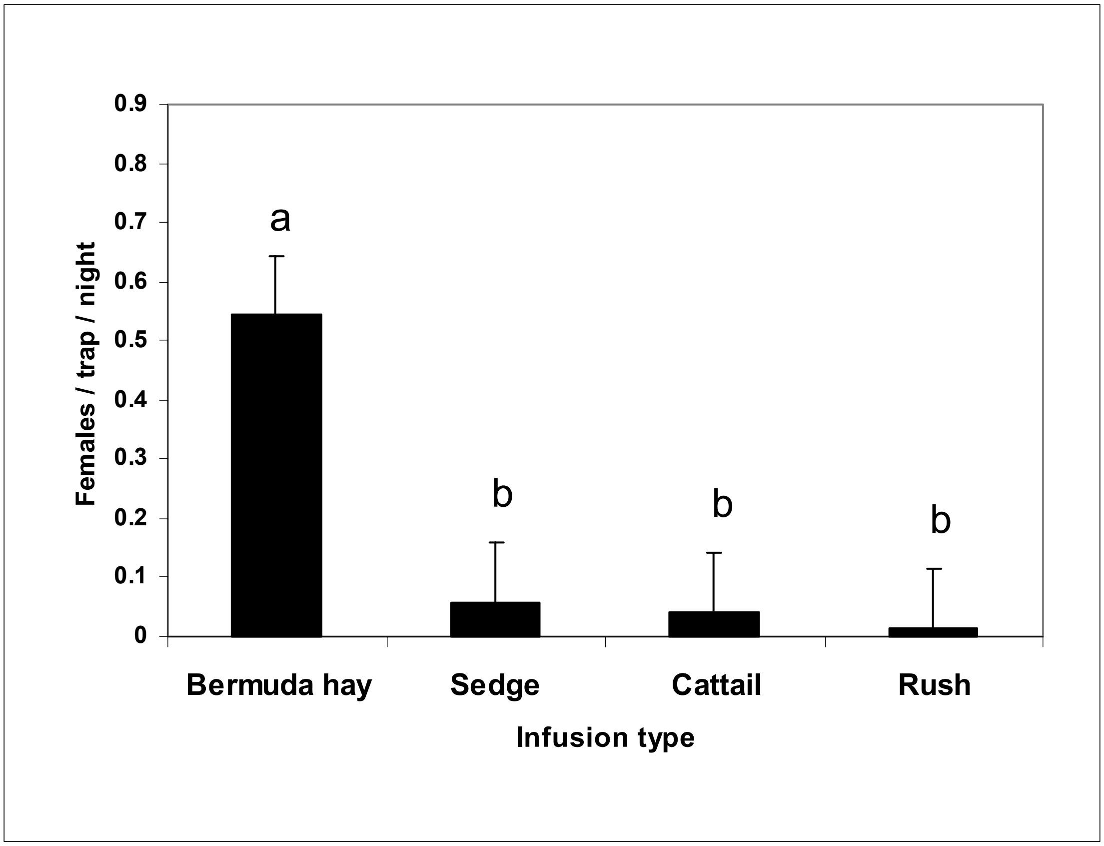

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Figure 2. Mean number of *Culex restuans* females collected by gravid traps with 4 different experimental infusions during 12 trapping occasions, Soils Bioremediation site, Lee Co., Alabama 2003.

trap. A list of mosquitoes and their respective totals collected by light traps at this site is provided in Table 6. *Culex quinquefasciatus* was collected by light traps on all 3 trapping sessions (8/1/2003, 9/5/2003 and 9/17/2003); *Cx. restuans* on 1 of 3 trapping sessions (9/17/2003); *Cx. nigripalpus* on 2 of 3 trapping sessions (9/5/2003 and 9/17/2003); and *Cx. erraticus* on 2 of 3 trapping sessions (8/1/2003 and 9/17/2003). *Culex salinarius* was not recovered from light traps at the Soils Bioremediation site.

2004 Field Evaluations

Due to low numbers of mosquitoes collected at the Tuskegee National Forest site in 2003, trapping was not performed at this site in 2004.

At the Soils Bioremediation site, 4607 mosquitoes were collected by gravid traps during a total of 4 trapping occasions for an average of 48 mosquitoes per trap per night. A list of mosquito species collected by gravid traps, together with the total numbers of females collected at this site, are provided in Table 7.

Culex spp. comprised 97% (4472/4607) of total collections. Twenty-four percent of the total *Culex* collections (1073/4472), were not identifiable to species due to damaged specimens.

Culex quinquefasciatus females accounted for >99% (n=3372) of the identified *Culex* collections and *Culex restuans* < 1% (n=27). Females of other *Culex* spp. were not collected by gravid traps at this site in 2004. Table 8 presents the total numbers of females captured by gravid traps with 4 infusion types for each species of *Culex* collected at the Soils Bioremediation site.

Table 6. Total female mosquitoes collected by carbon-dioxide-baited light traps during 3 trapping occasions at the Soils Bioremediation site in Lee Co, AL, 2003. Figures represent the number of specimens collected during a 24-hour trapping period.

Mosquito species	8/1/2003	9/5/2003	9/17/2003
Aedes albopictus	0	0	4
Aedes vexans	0	1	21
Aedes/Ochlerotatus spp.	0	0	1
Anopheles punctipennis	0	0	4
Culex erraticus	9	0	3
Culex nigripalpus	0	1	3
Culex quinquefasciatus	24	1	2
Culex restuans	0	0	1
Culex spp.	8	ca. 200	1
Psorophora columbiae	6	0	1
Totals	47	203	41

Table 7. Total female mosquitoes collected by gravid traps at the Soils Bioremediation site, Auburn, AL 2004. Figures represent actual specimens collected during twelve 24-hour trapping periods.

Mosquito species	Females collected
Aedes albopictus	134
Anopheles punctipennis	1
Culex erraticus	0
Culex nigripalpus	0
Culex restuans	27
Culex quinquefasciatus	3375
Culex salinarius	0
Culex territans	0
Culex spp.	1073
Total	78

Table 8. Total *Culex* females collected by gravid traps with 4 experimental attractants, Soils Bioremediation site, Lee Co., AL, 2004. Figures represent combined numbers of actual specimens collected during four 24-hour trapping periods.

Species	Cattail	Hay	Rush	Sedge	Totals
Culex quinquefasciatus	399	1239	894	839	2371
Culex restuans	0	1	7	19	27
Culex spp.	102	374	227	370	1073

Culex quinquefasciatus: 37% (1239/3372) of *Cx. quinquefasciatus* females were collected by gravid traps with hay infusion, 25% (839/3372) with sedge infusion, 12% (399/3372) with cattail infusion, and 27% (894/3372) with rush infusion (Figure 3). The variation among infusions was significant (overall $p < 0.0001$) when compared by the mean number of females collected by gravid traps with each infusion type. The mean number of females collected by traps with hay infusion was significantly greater ($p < 0.0001$) than that of traps with cattail infusion, marginally greater than that of rush infusion ($p = 0.0472$) and not significantly different than traps with sedge infusion ($p = 0.2176$). The mean number of females collected by traps with sedge infusion was significantly greater than that of cattail infusion ($p = 0.0244$). No other pair-wise comparisons of infusions showed significant difference.

Culex restuans: 70% (19/27) of *Cx. restuans* females were collected by gravid traps with sedge infusion, 26% (7/27) with rush infusion, 4% (1/27) with hay infusion, and 0% with cattail infusion (Figure 4). Variation among infusions was significant (overall $p = 0.0021$) when compared by the mean number of females collected by gravid traps with each infusion type. The mean number of females collected by traps with sedge infusion was significantly greater than that of cattail ($p = 0.0030$) and hay infusions ($p = 0.0071$), but not significantly different than that of rush infusion ($p = 0.1704$). No other pair-wise comparisons of infusions showed significant difference.

Light traps operated concurrently with gravid traps at the Soils Bioremediation site yielded 37 mosquito females. *Culex quinquefasciatus* was collected on 2 of 3 trapping sessions (6/8/2004 and 6/17/2004), *Cx. restuans*: 1 of 3 trapping sessions (6/8/2004); and *Cx. erraticus:* 1 of 3 trapping sessions (6/17/2004). *Culex salinarius* and *Cx. nigripalpus*

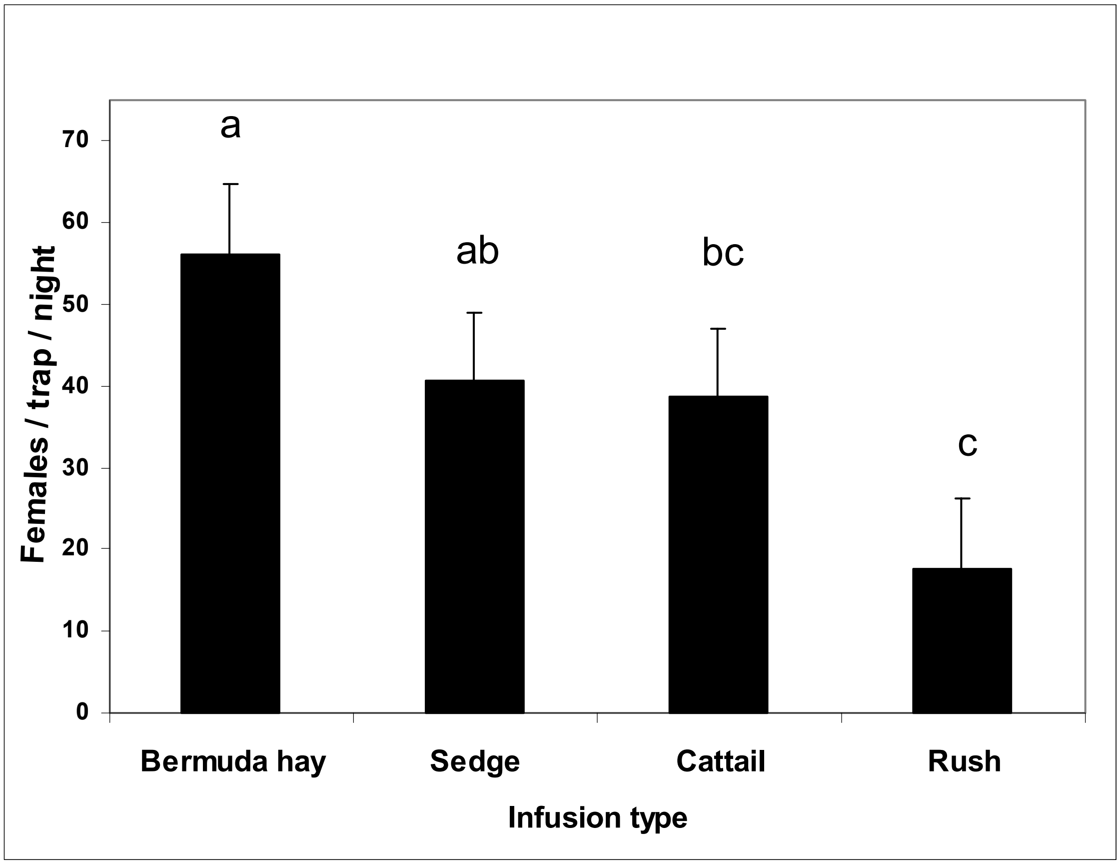

Figure 3. Mean number of *Culex quinquefasciatus* females collected by gravid traps with 4 different experimental infusions during 4 trapping occasions, Soils Bioremediation site, Lee Co., Alabama 2004.

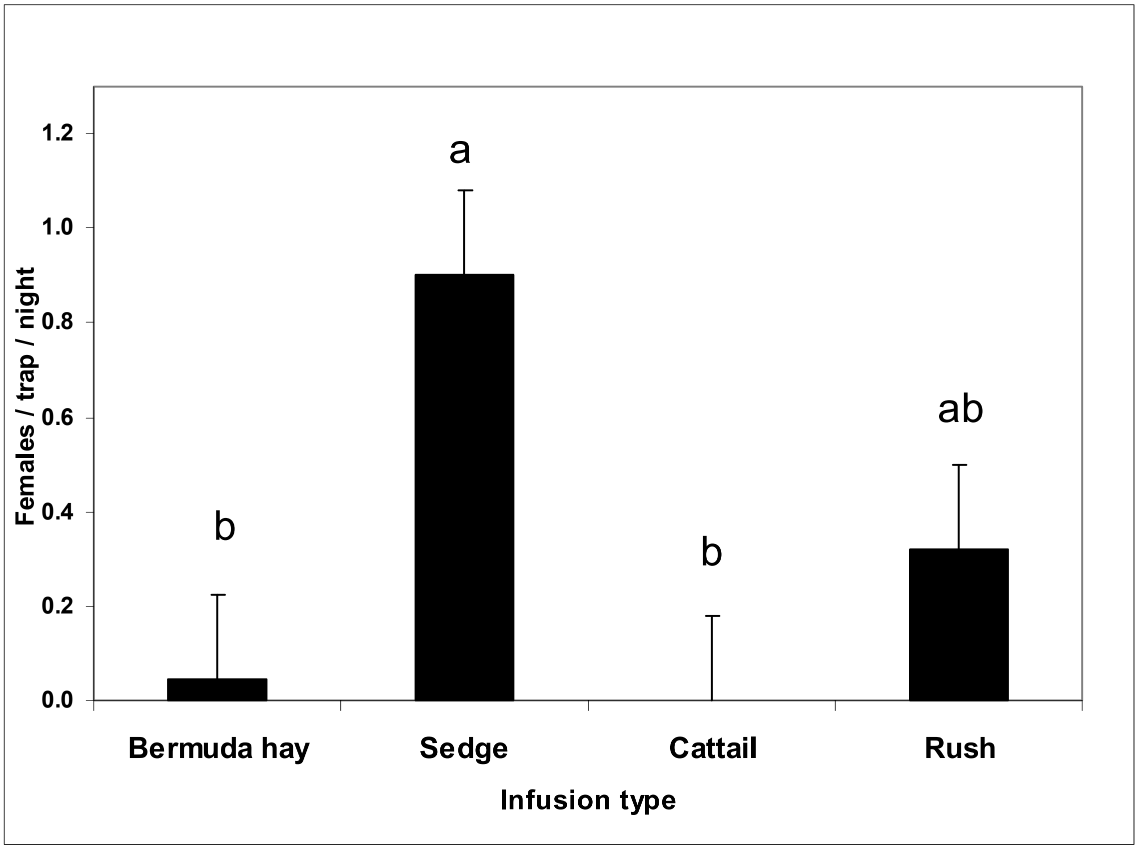

Figure 4. Mean number of *Culex restuans* females collected by gravid traps with 4 different experimental infusions during 4 trapping occasions, Soils Bioremediation site, Lee Co., Alabama 2004.

were not recovered from light traps at the Soils Bioremediation site in 2004. A list of the mosquito species collected by light traps at this site and their respective totals is provided in Table 9.

Discussion

The low numbers of female mosquitoes collected by traps at the Tuskegee National Forest site during the 2003 season were disappointing. The site was chosen based on the abundance and diversity of *Culex* larvae collected in ovitraps in preliminary experiments in the spring of the same year. Although initial gravid-trap collections were meager, work was continued at this site in the hope that the size of gravid-trap collections would increase as the season progressed. Unfortunately this did not happen. Because of the small numbers of female mosquitoes collected by gravid traps at this site, no meaningful conclusions could be drawn from these experimental data.

At the Soils Bioremediation site sufficient numbers of *Cx. quinquefasciatus* and *Cx. restuans* were collected in both 2003 and 2004 to show significant difference among infusions for their attractiveness to ovipositing females of these 2 species. Gravid traps with hay infusion collected significantly greater numbers *Cx. quinquefasciatus* than traps with any other infusion in both years (Figs. 1 and 3). In fact, the relative attractiveness of all 4 infusions to *Cx. quinquefasciatus* females did not change from 2003 to 2004, with the exception of the 2 least attractive infusions (cattail and rush). However, these 2 infusions were not determined to be significantly different from one another in either year.

Table 9. Total female mosquitoes collected by carbon-dioxide-baited light traps during 3 trapping occasions at the Soils Bioremediation site in Lee Co, AL, 2004. Figures represent the number of specimens collected during a 24-hour trapping period.

Mosquito species	6/8/2004	6/17/2004	6/22/2004
Aedes albopictus	3	6	0
Aedes atlanticus	0	0	1
Aedes vexans	3	0	0
Anopheles crucians	1	0	0
Anopheles punctipennis	0	1	3
Culex erraticus	0	1	0
Culex quinquefasciatus	0	3	14
Culex restuans	1	0	0
Totals	8	11	18

In 2003 traps with hay infusion collected the most females of *Cx. restuans* followed, in order, by sedge, cattail and rush (Fig. 2). In 2004 traps with sedge infusion collected the most females of *Cx. restuans* followed, in order, by rush, hay and cattail (Fig. 4). It should be noted that in 2004 the mean number of *Cx. restuans* females collected by traps with sedge, cattail and rush infusions did not differ significantly from 0. These extreme fluctuations in relative attractiveness from 2003 to 2004 probably can be explained best by the low numbers of female *Cx. restuans* (46 and 27 respectively) collected during the study as well as the infrequency and sporadic nature of those collections.

Although *Cx. nigripalpus* was occasionally encountered in gravid-trap collections, females of this species were not collected in sufficient numbers for conclusions to be drawn from the data. While both gravid-trap collected females were recovered from traps with cattail infusion (Table 5) at the Soils Bioremediation site in 2003, the single *Cx. nigripalpus* female collected at the Tuskegee National Forest site was from a trap containing rush infusion. Light-trap collections showed this species to be present during the 2nd and 3rd trapping occasions (9/5/2003 and 9/17/2003) when light traps were operated, although in low numbers (Table 6). The low numbers of *Cx. nigripalpus* collected by all traps during the entire season may reflect a small population size for this species at this field site.

Culex salinarius was not recovered from either gravid traps or light traps at the Soils Bioremediation site. Further investigation of the attractiveness of experimental infusions to *Cx. salinarius* and *Cx. nigripalpus* females will require identification of a suitable field site in which these species are more abundant.

In conclusion, gravid traps were found to be an effective tool for collective blood-fed females of some *Culex* spp. (particularly *Cx. quinquefasciatus*) and hay infusion was determined to be the infusion of choice for use in gravid traps. While this comparative field evaluation of attractants for collection of *Culex* females showed significant difference among the 4 infusions for their attractiveness to *Cx. quinquefasciatus* and *Cx. restuans*, efforts to identify attractants for other *Culex* spp. were largely unsuccessful.

III. FIELD COMPARISON OF GRAVID-TRAP INFUSIONS FOR CAPTURING CONTAINER-BREEDING *AEDES* AND *OCHLEROTATUS* FEMALES

Objective: To compare the attractiveness of infusions made from commercially available lawn and garden products to an oak-leaf infusion standard for attraction of ovipositing *Aedes* and *Ochlerotatus* mosquitoes in Alabama

Study Sites

Study sites to evaluate infusions for collecting *Aedes* and *Ochlerotatus* females were located in Lee County, AL. The first site, a wooded lot located in a suburban area within the Auburn city limits, was situated behind the Dean Road Recreation Center, 307 South Dean Road. The site was characterized by dense vegetation consisting of water oak; *Quercus nigra*, slash pine; *Pinus elliotti*, southern magnolia; *Magnolia grandiflora*, dogwood; *Cornus florida*, greenbriar; *Smilax laurifolia*, and muscadine vine; *Vitis rotundifolia.*

The second site was located in an Automobile Salvage Yard on county road 240 in Phenix City, AL. The land on which this facility was located sloped gradually towards the center of the property, where water drained over red clay and collected in a shallow depression. The area supported a variety of wetland plants including sweetgum, *Liquidambar styraciflua*; swamp willow, *Salix caroliniana*; red maple, *Acer rubrum*;

swamp tupelo, *Nyssa biflora*; broadleaf cattail, *Typha latifolia*; marsh rush, *Juncus effuses;* and sedges, *Rhynchospora* spp.

Materials and Methods

Evaluation of gravid-trap infusions for collection of ovipositing *Aedes* and *Ochlerotatus* females was conducted in spring and summer of 2004. Seven commercially available lawn and garden products were initially selected and used to produce infusions. After initial collections 2 additional materials were added to the original 7 and used to produce infusions. From these 9 infusions 3 were experimentally selected which showed to be the most attractive to ovipositing *Aedes* and *Ochlerotatus* females. These 3 infusions were then used in a field evaluation to compare their attractiveness with that of an oak-leaf infusion that has shown to be attractive to *Ae. albopictus* females (Trexler et al., 1998).

Initially infusions were produced from the following 7 commercially available lawn and garden products: organic humus, potting mix, pine mulch, dyed (red) hardwood mulch, pine straw, cypress mulch, and cedar shavings. Infusion preparation followed the protocol of Reiter (1983). Infusions were prepared in the first week of May. After a week of fermentation approximately 1 gallon of each infusion was poured into separate plastic containers and placed in each of 2 sites in Lee and Macon Counties, AL. Sites included a hardwood bottomland in the Choctafaula Creek drainage in Tuskegee National Forest in Macon Co. and a suburban residence on Ogletree Road, Auburn, Lee Co., AL. Larvae which hatched from eggs deposited by mosquitoes in these containers were collected and identified to species to help determine the infusions which were the most attractive to ovipositing *Aedes* and *Ochlerotatus* females. Larvae were collected twice at each site approximately 1 and 2 weeks after containers were initially set out. After some preliminary collections several problems became apparent with this method. (1) Larvae of *Toxorhynchites rutilus*, a species of mosquito which as larvae are predaceous upon other mosquito larvae, were frequently recovered from larval samples. These larvae have a voracious appetite and can consume many mosquito larvae through the course of their development. Efforts to remove and exclude them from collection containers before they preyed upon other mosquito larvae proved to be impractical. (2) Many females of *Aedes* spp. exhibit a behavior known as "skip oviposition" in which ovipositing females deposit eggs in several different sites rather than deposit them all in 1 place. It is difficult to know if conspecific larvae from a given collection container originated from the same or from different females. (3) Pupae were often present at the time collections were made. Identification of pupae to even the generic level is very difficult. As a result of these problems, no meaningful quantitative conclusions could be drawn. For these reasons alternative methods for selecting infusions for collecting female *Aedes* and *Ochlerotatus* were considered.

Composted horse manure and organic compost were added to the list of lawn and garden products used to produce attractants. Infusions were made from these 2 and the original 7 materials following the protocol outlined above. Nine gravid traps, each with a different infusion, were operated for 24- or 48-hour periods in a wooded lot located in a suburban area of the city of Auburn in Lee Co., AL. Six collections were made over the following 2-week period to identify the 3 infusions which appeared to be more attractive to *Aedes* and *Ochlerotatus* females.

The 3 materials selected from these preliminary experiments, along with oak-leaf litter, were used to produce infusions following the same protocol as previous experiments. These 4 infusions were then compared via gravid traps for their attractiveness to container-breeding *Aedes* and *Ochlerotatus* females at the 2 sites in Lee Co., AL outlined in "Study Sites."

Twenty-four gravid traps were run at each site. Each of the 4 infusion types were replicated 6 times for each trapping occasion at both sites. At the suburban wooded lot site traps were run overnight on 10 different occasions over an 8-week period (8/23 to 10/22/2004). At the Automobile Salvage Yard traps were run overnight on 7 different occasions over a 6-week period (8/31 to 10/14/2004). Fresh infusion was added to gravid traps for each trapping session. Trapping sessions were initiated in late afternoon and collections were retrieved approximately 24 hours later.

Carbon dioxide-baited CDC miniature light traps were operated at each site. Light traps were run concurrently with gravid traps during every trapping session. Landing counts (i.e., collecting female mosquitoes attempting to take a blood-meal) were also conducted to gain additional information on the activity of species of interest in this study. For two 2-minute periods at each site female mosquitoes landing on left leg below the knee of a human male were collected using a battery-powered aspirator.

Female mosquitoes collected by traps and aspirator were returned to the laboratory for identification.

Collection data were analyzed by ANOVA for variation among mean numbers of female mosquitoes collected by gravid traps with each of the 4 treatments (infusions).

Pair-wise comparisons of infusions were achieved via Tukey-Kramer adjusted statistical test at a 95% confidence level.

Results

Preliminary Studies: Larval Collections

At the Tuskegee National Forest site larvae of 6 mosquito species were collected from containers provided with the 7 experimental infusions: *Aedes albopictus, Ochlerotatus triseriatus, Culex territans, Culex restuans, Anopheles quadrimaculatus* and *Toxorhynchites rutilus.*

Aedes albopictus larvae were collected from containers with pine mulch, cypress mulch and cedar shavings (3 of 7) infusions, *Oc. triseriatus* from containers with potting-mix and dyed hardwood mulch (2 of 7) infusions, and *Tx. rutilus* from containers with organic-humus infusion (1 of 7). Numbers of larvae collected for each species and infusion type at the Tuskegee National Forest site are presented in Table 10.

At the Ogletree Road site larvae of 4 mosquito species were collected from containers with the 7 experimental infusions: *Ae. albopictus, Oc. triseriatus, Cx. territans,* and *Tx. rutilus.*

Aedes albopictus larvae were collected from containers with each of the 7 infusion types: organic humus, potting mix, pine mulch, pine straw, dyed hardwood mulch, cypress mulch, and cedar shavings; *Oc. triseriatus* with 5 of the 7 infusions: organic humus, potting mix, pine mulch, dyed hardwood mulch, and cypress mulch; *Tx. rutilus* with 6 of 7 infusions: potting mix, pine mulch, pine straw, dyed hardwood mulch, cypress mulch and cedar shavings (Table 11).

Table 10. Total immature mosquitoes collected from containers with 7 experimental infusions from 2 collections at a hardwood bottomland site in Tuskegee National Forest, Macon Co., Alabama, 2004.

Mosquito species	Organic humus	Potting mix	Pine mulch	Pine straw	Dyed hardwood mulch	Cypress mulch	Cedar shavings
Ae. Albopictus	0	0	5	0	0	3	9
Oc. Triseriatus	0	1	0	0	1	0	0
An. quadrimaculatus	1	10	1	1	0	3	9
Tx. Rutilus	2	10	1	1	0	3	7
Cx. restuans	25	37	113	7	24	0	1
Cx. territans	40	35	73	201	18	1	157
Pupae	3	2	9	12	0	0	0
Totals	71	95	202	222	43	10	183

Table 11. Total immature mosquitoes collected from containers with 7 experimental infusions from 2 collections at a residential area, Ogletree Road, Auburn, Lee Co., AL, 2004.

Mosquito species	Organic humus	Potting mix	Pine mulch	Pine straw	Dyed hardwood mulch	Cypress mulch	Cedar shavings
Ae. albopictus	55	10	20	11	3	25	9
Oc. triseriatus	14	8	2	0	7	1	0
An. quadrimaculatus	0	0	0	0	0	0	0
Tx. rutilus	0	1	1	3	2	6	10
Cx. territans	0	0	52	6	14	12	14
Totals	69	19	75	20	26	44	33

Preliminary Studies: Gravid-trap Collections

Adult females of 4 mosquito species (*Ae. albopictus, Oc. triseriatus, Cx. quinquefasciatus* and *Tx. rutilus*) were recovered from gravid traps containing 9 experimental infusions during 6 trapping sessions at the Dean Road site (Table 12).

Aedes albopictus, the most commonly collected mosquito species, was recovered from gravid traps on all 6 trapping occasions. A total of 460 *Ae. albopictus* females were collected during this preliminary study. Gravid traps with infusion of composted manure collected the highest number of *Ae. albopictus* females (79), followed by dyed hardwood mulch (55), then pine-straw and potting-mix (50 each) (Table 13).

During 3 of 6 trapping occasions, composted-manure infusion ranked first among all test infusions in the number *Ae. albopictus* females collected and ranked 4^{th}, 5^{th} or 6^{th} on other trapping occasions. Pine-straw infusion ranked 2^{nd} in number of *Ae. albopictus* females collected on 3 of 6 trapping occasions and ranked 3^{rd}, 5^{th} and 7^{th} on other trapping occasions. On 3 of 6 trapping occasions infusion of dyed hardwood mulch ranked 2^{nd} in number of *Aedes albopictus* females collected. This infusion ranked 5^{th}, 6^{th} or 8^{th} on other trapping occasions. Rankings for all infusions for the 6 trapping sessions are shown in Table 14. A ranking of 1 implies that a gravid trap with a particular infusion collected more females of *Ae. albopictus* on a particular trapping occasion than did traps with other infusions.

Based upon these results composted manure, pine straw and dyed hardwood mulch were selected to produce infusions for use in a field experiment comparing their attractiveness to that of an oak leaf infusion for collecting container-breeding *Aedes* and *Ochlerotatus* females.

Table 12. Total female mosquitoes collected from gravid traps with 9 experimental infusions from 6 collections at a suburban forested lot, Dean Road, Auburn, Lee Co., AL, 2004.

Mosquito species	Organic humus	Potting mix	Organic Compost	Pine mulch	Pine straw	Dyed hardwood mulch	Cypress mulch	Cedar shavings	Composted manure
Ae. albopictus	49	50	43	45	50	55	42	9	79
Oc. triseriatus	0	2	0	1	2	2	1	2	3
Cx. quinquefasciatus	14	3	2	23	2	6	0	1	2
Tx. Rutilus	0	0	0	0	1	0	1	0	2

Table 13. *Aedes albopictus* females collected from gravid traps with 9 experimental infusions from 6 collections at a suburban forested lot, Dean Road, Lee Co., AL, 2004.

Trapping period	Organic humus	Potting mix	Organic Compost	Pine mulch	Pine straw	Dyed hardwood mulch	Cypress mulch	Cedar shavings	Composted manure
21-23 July	9	13	9	21	8	24	16	9	37
27-29 July	9	9	9	5	5	11	5	6	14
29-30 July	6	4	5	6	6	7	11	4	4
2-4 August	12	11	13	1	14	10	6	10	16
4-5 August	2	11	6	6	9	3	1	7	6
5-6 August	11	2	1	6	8	0	3	4	2
Total	49	50	43	45	50	55	42	47	79

Table 14. Ranking of the number of *Aedes albopictus* females collected by gravid traps with 9 experimental infusions. A ranking of 1 indicates that traps with that infusion collected the highest total of females relative to traps with other infusions. Suburban forested lot, Dean Road, Lee Co., AL, 2004.

Trapping period	Composted manure	Pine straw	Dyed hardwood mulch	Pine mulch	Organic Humus	Potting mix	Organic Compost	Cypress mulch	Cedar shavings
21-23 July	1	7	2	3	6	5	8	4	9
27-29 July	1	5	2	5	3	3	3	5	4
29-30 July	5	3	2	3	3	5	4	1	5
2-4 August	1	2	6	8	4	5	3	7	6
4-5 August	4	2	5	4	6	1	4	7	3
5-6 August	6	2	8	3	1	6	7	5	4

Field Evaluation

At the suburban wooded lot site at total of 1327 mosquito females was captured by gravid traps. Combined collections were comprised of *Ae. albopictus,* 30.2% (401/1327); *Oc. triseriatus,* 1.4% (18/1327); *Cx. quinquefasciatus*: 54.5% (729/1327); *Cx. restuans,* 5.6% (74/1327); *Cx. nigripalpus,* 6.0% (80/1327). *Culex erraticus, Or. signifera* and unidentified *Culex* spp. Comprised the remaining 2.2% of collections. A complete list of mosquito species collected by gravid traps at this site is provided in Table 15, along with the total numbers of females collected.

Aedes albopictus: 27.7% (111/401) of *Ae. albopictus* females were collected by gravid traps with composted manure infusion, 27.7 % (111/401) with oak-leaf infusion, 22.7 % (91/401) with dyed (red) hardwood mulch infusion, and 21.9 % (88/401) with pine-straw infusion (Figure 5). The variation among infusions was not significant (overall p = 0.5228) when compared by the mean number of *Ae. albopictus* females collected by gravid traps with each infusion type.

Ochlerotatus triseriatus: 38.9% (7/18) of *Oc. triseriatus* females were collected by gravid traps with composted manure infusion, 33.3 % (6/18) with oak-leaf infusion, 16.7 % (3/18) with dyed (red) hardwood mulch infusion, and 11.1 % (2/18) with pine-straw infusion (Figure 6). The variation among infusions was not significant (overall p = 0.3075) when compared by the mean number of *Oc. triseriatus* females collected by gravid traps with each infusion type.

At the Automobile Salvage Yard a total of 1869 mosquito females was captured by gravid traps. The following mosquito species were collected in gravid traps: *Ae. albopictus,* 38.4% (718/1869); *Oc. triseriatus,* 0.4% (7/1869); *Cx. quinquefasciatus,*

Table 15. Total female mosquitoes collected by gravid traps at a suburban wooded-lot site, suburban forested lot, Dean Road, Lee Co., AL, 2004. Figures represent actual specimens collected during ten 24-hour trapping periods.

Mosquito species	Females collected
Aedes albopictus	402
Ochlerotatus triseriatus	18
Culex erraticus	1
Culex nigripalpus	80
Culex quinquefasciatus	729
Culex restuans	74
Culex territans	1
Culex spp.	17
Orthopodomyia signifera	5
Total	1327

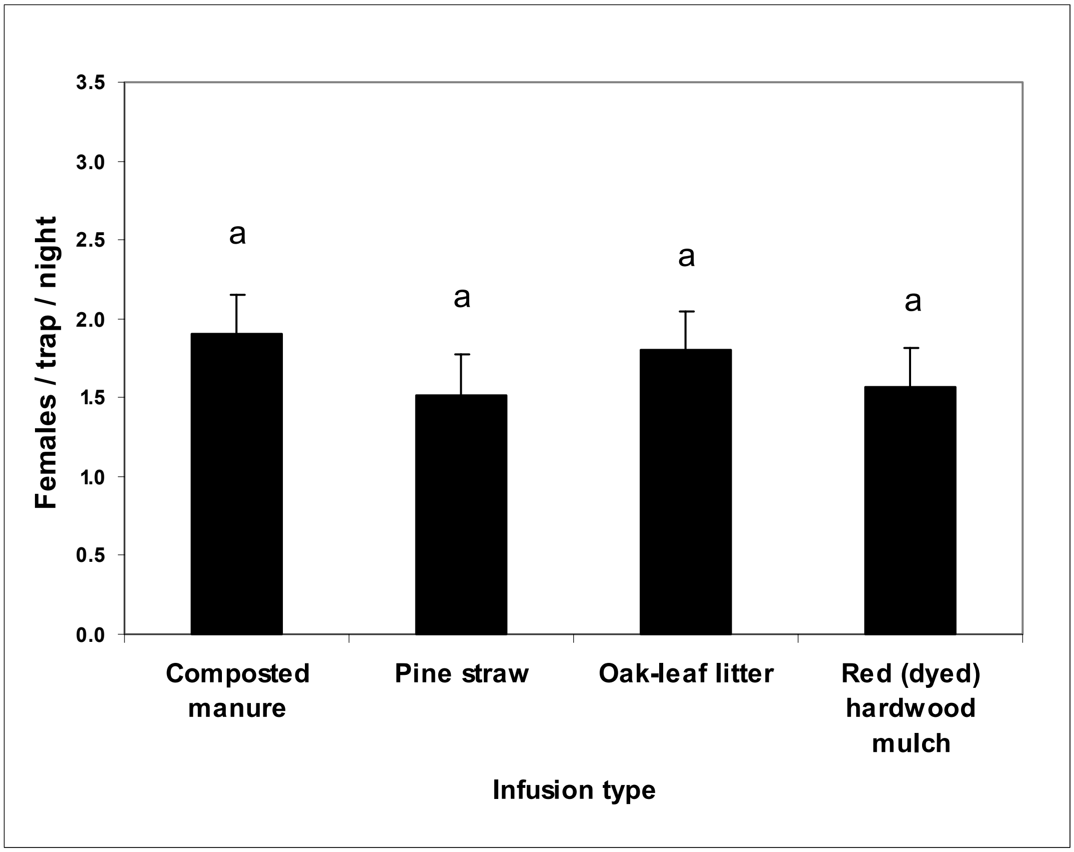

Figure 5. Mean number of *Aedes albopictus* females collected by gravid traps with 4 different experimental infusions during ten 24-hour trapping occasions at a suburban forested lot, Dean Road, Lee Co., Alabama, 2004.

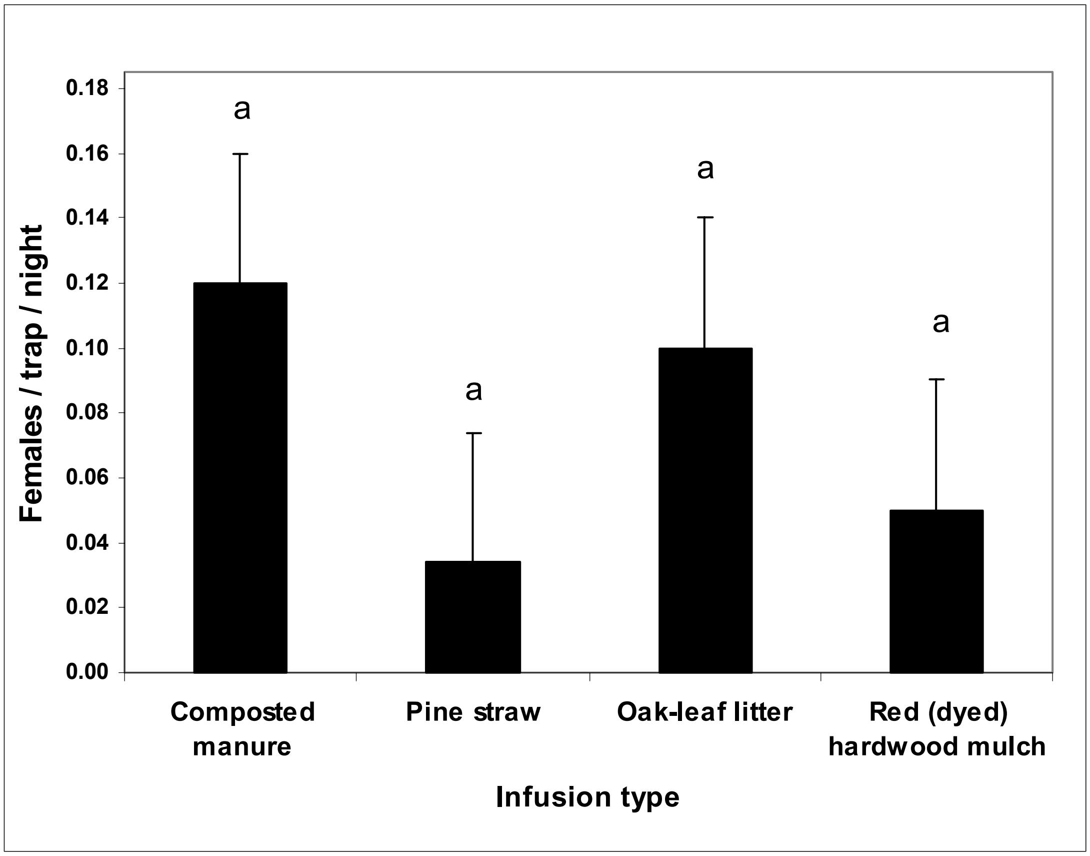

Figure 6. Mean number of *Ochlerotatus triseriatus* females collected by gravid traps with 4 different experimental infusions during ten 24-hour trapping occasions at a suburban forested lot, Dean Road, Lee Co., Alabama, 2004.

50.6% (945/1869); *Cx. restuans,* 1.8% (33/1869); and *Cx. nigripalpus,* 3.7% (69/1869). Females of unidentified *Culex* spp. represented 4.4% (83/1869) of the total mosquito collections. Females of *Ae. vexans, An. crucians, Cx. territans*, *Or.signifera, Ps. ferox, Ur. sapphirina* and unidentified *Culex* spp. accounted for the remaining 0.7% of collections. A complete list of mosquito species collected by gravid traps at this site is provided in Table 16 along with the total numbers of females collected.

Aedes albopictus: 28.6% (205/718) of *Ae. albopictus* females were collected by gravid traps with dyed (red) hardwood mulch infusion, 26.0 % (187/718) with pine straw infusion, 24.0 % (172/718) with composted manure infusion, and 21.4 % (154/718) with oak-leaf infusion (Figure 7). The variation among infusions was not significant (overall p = 0.6558) when compared by the mean number of *Ae. albopictus* females collected by gravid traps with each infusion type.

Ochlerotatus triseriatus: 28.6% (2/7) of *Oc.* *triseriatus* females were collected by gravid traps with oak-leaf infusion, 28.6% (2/7) with pine-straw infusion, 28.6% (2/7) with dyed (red) hardwood mulch infusion, and 14.3% (1/7) with composted manure infusion (Figure 8). The variation among infusions was not significant (overall p = 0.3075) when compared by the mean number of *Oc. triseriatus* females collected by gravid traps with each infusion type.

Landing Counts

At the suburban Dean Road site sixteen 2-minute-long landing counts yielded a total of 89 female mosquitoes of 2 species: *Ae. albopictus* and *Ps. ferox*. A total of 84 *Ae. albopictus* females was captured during landing–count collections, with an average of

Table 16. Total female mosquitoes collected by gravid traps at the Automobile Salvage Yard site, Lee Co., AL, 2004. Figures represent actual specimens collected during ten 24-hour trapping periods.

Mosquito species	Females collected
Aedes albopictus	718
Aedes vexans	3
Ochlerotatus triseriatus	7
Anopheles crucians	1
Culex nigripalpus	69
Culex quinquefasciatus	945
Culex restuans	33
Culex territans	3
Culex spp.	83
Orthopodomyia signifera	4
Uranotaenia sapphirina	3
Total	1869

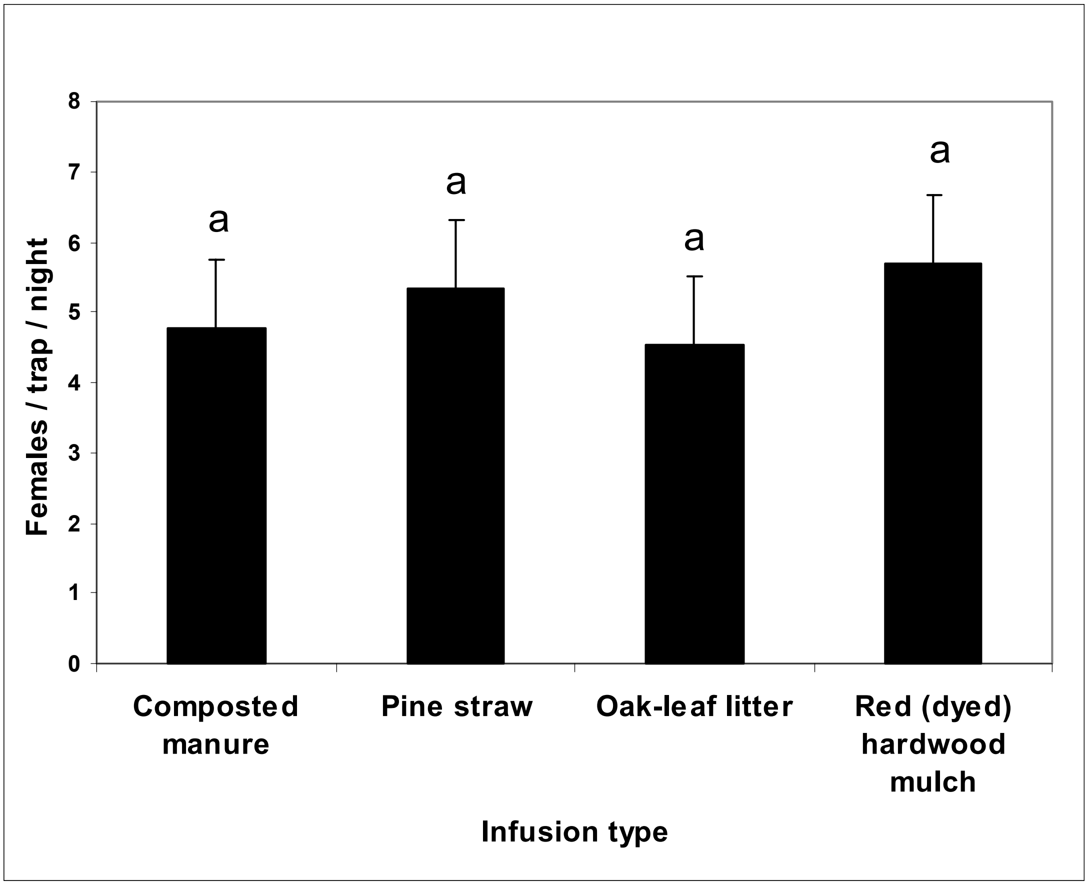

Figure 7. Mean number of *Aedes albopictus* females collected by gravid traps with 4 different experimental infusions during seven 24-hour trapping occasions at the Automobile Salvage Yard, Lee Co., Alabama, 2004.

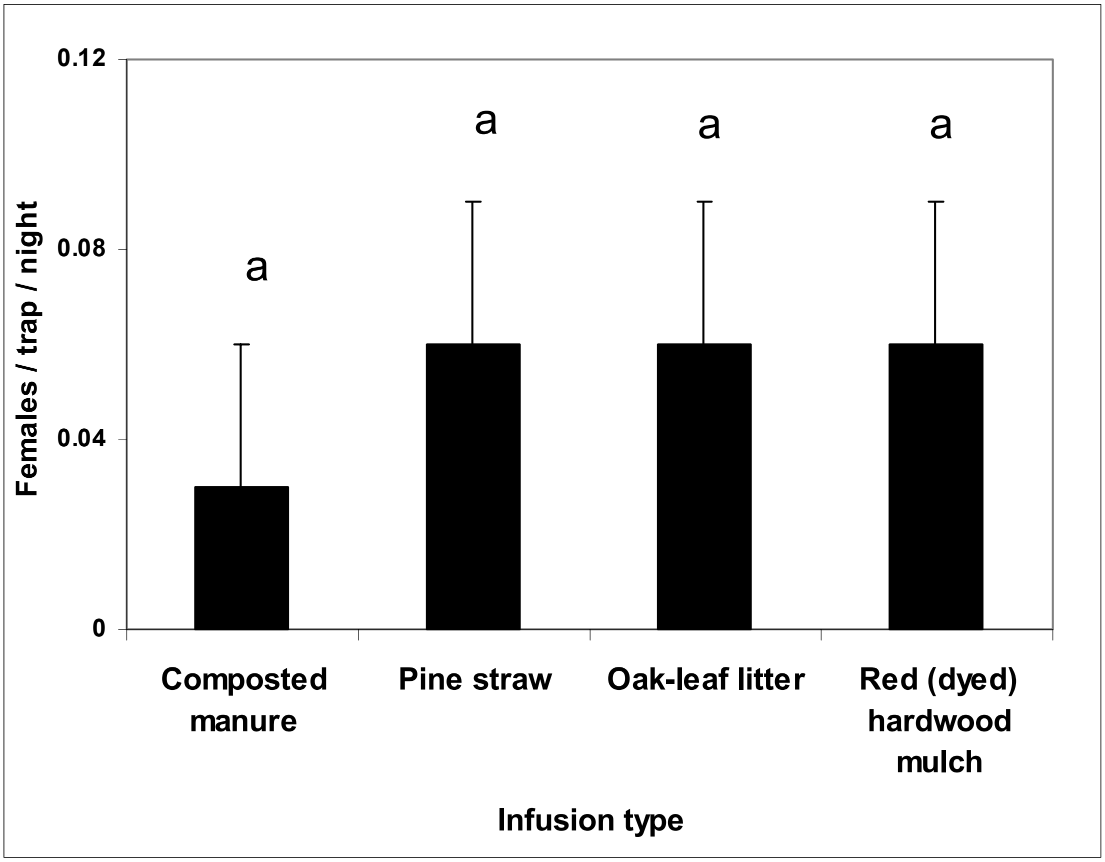

Figure 8. Mean number of *Ochlerotatus triseriatus* females collected by gravid traps with 4 different experimental infusions during ten 24-hour trapping occasions at the Automobile Salvage Yard, Lee Co., Alabama, 2004.

10.5 females per 2-minute period. The number of *Ae. albopictus* collected during landing counts ranged from 0 to 14; *Ps. ferox* from 0 to 1 with a total of 5.

At the Automobile Salvage Yard twelve 2-minute-long landing counts yielded a total of 160 female mosquitoes from 3 species: *Ae. albopictus*, *Oc. triseriatus* and *Ps. ferox*. A total of 158 *Ae. albopictus* females was captured during landing–count collections, with an average of 13.2 females per 2-minute period. The number of *Ae. albopictus* collected during landing counts ranged from 2 to 28. Only 1 specimen each of *Oc. triseriatus* and *Ps. ferox* was collected during landing counts at this site.

Discussion

Use of gravid traps for infusion selection in preliminary experiments alleviated problems associated with collection of larvae. Since *Tx. rutilus* is only predatory in its larval stage, its presence in study sites does not compromise gravid-trap collections. Problems associated with skip oviposition were also averted by collecting adults rather than larvae. An adult female mosquito collected by a trap constitutes a single data point and reduces difficulties associated with data interpretation.

At both the Dean Road site and the Automobile Salvage Yard landing counts demonstrated substantial populations of biting *Ae. albopictus* females. This, however, was not reflected in gravid-trap collections. The greatest mean *Ae. albopictus* females captured per trap in a single night during the entire study was a mere 5.7 collected from gravid traps with dyed (red) mulch infusion at the Automobile Salvage Yard. The greatest number of females recovered from any single gravid trap was 48. This seemed to be an isolated event, because the second greatest number of females recovered from a single trap was 20. This low trap productivity is in sharp contrast to collections of *Cx. quinquefasciatus* from the Soils Bioremediation site earlier the same year. During that study an average of 56.2 *Cx. quinquefasciatus* females were collected by gravid traps with hay infusion, whereas an average of only 0.44 *Ae. albopictus* females was captured by those same traps. The low numbers of *Ae. albopictus* females collected by gravid traps in sites that have relatively large populations of biting females may be due to several factors. (1) *Aedes* and *Ochlerotatus* females typically do not deposit all of their eggs at once as *Culex* females typically do. Instead, they may lay a few eggs at a time in various locations. (2) *Aedes* and *Ochlerotatus* females typically do not oviposit on the water's surface as *Culex* females do. Instead, they oviposit just above the water's surface in a suitable habitat or they may lay their eggs in a low, dry area that will flood at some time in the future. (3) While it is necessary for *Culex* females to locate a larval habitat which is ready for larval development, *Aedes* and *Ochlerotatus* females can oviposit in sites that will be suitable (wet) at some point in the future. These factors allow for a more flexible oviposition strategy and may help to explain why such low numbers of *Aedes* and *Ochlerotatus* females are captured by gravid traps. (4) Competition from other oviposition sites (e.g. automobile tires) may have contributed to the low numbers of container-breeding mosquitoes collected in gravid traps at the Automobile Salvage Yard. Based on the results of this work, with the infusions evaluated, gravid traps may not be an effective tool for collecting great numbers of blood-fed *Aedes* and *Ochlerotatus* females.

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APPENDIX

Infusion Source Characterizations

Composted manure: Garden Basics ® (Wal-mart) Composted Manure: Derived from composted pine fines, composted hardwood fines, chicken or cow manure and sand. Guaranteed analysis: Nitrogen (N) 0.05%, available phosphate (P_2O_5) 0.05%, soluble potash (K_2O) 0.05%.

Pine Straw: Commercially available unlabeled bale of pine straw.

Dyed (red) Hardwood Mulch: Garden Plus ® (Wal-mart) Decorative Soil Cover and Red Colored Mulch:. Mulch Magic (Iron-oxide based) PF Red Dye: Product Code PFM/RED. Hardwood mulch: >50% oak (*Quercus*), organic compost enriched. Sims Bark Co. Tuscumbia, AL.

Pine Mulch: Garden Basics ® (Wal-mart) mulched bark of *Pinus* spp. trees.

Organic compost: Organic ® Mushroom Compost: Derived from wheat straw, fibrous peat, dolomite, gypsum, crushed feathers, cottonseed meal, peanut meal and other ingredients. Guaranteed analysis: Nitrogen (N) 0.05%, available phosphate (P_2O_5) 0.05%, soluble potash (K_2O) 0.02%, Chlorine (Cl) < 0.01%. Packaged by Black Gold Compost Company, Oxford, FL.

Cedar shavings: Ozark ® Cedar Shavings: Shaved wood of cedar trees (Cupressaceae). Mount Ida, AR.

Potting Mix: Miracle·Gro® Moisture Control Potting Mix Enriched with Miracle·Gro® Plant Food: Formulated from 50-60% sphagnum peat moss, coconut husk fibers (coir pith), composted bark fines, perlite, a wetting agent, and fertilizer. Guaranteed analysis: Nitrogen (N) 0.18%, available phosphate (P_2O_5) 0.10%, soluble potash (K_2O) 0.10%. Derived from ammonium nitrate, ammonium phosphate, potassium nitrate, urea, calcium phosphate, potassium sulfate and methylene urea.

Cypress mulch: Robin Hood ®Premium Landscape Cypress Mulch: Mulched bark and wood of cypress (Cupressaceae). Hood Timber Company, Adel, GA.

Organic humus: Garden Basics® (Wal-mart) Organic Humus: No description provided with package; dark soil with high sand content.