AN EVALUATION OF REITER'S MEDIUM AND THREE DIFFERENT POOL SIZES FOR OVIPOOL SURVEILLANCE OF *CULEX TARSALIS*, *CULEX RESTUANS* AND *CULISETA INORNATA* IN MANITOBA.

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Lisa Marie Baspaly

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ABSTRACT

This study was conducted to determine whether hay infusion and smaller ovipools could replace current oviposition surveillance methods. Female *Culex tarsalis* given a choice of oviposition sites in the lab showed a preference for standing tap water over hay infusion (X^2 = 82.9; p<.05). Hay infusion (as described by Reiter in 1983) was not a suitable replacement for the conventional sod infusion in Manitoba. Field experiments were conducted to determine the minimum size of ovipool that could be used for surveillance of *Culex tarsalis*, *Cx. restuans* and *Culiseta inornata*. One point five per cent of egg rafts laid were collected from the smallest pools (10 cm X 10 cm X 15 cm), 47.8% were collected from the medium pools (30 cm X 15 cm X 15 cm) and 50.7 % were collected from the largest pools (40 cm X 30 cm X 15 cm). When frequency of oviposition events were calculated, there was no significant difference in the efficacy of the small, medium, large and traditional meter-square pools used.

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Section 1: General Introduction

Vector-borne pathogens are a major threat to human and animal health (WHO, 1997). Mosquitoes are important vectors of many pathogens, such as those that cause malaria, filariasis, dengue and encephalitis (Kettle, 1984).

There is greater taxonomic diversity in tropical arthropod-borne pathogens. Protozoan and nematode parasites, as well as arboviruses, are more common in tropical areas than in temperate areas (Roberts & Janovy, 1996). Temperate zones such as Canada and the northern United States tend to have less variety in arthropod-borne pathogens, which consist mainly of arboviruses such as Eastern and Western equine Encephalitis (WEE), Saint Louis Encephalitis virus (SLE), Venezuelan Encephalitis (VE), LaCrosse Encephalitis virus and Powassan Encephalitis virus (Artsob & Spence, 1979).

In Canada, during the last 25 years, there have been several outbreaks of encephalitis such as WEE, SLE and West Nile Virus (WNV). For all of those outbreaks, *Culex* spp. and *Culiseta* spp. were the primary targets of mosquito surveillance operations involved in transmission (Helson *et al.*, 1979).

Mosquito surveillance operations are an important part of predicting and controlling encephalitide outbreaks. They are necessary for monitoring the spread of arboviruses and other mosquito-borne pathogens. In Canada, these programs consist of collection and analysis of data from various types of traps including light traps, vertebrate-baited traps (sentinel bird flocks) and oviposition traps (Brust, 1976).

Oviposition traps are an important part of disease surveillance programs by virtue of the biological information they provide (Brust, 1982). Reiter (1983, 1986), Reisen & Meyer (1990) and Weber & Horner (1992) have examined ways of increasing oviposition trap efficiency by searching for a medium that will render an artificial site more attractive than surrounding natural oviposition sites. Oviposition attractants are used in surveillance operations to encourage females to lay eggs in artificial aquatic habitats.

Newly laid eggs are white, but turn black or brown within one or two hours. A rigid, proteinaceous chorion protects the egg. It minimises water loss and is permeable to gases. Females of raft-laying species will lay 30 to 300 eggs per raft, depending on the species, overall nutrition, stage in gonotrophic cycle, and size (Clements, 1999).

Oviposition pools are especially important where *Culex restuans* Theobald, a species of concern for surveillance operations due to its potential capacity to transmit pathogens, is not reliably collected in light traps (Brust, 1990). In Canada, sod infusions contained in 1 m X 1 m X 15 cm pools have been used to monitor *Culex* and *Culiseta* spp. (Brust, 1982; Brust 1990; Buth *et al.*, 1990). There are a number of problems associated with this combination of pool size and infusion. Areas located away from humans, but adjacent to populated areas, are generally chosen as surveillance sites to decrease the risk of tampering. The meter-square frame is large, so it is difficult to carry one or more into an unpopulated area, usually located some distance away from roads

or people. Also, a pool of that size requires approximately 75L of water to fill it to a level where it covers the sod (approximately 5 cm from the top of the ovipool). Most relatively remote areas do not have tap water available nearby, and even many outside urban locations are not so equipped. Seventy-five liters is a large amount of water to transport into a site, and is difficult for one person to carry. Road access or the use of ATVs are possible solutions to this problem, but road access will also allow access to the general public, increasing the risk of tampering. Surveillance operations in Canada are typically limited in terms of money and resources, so the use of ATVs may not always be a feasible option. There are also the logistical problems associated with transporting an ATV and possibly fuel out to the limit of the road access in a remote area. Although surveillance is primarily concerned with mosquitoes in proximity to humans, mosquito populations outside urban areas must also be monitored to identify bridging vectors, amplifiers and the presence of any virus.

There are also problems associated with the sod. A large piece of sod has to be cut and carried into remote areas when setting up pools. Smaller pieces of sod tend to float in large pools. Alternatively, the sod can be dug up on site, but this is not legal or desirable in many areas such as City and Provincial Parks. Also, purchasing sod from a local garden centre can be a problem, as the commercial sod may be over-fertilized to the point where it creates a thick algal bloom in the pools after only a few days.

This research was conducted to examine ways in which mosquito ovipool surveillance in Manitoba could be streamlined. The focus for this study was on ovipools, as this was considered to be the most problematic area at the time this research began. The objectives of this research were:

1) to determine whether gravid female *Culex tarsalis* Linnaeus are more likely to oviposit in a hay infusion medium (as described in Reiter, 1983) vs. distilled / standing tap water, and

2) to determine the minimum size ovipool that can be used for surveillance of *Cx. restuans*, *Cx. tarsalis,* and *Culiseta inornata* Williston in Manitoba.

Section 2: Review of Pertinent Literature

The family Culicidae is divided into three subfamilies: the Culicinae, the Anophelinae and the Toxorhynchitinae (Lane & Crosskey, 1993). Worldwide there are about 3200 species of mosquitoes distributed in 38 genera (Harbach & Kitching, 1998).

Location and selection of an oviposition site is an essential part of the mosquito life cycle. Before an oviposition site is selected, a blood meal is needed for eggs to develop in anautogenous species. Autogenous species may lay their first batch of eggs without a blood meal, relying on nutritional reserves left over from earlier life stages. The blood meal is where most pathogens are acquired. Thus, pathogen transmission requires the completion of one oviposition cycle before pathogen transfer can occur with a subsequent blood meal. Oviposition is an important component of the surveillance of mosquito-borne pathogens (Bentley and Day, 1989). It should be mentioned that for host-pathogen interactions where transovarial transmission occurs, transmission occurs from an infected female to her progeny. These progeny are then sometimes able to infect with their bite, and / or continue the cycle of transovarial transmission (Turell, 1988).

In this chapter, I will broadly examine mosquito oviposition strategies, with a focus on the species that lay their eggs as rafts on the surface of water. These species are of particular importance to disease surveillance in Manitoba. It is assumed here that readers will have a general familiarity with culicid biology

and only short reviews are provided for the more pertinent aspects of their life cycle.

BASIC LIFE HISTORY

The major events in the life of a female mosquito are hatching, larval development, pupation, emergence as an adult, mating, feeding and oviposition. The rate of embryonic development is dependent on temperature but larvae usually take a few days to several weeks to hatch (Lane & Crosskey, 1993). Eggs laid on the surface of water will hatch as soon as embryonic development is complete. Larvae have modified mandibles / maxillae to obtain food from the water column or the substrate. The larvae of most species eat microorganisims (such as protists), detritus (such as decomposing leaves), algae, and dead as well as living invertebrates. Some species also consume other mosquito larvae (Clements, 1999). During pupation, larval structures and organs eventually degenerate and are replaced with the pupal forms from undifferentiated cells in the imaginal discs. When metamorphosis is complete within the pupal cuticle, air is inhaled to increase the internal pressure. This pressure causes the cuticle to split along the ecdysial sutures. The adult then slowly emerges from this split (Lane & Crosskey, 1993; Clements, 1999).

Mosquitoes have diverse life history patterns. There are several traits commonly used to categorize these life histories: the stage in which diapause occurs, number of generations per year (voltinisim) and type of larval habitat

(Lane & Crosskey, 1993). Larval habitat, though not a life history trait in the classical sense, is widely used to categorize life histories. Larval habitats are especially important to identify during surveillance operations, as they will guide the use and placement of ovipools, and direct control efforts such as larviciding. It is also important to be aware of the preferred larval habitats for vector species, as this will be valuable information for any mosquito control / abatement programs. Larval habitat is the only trait used to describe life history that will be discussed here.

LARVAL HABITATS

Larval habitats are determined by where the female mosquito oviposits (Laird, 1988). Clements (1999) divided mosquito oviposition sites into the following six categories: water surfaces of permanent, semi-permanent, or transient ground waters; soil surfaces subject to transient flooding; leaf surfaces of aquatic plants; water or plant surfaces of phytotelmata; other natural cavities; artificial containers. Only those habitats that are relevant to mosquitoes that lay egg rafts, *i.e.* water surfaces, phytotelmata, and natural or artificial containers, will be discussed here.

The term "ground waters" includes ground pools, rock pools (saline and fresh), puddles, hoof prints, stream or river edges (Clements, 1999), rice fields, saline and fresh water marshes (predominantly vegetation with abundant water), saline and fresh water swamps (predominantly water with abundant vegetation),

ponds, ditches, polluted waters, wells and subterranean waters (caves, flooded cellars, etc.) (Lane & Crosskey, 1993). The main differences among ground habitats are size, permanence, exposure to sunlight or shade, chemical composition and aquatic flora. These differences will affect such things as water chemistry and will be a function of environmental factors such as elevation or latitude.

Permanent ground habitats (marshes, swamps, exposed ponds, forest ponds, ditches) usually have permanent vegetation of some sort and are colonized by a variety of mosquito genera, including *Culex, Aedes* Meigen, *Anopheles* Meigen and *Coquillettidia* Edwards (Lane & Crosskey, 1993).

Temporary water is used as an oviposition site for some mosquito species. Small ponds, ruts, hoof prints and puddles are examples of this type of habitat and they are used mainly, but not exclusively, by aedine mosquitoes. These mosquitoes have adapted to these temporary habitats by developing eggs that can resist desiccation (Clements, 1999; Lane & Crosskey, 1993).

Some mosquitoes are found in slow flowing water. In Australasia, larvae of *Culex starckeae* Stone & Knight are found in flowing water, and other species, such as *Anopheles punctipennis* Say, inhabit the edges of shallow streams (Lane & Crosskey, 1993).

Rice fields are another common oviposition site for *Anopheles*, *Culex,* and *Psorophora* species mosquitoes. Rice fields may prolong the developmental season for some species. *Anopheles gambiae* Giles will oviposit in rice fields

during the dry season when transient ground pools are unavailable (Jones & Schreiber, 1994).

Polluted waters are used as oviposition sites by *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar and some others. Polluted waters range from ponds containing decomposing wood from logging operations, cesspits, cesspools and latrines, to ditches in both urban and rural areas. These species are making use of a habitat where there is less competition, as most other species cannot tolerate even a moderate level of organic pollution (Lane & Crosskey, 1993).

Mosquitoes also colonize subterranean waters (Kay *et al.*, 2000). *Culex pipiens molestus* Linneaus breeds in dark underground waters such as cellars and mine shafts in India and Hungary, and possibly elsewhere. *Culex quinquefasciatus* has also been found in mines, while *Anopheles smithii* Theobald is sometimes found in African caves, along with other *Anopheles* species (Lane & Crosskey, 1993; Beaty & Marquardt, 1996). A few species, such as *Anopheles stephensi* Liston are sometimes found in wells (Lane & Crosskey,1993).

Another category of habitat utilized by raft layers is the phytotelmata (Clements, 1999). These include tree holes, leaf axils of bromeliads and of other plants, floral bracts, bamboo, pitcher plants, leaf pools, fallen bracts and fruit husks.

A natural container habitat is a very small, isolated pool containing some sort of aqueous medium. Tree holes are the most widely used container habitat and certain species of *Toxorhynchites* Theobald, *Ochlerotatus, Sabethes* Robineau-Desvoidy, *Orthopodomyia* Theobald, *Aedes*, some *Anopheles* and a few *Culex* use them. Certain mosquitoes show a preference for a specific type of tree hole. Some, such as *Sabethes chloropterus* Von Humboldt, prefer tree holes with narrow apertures while others utilize a specific tree species (Lane & Crosskey, 1993). Specific preferences, if any, vary among species.

Ground containers such as cocoa pods and coconut husks are utilized by *Anopheles* and *Eretmapodites* Theobald (in Africa) and *Armigeres* Theobald (in Asia) (Lane & Crosskey, 1993). Large fallen leaves, palm fronds, and banana leaves are all utilized by some *Aedes, Culex* and *Eretmapodites* species (Lane & Crosskey, 1993; Clements, 1999).

An additional category used by Clements (1999) to classify mosquito oviposition sites / larval habitats is other natural cavities which includes rock holes, crab holes, and discarded gastropod shells. Empty snail shells are used by *Aedes calceatus* Edwards, *Eretmapodites quinquevittatus* Theobald and *Eretmapodites silvestris* Ingram & De Meillon (Clements, 1999). Rock holes are utilized by *Aedes vittatus* Bigot and *Deinocerites* Theobald breed in tunnels dug by land crabs. Certain species of *Aedes* and *Culiseta* will also breed in this specialized habitat.

The last category relevant to raft layers is artificial containers (Clements, 1999). Man-made containers such as water tanks / cisterns, latrines, septic tanks, water storage pots, discarded tires, cans and bottles can function as oviposition sites for mosquitoes (Lane & Crosskey, 1993; Clements, 1999). *Culex quinquefasciatus* and *Cx. stigmastomata* are just some examples of these kinds of breeders (Bentley & Day, 1989; Clements, 1999).

FEEDING

Most species of mosquitoes are anautogenous, meaning they must take a blood meal to lay eggs. However, some species can use protein they accumulated during the larval stages to provide enough energy to lay the first batch of eggs. These species are called autogenous (Lane & Crosskey, 1993; Clements, 1999). Autogeny is an important factor in vector biology as it can decrease the rate of pathogen transmission. Autogenous females are older when they take their first blood meal. Female mortality increases with age (Reeves *et al.*, 1994), so fewer of these mosquitoes will survive to take multiple blood meals, thus potential for pathogen transmission is lower.

Autogeny is a genetically determined trait among mosquitoes but expression of autogeny is often controlled by environmental factors (Fox, 1994). Poor larval nutrition caused by environmental factors may reduce protein reserves for some individuals. The resulting adult mosquito will be unable to lay the first batch without a blood meal (Gillies, 1972; Brust, 1991).

Mosquitoes feed on plant nectar or other sources of carbohydrates (Nayer & Sauerman, 1975). Female mosquitoes require a blood meal for each batch of eggs they lay, except in the case of autogenous females, which may lay their first batch of eggs without blood. It is this blood feeding that is significant to pathogen transmission, as this is the point where pathogens are transferred to or from hosts.

Once a mosquito has found a suitable feeding site on the host and penetrated the skin with piercing stylets, it will insert its stylet bundle (which forms the salivary ducts and food canal) into the skin. Saliva is injected into the feeding site, along with an anti-coagulant like substance, in some cases, during probing. This injection of saliva is the primary means of pathogen introduction into a vertebrate host. Chemoreceptors on the stylet bundle detect the presence of blood and direct probing into the capillary, or into the haematoma caused by probing. The pumping structures in the head are activated, and blood is sucked into the midgut. A female mosquito can consume up to four times her weight in blood in just a few minutes (Lane & Crosskey, 1993). Feeding is terminated by feedback from stretch receptors in the midgut that respond to distension. Females then digest the blood and develop eggs. If not enough blood was obtained to initiate egg development, the female must search for another host. If the volume of blood obtained was sufficient, host-seeking and biting behaviour are inhibited fully (Klowden, 1986).

In terms of blood-feeding patterns, mosquitoes range from being generalists or specialists (Bentley & Day, 1989). A generalist will feed on a variety of hosts, while a specialist is limited to feeding on specific hosts. This is an important factor in assessing vector potential. Host use patterns may vary seasonally, between geographic regions and as the relative abundance of different hosts changes in an area (Reisen *et al.*, 1997).

OVIPOSITION

Once a blood meal large enough to stimulate ovarian development has been obtained and the eggs have developed, the female must locate a suitable oviposition site.

Nutritional status is known to affect mosquito oviposition (Foster, 1995). Prolonged exposure to sugar inhibits oviposition in the laboratory. Carbohydrate-starved *An. gambiae* females laid an average of 49 eggs per raft, while sugar-fed females laid only 22 eggs per raft (Gary & Foster, 2001). Sugar availability in the field may be important in influencing oviposition behaviour of gravid females. Depleted nutritional reserves may force females to lay eggs in poor or overcrowded habitats (Wallace & Merritt, 1999; Gary & Foster, 2001).

Insemination is known to influence mosquito oviposition behaviour. During copulation, the male introduces a substance produced by his accessory gland, called matrone (Klowden, 1999). Matrone will render the female refractory

to any further insemination. This substance is known to be an oviposition stimulant in several mosquito species (Klowden 1999; Lee & Klowden, 1999).

Mosquito egg-laying has been divided into two distinct behaviours: pre-oviposition and the actual deposition of the eggs on the appropriate substrate. Pre-oviposition behaviour is any behaviour involved in the attraction, location, recognition of, and acceptance of an oviposition site. Pre-oviposition behaviour is induced by a hemolymph-borne substance in *Ae. aegypti* (Klowden and Blackmer, 1987). The behaviour can involve extensive searching flights, which often occur during twilight. The initiation of a pre-ovipositional flight is linked with environmental factors, such as rainfall, relative humidity, temperature and wind speed (Bentley & Day, 1989). Where the species exhibit two crepuscular biting periods, two pre-oviposition flights may occur.

Factors affecting site selection

Gonotrophic development may take two to seven days (Clements, 1999), after which a female searches for an oviposition site. Mosquito oviposition behaviour is regarded as similar to host-seeking behaviour. Both require the integration of complex physical and chemical cues. Long-range cues, probably involving vision, allow mosquitoes to identify different habitats and specific host and oviposition site characteristics (Weber & Horner, 1992). As the distance decreases, other cues become more important. Olfactory cues help mosquitoes to identify volatile factors at the oviposition site. These volatile compounds will

be discussed in detail later in this chapter. Once an oviposition site has been identified, short-range cues such as temperature and chemical signals received by contact chemoreceptors probably become more important (Weber & Horner, 1992; Weber & Tipping, 1990).

Selection of an oviposition site is a critical factor in mosquito survival and population dynamics. Some species of mosquito will oviposit in almost any aqueous habitat, but others are quite specialized when it comes to site selection. For example, *Culex nigripalpus* Theobald egg rafts have been found in a great variety of habitats, while *Culiseta melanura* Coquillett will only oviposit in cedar or bay-head swamp potholes (Bentley & Day, 1989).

Female site choice does not appear to be driven by larval survival in some *Culex* species. For example, Roberts (1996) found that *Cx. quinquefasciatus* larvae, which are not normally found in brackish water, survived well in concentrations of up to 25% sea water. Gravid *Cx. quinquefasciatus* almost always chose to oviposit on fresh water in the lab trials. Survival in *Culex sitiens* Weidemann larvae was highest in saline water (66% sea water), but oviposition was greatest in concentrations of only 28% sea water.

Oviposition site selectivity is considered to be species dependent but there may be some overlap in habitat preference (Bentley & Day, 1989). Mosquitoes invest considerable energy in selecting an appropriate oviposition site and it is evident from the results of several studies that this selectivity is what determines

larval distribution (Bates, 1940). There is no evidence that a species that is selective in oviposition sites is also selective when choosing hosts.

For many species, chemical substances have an effect on oviposition site selection. The origins of these substances are wide-ranging and their chemical structures are varied (Bentley & Day, 1989). An oviposition attractant is any chemical that causes a gravid female mosquito to make oriented movements toward the source. An oviposition stimulant is any chemical that elicits oviposition. Conversely, an oviposition repellent is any chemical that causes the insect to make oriented movements away from the source, while an oviposition deterrent will inhibit oviposition by an insect in an area where oviposition would have occurred in the substance's absence (Bentley & Day, 1989). Only oviposition attractants and stimulants will be discussed in this chapter.

Physical and chemical factors interact to affect oviposition site selection. Physical factors include water qualities (such as depth or movement), visual cues, landscape, soil quality and physical obstructions (Bentley & Day, 1989; Clements, 1999). Chemical factors include substances of egg, larval and pupal origin, vegetable matter, organic compounds, inorganic salts, and presence of predators/parasites (Isoe & Millar, 1995; Isoe *et al.*, 1995).

A mosquito's response to certain visual cues such as colouration (wavelength and frequency) and shade intensity will influence the selection of an oviposition site (Dhileepan, 1997). Colouration is an important cue and can elicit pre-oviposition behaviour from a responsive female (Belton, 1967). Colour

preference varies greatly among species. To some day-laying species, such as *Wyeomyia mitchellii* Theobald, colouration of the oviposition medium is important for accepting or rejecting a site (Frank, 1986). Mosquitoes are using wavelength discrimination to select coloured backgrounds (Clements, 1999; Beehler & Mulla, 1993). Still water often has a mirror-like surface that can reflect brightly (*i.e.* has a specular reflection). Females that reject light-coloured oviposition media on dark backgrounds will readily oviposit on a reflective site. The mechanism by which females recognize a specular reflection is not understood.

For certain species, visual cues are more important than chemical cues. *Anopheles darlingi* Root will die without ovipositing when kept in complete darkness, while other species, such as *Cx. quinquefasciatus,* will oviposit regardless of light conditions (Zulueta, 1950).

Shade is an important visual cue for oviposition site selection for many species but is less or not important for others. Shade is defined here as protection from direct sunlight or moonlight. *Culex restuans* will oviposit mainly in shady pools, while *Cx. tarsalis* will oviposit mainly in pools exposed to sunlight (Brust, 1990). In a tire dump in Indiana, Beier *et al.* (1983) found that 21.6% of the larvae in exposed tires were *Aedes atropalpus* Coquillett, while the same species was only present in 1.2% of shaded tires. Conversely, *Aedes triseriatus* Say comprised 14.0% of those in exposed tires and 61.9% of those found in shade. Removing the vegetation that overhangs ponds and streams, thus

denying ovipositing females shade, has been an effective control strategy for *Anopheles leucosphyrus* Doenitz in Borneo (McArthur, 1947). The presence or the absence of shade may not be directly affecting the gravid female, but may instead be acting on the water temperature or chemical composition of the medium as a result of leaf fall. On the other hand, some species that oviposit at night will only oviposit in areas shaded from direct starlight (and presumably sunlight during the day) (Clements, 1999).

Elevation of an oviposition site can affect its suitability to a particular mosquito species (Dhileepan, 1997; Jones & Schreiber, 1994). Some species that oviposit in tree holes show a preference for a particular height (*e.g. Toxorhynchites rutilus* Coquillett) (Jones & Schreiber, 1994), while others do not *e.g. Ae. triseriatus* (Wood *et al.* 1979). Different elevations can mean a different brightness (from reflected sun) and / or chemical composition of the oviposition medium or a different humidity. Wetter areas will have more ground-level sites to exploit and the elevation preference for a single species can vary with habitat, *i.e.* grassland versus woods (Downs & Pittendrigh, 1946).

Gravid female mosquitoes are responsive to water and water vapour (Kennedy, 1942). The optical characteristics of water can act as cues to its presence. It has also been hypothesized that mosquitoes may use humidity gradients to find water, but in further studies in the field, researchers have shown that this seems unlikely and clear evidence of this has not yet been demonstrated (Clements, 1999).

The presence, size and type of water body will affect oviposition (Lothrop & Reisen, 2001; Weber & Horner, 1992). Chemical constituents found in water are also an important factor in selecting an oviposition site for most species. In most lab trials, females will choose a natural substance resembling the type found in nature over distilled or tap water (Gjullin *et al.*, 1965; Wilton, 1968; Ahmadi & McClelland, 1983).

Water movement will also affect site selection. Most mosquito species oviposit only in still water, but a few prefer the edges of streams or rivers. Water on the edges of rivers and streams moves much slower than water flowing in the centre. Many species will choose to oviposit in still water in lab experiments, despite their habit of ovipositing on flowing water in the field. In contrast to this, *Anopheles minimus* Theobald, though it will readily oviposit on still water in the lab, will refuse to oviposit in small still pools formed when its natural stream habitat is drying up. The rejected pools were suitable for larval development, as they had previously contained many larvae (Clements, 1999). Assuming there were alternative stream-type oviposition sites in this study, it appears that moving water has some characteristic that is highly desirable to this species, but in the absence of that particular cue, stillness becomes the most desirable trait (demonstrated in lab experiments).

Species that oviposit on soil (*Aedes* and *Psorophora* Robineau-Desvoidy) evaluate site suitability based on its moisture content, its texture, and the soil

type. While there is some overlap, most species have a particular set of soil characteristics to which they are attracted (Strickman, 1980).

Physical obstructions can also be an important characteristic of the landscape that affects oviposition for some species. *Anopheles culicifacies* Giles will oviposit in ricefeilds only until the plants reach 0.3 metres in height, then oviposition activity decreases. It has been shown that this species is sensitive to the mechanical obstruction caused by the taller plants. In these experiments, it was determined that shade was not a significant factor (Russell & Rao, 1942). The taller plants may impede the pre-oviposition behaviour, which includes a set flying pattern. Other species, such as *An. gambiae*, have been shown to be less sensitive to physical obstructions (Chow, 1948), while *Culiseta antipodea* Dobrotworsky is noted for having to pass through very dense vegetation to reach its preferred oviposition sites (Dobrotworsky, 1965).

Dadd and Kleinjan (1974) found water that had previously contained egg rafts was more attractive to *Cx. pipiens* egg-laying females that distilled water. This attraction is due to a pheromone, 5R 6S-erythro-6-acetoxy-5-hexadecanolide, secreted in the apical droplet (Laurence & Pickett, 1982, 1985). A number of raft layers (*e.g. Culex, Culiseta, Uranotaenia* Lynch Arribalzaga) have an apical droplet attached to their eggs, but in lab trials, only that of *Cx. quinquefasciatus* was a strong attractant. This kairomone was also an attractant for *Cx. tarsalis*. The apical droplet pheromone released by *Cx. tarsalis* appears to be less attractive than that of *Cx. quinquefasciatus*, but more attractive than

that of other *Culex* species (Bruno & Laurence, 1979). *Culex pipiens* Linnaeus has neurons that respond to this egg raft pheromone, but behaviourally, there is no response (Davis & Bowen, 1994).

Interestingly, Millar *et al.* (1994) found in their experiments with *Cx. quinquefasciatus* that there was no significant attraction to water containing egg rafts, but there was an additive effect to 3-methylindole, a chemical that is often present in polluted waters (Blackwell *et al.*, 1993; Mboera *et al.*, 1999). This chemical is believed to be an oviposition aggregation pheromone and could possibly act as an oviposition stimulant.

Many results obtained in the lab have been an indication that there is a preference for oviposition media that has previously contained larvae, pupae, exuviae or emerging adults. Dadd and Kleinjan (1974) found water that had previously contained larvae or pupae was more attractive to egg-laying *Cx. quinquefasciatus* females than distilled water. Reisen and Meyer (1990) found that water in which larvae or pupae had been reared was unattractive to *Cx. pipiens* and *Cx. tarsalis* in the field. This discrepancy is probably due to differences in experimental design, while the true attraction is probably to the metabolic wastes and other chemical products produced by larvae and pupae (Bentley *et al.*, 1976; Reisen & Siddiqui, 1978; Horner & Weber, 1992).

In recent studies, it has been shown that it is the bacteria associated with the pupae that are attractive to ovipositing females (Dadd & Kleinjan, 1974; Blaustein & Kotler, 1993; Rejmankova *et al.*, 1996). When the bacteria were

removed from larval media, water containing washed larvae were still attractive to gravid females. This implies that there may be a larval secretion that is acting as an attractant (Benzon & Apperson, 1988).

A number of organic matter infusions have been shown to attract gravid female mosquitoes of one or more species. Some examples of infusion materials are: alfalfa hay (Hazard *et al.*, 1967; Chadee *et al.*, 1993), sod (Lampman & Novak, 1996a & b), Bermuda grass (Gjullin *et al.*, 1965; Du & Millar, 1999b; Mboera *et al.*, 2000), bulrushes (Du & Millar, 1999a), fruit rinds (Lounibos, 1978), rice hulls (Ikeshoji *et al.*, 1975), birch wood (Bentley *et al.*, 1979) and manure (Kramer & Mulla, 1979; Reisen & Meyer, 1990). All of these infusions simulate natural oviposition sites that contain decaying organic matter.

An infusion that is attractive to one species can be neutral or repellent to another species. For example, a hay infusion prepared with yeast and lactalbumin is attractive to *Ae. albopictus* but is an oviposition repellent to *Ae. aegypti* (Allan & Kline, 1995). To some species, the presence of vegetable matter is not necessary for oviposition. *Wyeomia vanduzeei* Dyar & Knab naturally oviposits in the infusions contained in the leaf axils of bromeliads, but showed no preference for this infusion over tap water in laboratory trials (Frank *et al.*, 1976).

Fatty acid esters tend to induce negative responses from most female mosquitoes but will act as an attractant to *Ae. aegypti* and *Cx. quinquefasciatus*. It has been shown in the lab that the presence of fatty acids can be lethal to

larvae, so this may be why most species avoid them (Clements, 1999). Interestingly, fatty acids have never been isolated from a natural oviposition site (Davis & Bowen, 1994), but they are probably present in the polluted waters where *Cx. quinquefasciatus* oviposits. Some ketones are also attractive to *Cx. quinquefasciatus* (Ikeshoji & Mulla, 1974) and *Ae. aegypti* (Knight & Corbet, 1991).

Phenols have also been examined as possible oviposition attractants. *Ochlerotatus triseriatus*, *Ae. aegypti* and several species of *Toxorhynchites* have all been shown to oviposit more on oviposition sites containing 4-methyl-phenol. Since all *Toxorhynchites* oviposit from the air, the phenol must be acting as an attractant in the vapour phase (Linley, 1989).

An indole, 3-methylindole, has been shown to be an oviposition attractant for *Cx. quinquefasciatus*, *Cx. tarsalis* and *Cx. stigmatosoma* (Beehler *et al.*, 1994; Mboera *et al.*, 2000). 3-Methylindole is often present in human and animal faeces. Although mosquitoes may be attracted to low concentrations, higher concentrations of the same organic metabolites can act as a repellent, indicating a polluted oviposition site (Millar *et al.*, 1994; Beehler & Mulla, 1995).

Proteinaceous hydrolysates are common constituents of attractive infusions. For example, egg albumin is attractive to *Cx. quinquefasciatus* (Clements, 1999) and proteins added to straw infusions were attractive to ovipositing *Culex* females (Murphey & Burbutis, 1967; Murphy *et al.*, 2001).

However, attraction by proteins may be confounded by microbial fermentation / contamination. Fermented or polluted water interacts differently with the oviposition pheromone found in *Cx. quinquefasciatus* (Laurence & Pickett, 1985) by increasing the oviposition site's attractancy when diluted but reducing its attractancy when the water is undiluted (Blackwell *et al.*, 1993).

The salinity of an oviposition site can be an important factor in oviposition site selection. Female mosquitoes use chemosensilla on the tarsi, labrum and labellum to assess salinity. All freshwater species can tolerate some degree of salinity in the lab, and this should not be surprising given that most natural oviposition sites contain some measure of inorganic salts (Clements, 1999). Species that do breed in saline environments often choose slightly lower salinities than seawater in the lab (Petersen & Rees, 1967). It has been hypothesized that this is to avoid ovipositing in an area where the salts may become too concentrated. Saline breeders do not all prefer the same salinity. This may be a mechanism to reduce competition (Roberts, 1996; Clements, 1999).

From data collected in the field, it seems that females of certain species will avoid ovipositing in the presence of predators or parasites. Ritchie & Laidlaw-Bell (1994) found that *Aedes taeniorhynchus* Weidemann, avoided ovipositing in sites inhabited with larvivorous fish. *Anopheles punctipennis* avoided ovipositing where copepods (Torres Estrada *et al.*, 2001), bluegill fish (*Lepomis macrochirus* Rafinesque) and tadpoles were present (Petranka &

Fakhoury, 1991). It is possible that the reduction in rafts collected was caused by predation by the bluegills, not a repellent effect on the gravid females. It is also possible that while the tadpoles were not predacious, they would compete with larvae for food resources. Their presence could also affect the organic composition of the oviposition site.

In field studies, there were fewer *Cx. pipiens* egg rafts laid in ponds where notonectids (Hemiptera) were present (Clements, 1999). Chesson (1984) speculated this might be due to the notonectid behaviour of breaking up the rafts once they are laid.

It is possible that females of some species of mosquitoes do respond negatively to chemical odours or cues given off by predators. In controlled experiments with *An. punctipennis*, fish were detected by the females chemically rather that visually (Ritchie & Laidlaw-Bell, 1994).

The presence of parasites appears to have an effect on oviposition for *Ae. aegypti.* It was noted in a lab study that female *Ae. aegypti* oviposited less often in water that had previously held larvae which were parasitized by the trematode *Plagiorchus elegans* Rudolphi (Lowenberger & Rau, 1994). The number of eggs that were laid on this medium decreased as the medium was replaced with one exhibiting an increased intensity of larval infection. It was noted that the presence of the trematode itself in the oviposition site did not affect oviposition (Zahiri *et al.*, 1997).

Oviposition strategies

There are four main oviposition strategies used by culicine mosquitoes. The first, egg dropping, entails depositing individual eggs on the water surface, usually without any contact with the water. *Anopheles*, *Sabethes*, *Toxorhynchites* and *Wyeomyia* species all use this strategy. *Culex, Culiseta* and *Coquillettidia* lay egg rafts directly on the water surface. Some *Aedes* spp. and *Psorophora* spp. deposit individual eggs on a substrate at or above the water line. Some species attach their eggs to aquatic vegetation, usually below the water line. This strategy has been adopted by species of *Aedeomyia* Theobald and by some *Culex, Mansonia* Blanchard and *Anopheles* species (Bentley and Day, 1989). Only the second strategy mentioned, laying egg rafts, as it is applied within the sub-family Culicinae, will be discussed further as this is the strategy used by the primary arbovirus vectors in Manitoba.

The subfamily Culicinae is the largest of the three sub-families. The eggs of this subfamily vary greatly in shape and method of oviposition. Eggs of *Aedes* spp. and *Psorophora* spp. are laid singly above a water line, and can withstand long periods of desiccation. Eggs of *Culex*, *Coquillettidia*, and *Culiseta* spp. are laid in long, boat-shaped rafts on the water's surface, and cannot withstand desiccation. *Mansonia* spp. eggs are attached to the underside of floating vegetation in a jelly-like mass.

The Culicinae is divided into either two or nine tribes (Lane & Crosskey, 1993). For the purpose of this discussion, I will adopt the two-tribe model as it

lends itself nicely to the breakdown of oviposition strategies. This entire subfamily contains 24 genera and almost 3000 species, divided among the Sabethini and the Culicini (Harbach & Kitching, 1998). The Sabethini are not of great medical importance, and will not be discussed further here.

The tribe Culicini is large and of major medical importance, due to the number of species that are vectors of pathogens to animals and humans. This tribe can be divided into four main groups, based on oviposition strategies.

The first group includes the aedine genera or floodwater mosquitoes: *Aedes*, *Psorophora*, *Ochlerotatus*, and *Opifex* Hutton spp. The eggs of these species are usually deposited singly and can withstand desiccation for years, if necessary. Their larval habitats are diverse, including any ground collection of water or container-type habitat. Examples are flooded meadows, ponds, rock pools, leaf axils, tree-holes, bamboo, and man-made containers such as tin cans or tires. Most habitats are temporary and are liable to dry out for parts of the year. In northern regions, this can include pools that are covered by snow and ice in the winter (Kitron *et al.*, 1989).

The second group is called the Quasi-Sabethine group by Lane & Crosskey (1993). It consists of the following genera: *Haemagogus* Williston, *Heizmannia* Ludlow, *Zeugnomyia* Leicester, *Armigeres*, *Udaya* Thurman, and *Eretmapodites*. These genera exhibit a mixture of sabethine and aedine characteristics with regards to breeding. They are primarily container breeders, and often oviposit in tree holes, bamboo, leaf axils, bromeliads, rock pools,

empty snail shells or coconut husks. The eggs can withstand partial desiccation, and are usually deposited individually on the surface of the water.

The third group contains species that seek vegetation, and includes the following genera: *Aedomyia*, *Coquillettidia*, *Ficalbia* Theobald, *Hodgesia* Theobald, *Mansonia*, *Mimomyia* Theobald, and *Uranotaenia*. Species of these genera breed in habitats with dense vegetation. For *Mansonia*, *Coquillettidia*, and a few species of *Mimomyia*, this is necessary as the larvae obtain their supply of oxygen by piercing the stems of aquatic plants (Lane & Crosskey, 1993).

The fourth group is the miscellaneous group, and includes the following genera: *Culex*, *Culiseta*, *Deinocerites*, *Galindomyia* Stone & Barreto, and *Orthopodomyia*. Due to their medical importance, a more detailed look at these genera will follow.

Culex is the largest and most important genus. *Culex* spp. are found in almost all geographical locations, except the extreme north. Most *Culex* species lay their eggs in rafts on the water's surface, but there are a few that deposit their eggs on vegetation, and an even smaller number that lay their eggs individually. *Culex gaudeator* Dyar & Knab (South America) lays its eggs in a gelatinous mass on the water surface found inside bromeliads (Clements, 1999). Most *Culex* eggs cannot withstand desiccation, and hatch within a few days. Because the eggs cannot tolerate desiccation, there may be virtually continuous reproduction when environmental conditions permit. They typically breed in ground collections

of water such as ditches, small pools and ponds, but some can also be found in marshes, rice fields, bamboo, tree-holes, and container habitats. A few, such as *Cx. quinquefasciatus* can even be found in the polluted waters of cesspits (Mboera *et al.*, 2000; Millar *et al.*, 1994).

There are several subgenera of *Culex: Melanoconion, Culiciomyia, Eumelanomyia, Lophoceraomyia*, and *Culex*. The subgenus *Culex* has an almost worldwide distribution and contains most of the medically important species. Female *Culex* usually bite at night. Most species feed primarily on birds, a few on amphibians and reptiles, a few on mammals, and some on both birds and mammals (Lane & Crosskey, 1993). In cold climates, females overwinter in shelters such as garages and caves, surviving off a large fat reservoir (Bentley & Day, 1989).

The genus *Culiseta* contains 35 species (Knight & Stone, 1973). Most have a temperate distribution, but a few extend into northern or sub-arctic areas (Wood *et al.*, 1979). Only seven species occur in tropical areas. In most species, eggs are laid as rafts on the surface of the water (Lane & Crosskey, 1993). *Culiseta morsitans* Theobald rafts are also deposited on leaf litter just above the water line. *Culiseta* eggs cannot withstand desiccation, except in the case of *Cs. morsitans*, where the eggs can remain viable for up to six months on a damp substrate (Clements, 1999).

There is some evidence that certain species of *Culex* drink prior to oviposition. Weber & Tipping (1990) showed that this behaviour occurs in *Cx.*

restuans. They believe there is no evidence that the female is evaluating the oviposition site by taste, but rather it is probably drinking to build up the pressure needed to eject its eggs.

Culiseta larvae are usually found in ground collections of water with submerged vegetation, *i.e.* ponds, ditches, swamps, rice fields. *Culiseta fraseri* Edwards (Africa) is found in tree holes and *Culiseta longiareolata* Macquart (Europe) is found in wells and rock pools. The few tropical species breed throughout the entire year but the more temperate dwellers overwinter as larvae (for example *Cs. annulata*) or adults, or in the case of *Cs. morsitans*, as eggs on a substrate. Several species feed on birds, others on mammals, and a few on reptiles (Clements, 1999; Lane & Crosskey, 1993).

The genus *Deinocerites* contains 18 mainly Neotropical species; however, a few do occur in the United States (Lane & Crosskey, 1993). All species of this genus oviposit in crab holes. The eggs are laid above the water line on the tunnel walls, and after hatching, the larvae wriggle down to the water. These eggs cannot withstand desiccation. Adults of this genus feed on many different birds and mammals, including man (Clements, 1999).

The genus *Orthopodomyia* is a small genus with representatives found in Neotropical and Oriental areas. There are a few representatives in North America (*Orthopodomyia alba* Baker and *Orthopodomyia signifera* Coquillett), as well as a few in the Afrotropical and Palaearctic Regions. Larvae of this genus are found in tree-holes and bamboo; however, the North American species

oviposit in bromeliads and in the spathes of *Heliconia* sp. plants (Lane & Crosskey, 1993). Eggs are surrounded by a longitudinal gelatinous phalange which helps them adhere to the inside surfaces of tree holes just above the water line. In northern climates, this genus will overwinter as adults, larvae, or eggs (Clements, 1999). Because they are relatively rare, little is known about their feeding habits and life history.

DISCUSSION

Oviposition strategies used by female mosquitoes vary greatly within each sub-family, tribe, and even within a genus. There are advantages associated with each strategy.

For raft layers, eggs are arranged in vertical rows to couple intrachorionic spaces to the water column. This coupling is believed to prevent desiccation of the eggs and allows hatching larvae to emerge directly into the water. The eggs are fastened together mechanically with chorionic projections. Eggs that are dislodged and lie horizontally, or sink, show reduced hatchability (Weber & Tipping, 1992).

The advantage of rafts is that they are self-righting and resistant to being sunk by raindrops (Bentley & Day, 1989). In field studies, imperfect rafts were not often collected after windy or rainy days. This is because they were broken up and trimmed off. Rafts may, in specific circumstances, also offer increased

protection from predators (Weber and Tipping, 1992), in that if the eggs are found all may not be eaten.

Species that are generalists in terms of site selection appear to have an advantage over the specialists, as the specialist's range for oviposition is limited by the availability of those particular oviposition sites (Bentley & Day, 1989). On the other hand, a species that uses the specialist strategy may be exploiting resources (such as crab holes) where there are distinct advantages (*e.g.* competition is reduced, or they are more well adapted).

Eggs laid above the water line of a temporary pool are resistant to desiccation. These eggs can survive for many months, and in some cases years, until flooding stimulates hatching and provides a suitable aquatic environment for the larvae (Laird, 1988). Because mosquitoes that use this strategy do not need an immediate larval habitat, they can oviposit as soon as an egg batch is developed and an oviposition site has been located (Bentley & Day, 1989). Mosquitoes that oviposit in bodies of permanent water can also lay their eggs as soon as they have developed (Lane & Crosskey, 1993). Females that lay individual eggs or rafts in temporary pools, or that attach their eggs to vegetation must spend time locating and evaluating oviposition sites that will be immediately suitable for their offspring (Clements, 1999). In the absence of a suitable oviposition site, these females do not lay their eggs.

When eggs laid above the water line of a temporary pool are exposed to the appropriate stimuli, hatching will occur over a prolonged period of time. This

is called a staggered hatch. When a female *Aedes* oviposits, the egg surfaces become colonized by bacteria that lower the oxygen concentration in the microenvironment around the egg. This lowering of the oxygen concentration can stimulate eggs to hatch. Eggs where more bacteria have colonized will hatch first, and these larvae will feed on the remaining bacteria around the other eggs. This removal of the bacteria may delay the hatching stimulus for the remaining eggs. A staggered hatch is clearly advantageous in habitats where there is competition for food. Eggs can also be stimulated to hatch by repeated wettings. Eggs laid early in the season will only require one soaking to hatch almost all of the eggs. Later in the season, more soakings will be required to induce fewer eggs to hatch (Bentley & Day, 1989). The combination of delayed hatching of these eggs on prolonged submersion in water and the necessity for several soakings and desiccations to promote hatching of some eggs clearly increases the probability of survival for mosquitoes living in temporary habitats that are likely to dry out (Lane & Crosskey, 1993). Substrate laying is an excellent strategy for cooler locations where seasonal temperatures may only allow for a single generation of mosquitoes each summer. It is important to note though, that many univoltine species in temperate climates exhibit obligatory diapause, which is determined by genetics, not temperature (Mitchell, 1988).

Eggs laid in saline or polluted environments have the advantage that it is less likely that competing larvae will be present, as relatively few species exploit these environments. There may be some conspecific competition if saline

oviposition sites are limited. Among saline-breeding species, there are different preferences for specific salinities (although there is some overlap) (Clements, 1999). This may be a device to help reduce competition for a very specific resource.

Permanent collections of water are persistent and well suited to species that take longer to develop. However, permanent collections of water usually support many predators, as well as developing larvae. Predators include fish, tadpoles, turtles, other mosquito larvae, predacious birds, lycosids (terrestrial spider that will streak out on to the water surface to seize emerging mosquitoes), dragonflies, beetles, chaoborids, and hemipterans. Adult flies, such as Dolichopodidae, Scathophagidae, Anthomyiidae and Muscidae feed on larvae and pupae at the water's surface, as well as on adults (Chesson, 1984). Cockroaches, mites and ants will all devour any eggs that wash into their reach. Bacteria and viruses, protozoans, fungi, and nematodes also attack mosquito larvae (Chesson, 1984; Lane & Crosskey, 1993; Clements, 1999).

The larvae of container breeders, such as *Cx. restuans*, are relatively predator free. The mosquito populations in these habitats are mainly limited by competition for resources. Most species breeding in artificial and natural containers (*i.e.* phytotelmata) exhibit drought–resistant eggs and rapid larval development (Bentley & Day, 1989). These adaptations allow them to exploit these transient habitats.

Geography and weather can affect which oviposition strategy is more advantageous (Fraser & Brust, 1976). For example, *Aedes* spp. that live in more temperate areas have adopted the substrate laying method, allowing them to take advantage of snowmelt and flooding. Eggs can overwinter under the snow until the spring snow melt, when they will hatch (Clements, 1999). The life stages of these *Aedes* spp. are attuned to seasonal and environmental changes. In more tropical areas, the same species mentioned above are often found as temporary ground water or container breeders (Lane & Crosskey, 1993). Some northern univoltine species are multivoltine further south (Clements, 1999).

Culex tarsalis is known to breed in temporary ground pools in northern prairie latitudes, but will breed primarily in tree holes further south (Reisen *et al.*, 1997). They are not known to breed in tree holes in Manitoba (Wood *et al.*,1979). This is probably because fewer trees are available for breeding in the prairies, and the more arid climate limits, to some extent, the use of temporary pools. Species that live in more tropical areas are able to exploit temporary oviposition sites such as hoof prints or fruit rinds, due to their presence and longer persistence in those climates (Clements, 1999). In the rainy season, many species will exploit a particular oviposition site, but when the environment begins to dry, they will move to another more permanent body of water (Gary & Foster, 2001).

An examination of Culicidae phylogeny brings to light certain patterns. Harbach & Kitching (1998) believed the subfamily Anophelinae to be the most

primitive. The Culicinae branch off early, and subsequently give rise to the Toxorhynchitinae (see Figure 1).

Most of the primitive species (Anophelinae) are ground water breeders, with some species exploiting phytotelmata. Artificial container breeders are a relatively recent development but some of the more primitive species have adapted to this resource. The most primitive genera are single egg layers, so raft laying seems to be a trait derived later in certain species. It would not be beneficial for those species which breed in smaller tree holes, flower bracts, or any small oviposition site to lay rafts, as the small size of the oviposition site could not support more than a few larvae. On the other hand, if a species was to breed habitually in larger tree holes, ditches, ponds or larger bodies of ground water, raft laying may allow them to minimise the time spent looking for a suitable oviposition site in that only one must be located. Although the larvae will be competing for food resources, perhaps the benefit of less time spent searching (which may allow for an extra batch of eggs) outweighs this. An examination of Culicidae phylogeny may illuminate the behaviours of primitive mosquitoes but it is an unreliable gauge of oviposition strategy.

Studies on oviposition site preference are important in the planning of a vector control program, especially in the surveillance and monitoring phases (Yap *et al.*, 1995). They provide key information that, when evaluated with other data, can help form an epidemiological picture. There are two types of ovitraps commonly used in disease surveillance programs.

Gravid traps are useful for arbovirus surveillance programs because they attract mostly gravid, blood-fed females, which are more likely to be infectious (Bellini *et al.*, 1996). These traps vacuum up gravid females as they alight on the infusion surface to oviposit. The females are collected alive in a mesh bag, and can be analysed in the lab for the presence of arboviruses or other pathogens (Reiter, 1983; Beehler *et al.*, 1994). Other traps, such as CO_2, light, or vertebrate-baited traps will collect host-seeking females, most of which have not taken their first blood-meal, and so are less likely to be infectious (Millar *et al.*, 1994). Gravid traps will also provide information as to where in the arboviral / pathogen transmission cycle a community currently is, *i.e.* females are present in the gravid traps, therefore a blood meal has already been taken.

Ovipools are artificial oviposition sites, placed in the field, where egg rafts can be collected and reared for identification purposes (Reiter, 1986). Ovipools can also provide an estimate of when blood-feeding is occurring (an important piece of epidemiological information), by extrapolating backward from the time the egg rafts are collected (Madder *et al.*, 1980).

Ovipools and gravid traps are usually baited with an infusion (Reiter et al., 1991; Reiter, 1983; Ritchie, 1984), the most popular in Manitoba being grass cuttings or sod infusions (Brust, 1990; Brust, 1976; Brust & Ellis, 1976). The purpose of the infusion is to render the artificial site attractive to ovipositing females. Infusions are cumbersome to work with and must be replenished or replaced frequently. Also, their attractiveness changes constantly, at an

unpredictable rate (*i.e.* dependent on temperature, rainfall, etc.) (Reiter *et al.*,1991; Beehler *et al.*, 1994). Infusions are impossible to standardise because the chemical constitution and microbial fauna change in an unpredictable way. Identifying standardised infusions for *Culex* mosquitoes would be very useful (Millar *et al.,* 1994; Steinly *et al.*, 1991).

There are many contradictory results in the literature on infusions if one is looking to identify a standardised bait. Experiments that do not account for the different definitions of an "attractant" and a "repellent" may be responsible for a great deal of confusion (Bentley & Day, 1989).

Ovitraps, in terms of surveillance programs, are an important component of data-gathering procedures. When used in conjunction with information from light traps, vertebrate, or sentinel-baited traps, and landing or biting counts, the ovitraps help to illuminate the epidemiology of the disease, and hopefully allow time for preventative measures to be taken.

Sampling the blood-fed component of a mosquito population will increase the probability of detecting pathogens during a field surveillance program. Collections of blood-fed or gravid females produce higher arbovirus isolation rates than collections of unfed females attracted to light, CO_2 or vertebrate baited traps (Reeves *et al.*, 1961). Searches for gravid, resting females in the field are labour intensive and usually yield few specimens (Reisen & Pfuntner, 1983). It is for this reason that ovipools are a part of any arbovirus surveillance program.

Ovipools generally will only attract egg raft layers. This is significant right now in Canada (and Manitoba), because the majority (but not all) of our major vector species are raft layers. The ovipool collections, when maintained throughout the summer, can be used to determine which raft layer species are prevalent in an area and where in the virus transmission cycle a particular cohort is. For example, when *Cx. tarsalis* egg rafts are collected an assumption can be made that if an arbovirus is present in the reservoir host population, it would be possible, in the right conditions, to see transmission to human and animal hosts within a week of the egg raft collection, depending on the incubation period of the virus and providing vector densities are suitable (Bres, 1988). This is because females of the egg laying cohort will now be seeking their next blood meal. If they are infected, they may transmit the virus, provided the extrinsic incubation period is complete. Rafts collected in ovipools can be brought to the lab, where hatched larvae can be identified. These larvae can also be used for virus testing in cases where transovarial transmission is suspected, in conjunction to the testing of males caught in light traps. The relative abundance of *Culex* egg rafts, as well as the timing of the first and final oviposition are important surveillance data (Brust, 1990).

Ovipools are not an exact indicator of species density, but can provide a rough sense of relative abundance. They are better used to estimate potential transmission rates. At any given time, there is a myriad of natural oviposition sites that will also be utilized by ovipositing females, and as for New Jersey and

CO_2 traps, only a small proportion of the species may be represented in these pools (i.e. only the gravid females).

CONCLUSION

It appears that primitive mosquitoes may have been single egg layers, which oviposited in permanent ground pools. As time went on, new geographical locations with different climates were colonized, allowing for the exploitation of new oviposition sites. Many species became specific in their site selectivity, while others did not. Over time, the diverse range of oviposition strategies that we see today arose. For an overview of genus, larval habitat and oviposition strategy, see Figure 2.

There are many substances that are attractive to mosquitoes. Because their strategies are so varied, there will be no one common bait, even within a single genus, or sometimes even a single species. Most species have evolved slightly different oviposition strategies. These strategies are broadly determined by the biology of the species, physical geography and climate, and inter- and intra-specific competition. Each of these factors will affect a given species to varying degrees. Specific behaviours and responses to different physical and chemical cues have evolved to facilitate each individual strategy. Seeking to classify these strategies by the use of systematics will only work in the very broadest sense, and with many exceptions. Undoubtedly, convergence has occurred repeatedly in distantly related species.

Ovitraps are a useful tool for pathogen surveillance, but the efficacy may vary depending on species and location. Once species composition has been determined, ovitraps can be tailored to the particular strategy of the vectors in an area. Research should be conducted to examine particular infusions that are attractive to individual vector species.

Section 3: The evaluation of Reiter's medium and three different ovipool sizes for *Culex* and *Culiseta* spp. surveillance in Manitoba.

Surveillance is an important step in controlling the spread of mosquito-borne pathogens. Oviposition attractants are used in surveillance operations to entice females to lay eggs in artificial pools. Historically, sod infusions have been used in Canada. These infusions require considerable time and effort to prepare and use. Hay infusion is more easily prepared than sod infusion. Reliable data are needed to show how surveillance programs can be streamlined.

Researchers conducting field trials looked at the minimum size of ovipool that could be used for surveillance of *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata*. Traditional surveillance programs conducted in Manitoba use 1 m by 1 m by 15 cm pools, which are cumbersome to set up and time-consuming to maintain.

The objectives of this research were to determine whether gravid female *Cx. tarsalis* are more likely to oviposit in a hay infusion medium (as described in Reiter, 1983) vs. distilled / standing tap water, and, to determine the minimum size ovipool that can be used for surveillance of *Cx. restuans*, *Cx. tarsalis* and *Cs. inornata* in Manitoba.

MATERIALS AND METHODS

Rearing of mosquitoes

Egg rafts and third instar larvae of *Cx. tarsalis* were obtained from Dr. William Reisen at the University of California, Davis. Two strains were received,

one that was well adapted to lab conditions (BFS = Bakersfield strain) and one that had been selected for susceptibility to Western Equine Encephalitis (HVP = high virus potential strain). These strains were reared separately, and were used separately during all experiments. After several months, it was apparent that the HVP strain had better survivability, and so it was chosen for all subsequent lab trials.

Upon arrival, the mosquitoes were transferred into 30 X 20 X 6.5 cm pans filled with 3 L of distilled water. The eggs and larvae were placed in an incubator that was set to maintain temperature at 23^0C and 70% RH. Eggs and larvae were kept at 18:6 L:D photoperiod, with a dusk / dawn step down from eight six-foot fluorescent light bulbs to a 20 watt bulb on a timer. The 20 watt bulb was on for 2 h after the main lights had shut off, and came on 2 h prior to the main lights. Larvae were initially fed a beef liver powder slurry; however, due to high mortality of fourth instar larvae, soft fish food (Tetra Nature's Delica® whole brine shrimp in a nutrient rich gel) plus one Hagen® original blend guinea pig (alfalfa) pellet per pan (avg. weight 0.33 g per pellet) was used.

The surface of the water in larval rearing pans was skimmed periodically to remove any surface film that was forming. Pupae were removed and placed into a deep-bottomed petri dish (8.75 cm diameter X 2.25 cm depth). The dish was then set inside a 25 cm X 25 cm x 25 cm plastic cage, along with a 50 ml beaker of water and a 50 ml beaker of sucrose. Each beaker held a wick, made

of rolled up paper towels, and saturated with the appropriate solution. Pupae were collected by cohort and emerged as adults directly into the holding cages.

Mated females were transferred to a 2.5 cm X 2.5 cm X 10 cm feeding cage using a battery-powered aspirator (Hausherr's Machine Works, New Jersery). These cages were constructed from clear plastic and were mesh on two sides. The cage was then held either between my forearms or was strapped to the side of my calf (lower leg). Fed mosquitoes were returned to the rearing cage. Two days after feeding, a deep-bottomed petri dish filled with distilled water was placed inside the rearing cage. Egg rafts were gently transferred with an ovilifter (small sieve) to a rearing pan. No more than four large egg rafts (approximately 150 eggs per raft) were placed in a pan; 6-7 smaller egg rafts (approximately 30 eggs per raft) were placed in a single rearing pan.

Hay infusion preparation

Hay infusion was prepared for use in the laboratory and in field trials, modified slightly from Reiter (1983). The infusion consisted of 102 g of timothy hay (obtained from Dr. K.W. Wittenberg, Animal Science Dept., University of Manitoba), 1 g of lactalbumin, 1 g of dried brewer's yeast and 20 L of tap water. Batches of 20 L were made for field use, and batches of 2L were made for lab use. The recipe was modified so that the ingredients would be maintained in the correct proportions. The appropriate amount of hay was placed in a mesh bag, so it could be easily removed. The hay infusion was then allowed to stand for

three days, in a covered bucket, at approximately 17^{0}C. Any large, floating pieces of debris were removed before use in experiments. This was done to ensure that the clarity of the infusion matched that of the water as closely as possible. The same bale of timothy hay was used to prepare all of the infusion used in this study.

Lab trials

All mosquitoes were reared at 23^{0}C and were used no earlier than their fifth day after emergence. This was to ensure that all females had sufficient time to be mated. All mosquitoes used for one treatment had pupated in the same 24 h period, thus ensuring that all females used in one treatment were approximately the same age. Female age differed from treatment to treatment, ranging from four to seven days post emergence. However, only gravid, nulliparous females were used in all treatments.

In a fridge-sized laboratory incubator, eight 25 cm X 25 cm X 25 cm cages were arranged to house 10 blood-fed *Cx. tarsalis* females each. Each cage contained a 50 ml beaker of 10% sucrose with a wick, and a 50 ml beaker of water with a wick. The sucrose served to nourish the mosquitoes while the water served to increase the humidity to approximately 70%. Two petri dishes were placed in each cage, one containing 50 ml of tap water, one containing 50 ml of hay infusion. When 50 ml of fluid were added, the petri dish was filled to 1 cm in depth. The location of the dishes in each cage followed a simple Latin square

design, with the position of the first dish being randomly determined. The location of each cage inside the incubator also followed a Latin square design.

Females were fed 48 h before the trial began. During that time, they remained in the rearing cages, but were denied a suitable oviposition site. After 48 h, females were placed into the experimental cages. After 24 h, egg rafts were collected and counted. After an additional 24 h, egg rafts were again collected and counted, and females were removed from the cages. All eggs, regardless of the substrate in which they were laid, were then placed back into rearing pans to provide the next cohort.

At the beginning of these trials, four control cages and four experimental cages were set up. The control cages had either two petri dishes of tap water, or two petri dishes of hay infusion. The purpose of this preliminary trial was to establish that females would lay rafts on each medium in the absence of a choice. It was important to determine this, as females may reabsorb their own eggs in the absence of a suitable oviposition site. A total of five trials were run, with eighty females used per trial (10 females per cage).

Field trials, 2000

Oviposition preferences were monitored from the end of May to 31 August, 2000. Egg rafts were collected from two kinds of oviposition pools, established at four locations around Winnipeg. Pools were placed at the Assiniboine Park Zoo, at the Apiary on the University of Manitoba campus, at the

Charleswood sewage lagoon and at the McPhillips Sludge Beds. Pools were placed so that they were both shaded and exposed to sun at varying times each day (approximately 2 hours of direct sun, 2 hours of complete shade).

Assiniboine Park. Four white plastic 30 cm X 20 cm X 6.5 cm pools, lined with black plastic, and containing 2 L of hay infusion were placed at the north, south, east and west edge of the site at Assiniboine Park on 30 May. When no oviposition was seen by 30 July, the plastic pools were replaced with 40 cm X 30 cm X 5 cm wooden pools, lined with clear plastic. Pools were removed on 1 August and the site was abandoned for this project. Plastic pools were set up at the Assiniboine Park Zoo location only. The wooden pools used for the remainder of this study are described in a following section. Each pool was filled with hay infusion.

Apiary – U of M. Two wooden 40 cm X 30 cm X 15 cm pools lined with clear plastic were placed at this location on 15 June until 30 August.

Charleswood Sewage Lagoon. According to City of Winnipeg data, there were large numbers of *Culex* spp. mosquitoes at this location throughout the summer of 2000. A white plastic 20 cm X 30 cm X 6.5 cm pool, lined with black plastic and filled with hay infusion was set up on 1 August, 2000 to monitor for oviposition until 30 August.

McPhillips Sludge Bed. This area became the new project site on 1 August, 2000 based on the large numbers of *Culex* spp. mosquitoes collected in City of Winnipeg Insect Control Branch light traps. Plastic 20 cm X 30 cm X 6.5

cm pools were set out to monitor for oviposition on 1 July. Six wooden ovipools of three different sizes were set up following a simple Latin square design. Pools were lined with either black or clear plastic.

All pools were checked daily for egg rafts. The number of eggs per raft was determined from the mean of two counts of each egg raft. All eggs were allowed to hatch in 250 ml plastic cups kept at room temperature. The first instar larvae were identified using the taxonomic keys of Dodge (1966).

Field trials, 2001

Although the objective of the field trials in 2001 was the same as in 2000, it was necessary to modify the experimental design to ensure the maximum number of experimental units was observed and collected. The only experimental site in 2001 was the Glenlea Research Station. At Glenlea, an open field was chosen, bordered in the east by the Red River (500 m away), in the west by several experimental ponds (700 m away), which were dry for the first part of the season, and then sampled regularly to determine the extent they were being used for breeding. No breeding was found in these pools, probably due to the murky water quality and algal blooms that appeared in August. To the north, 700 m away were several farm buildings and to the south of the site was a stand of trees that extended down to the river. The pools were placed along this stand of trees. Pools and pool layout did not change except for the addition of two 1 m X 1 m X 15 cm wooden experimental pools for comparison purposes in

August. Pools were exposed to both sun and shade each day. Based on field and lab results in 2000, I used sod rather than hay infusion as an oviposition attractant.

Pool construction

Pools were made by hand and consisted of a wooden frame, lined with black plastic. The plastic was held in place by using a rubber band around the upper edge of the pool. There were three sizes of experimental pools: 40 cm X 30 cm X 15 cm, 30 cm X 15 cm X 15 cm, 10 cm X 10 cm X 15 cm. These sizes were chosen to cover the greatest range of probable sizes, while minimizing the volume of fluid that would require transportation to the experimental site. A larger pool, 1 m X 1 m X 15 cm had already been made for previous University of Manitoba oviposition experiments (Brust, 1990; Brust, 1991; Buth *et al.*, 1990).

Sod infusion

The sod infusion consisted of commercially grown sod, obtained from a local garden supply centre and regular tap water. The bottom of the pools were lined with black plastic, and then had a layer of sod placed so as to cover the bottom of each pool. The sod was then covered to a depth of 10 cm by tap water.

Walk–in Incubator trial

Oviposition was examined in two identical medium-sized pools, one filled with tap water, the other with hay infusion. These trials were similar to the lab choice experiments, but field pools were used and were more widely separated than the petri dishes were. Also, female mosquitoes would have an increased space in which to sense the cues associated with each oviposition medium.

A standard walk-in incubator (L X W X H) 2.75 m X 2.80 m X 2.85 m was set on a 6:18 dusk: dawn cycle, with 3 gradual steps in light reduction / addition. The purpose was to mimic field light conditions as closely as possible. Two medium-sized pools (30 cm X 10 cm X 10 cm) were placed in opposing corners of the space, and were filled with either hay infusion or standing tap water. Sixty to eighty females, fed 48 hours in advance, were released. Egg rafts were removed each morning until no more rafts were laid (approximately nine days), and were allowed to hatch. There were five trials conducted, with varying numbers of females (45 – 97), depending on the number of females available from the lab colony.

RESULTS

Lab trials

Laboratory trials ran from February, 2000 to November, 2002. There were some difficulties with the maintenance of the culture, and two additional shipments of eggs and larvae were received from California. A total of 334 egg

rafts were collected in lab trials. Of those collected, 68 were laid in the hay-infusion, 266 in tap water. In preliminary experiments, there was no preference for distilled water over tap water in the lab. This was later confirmed with experimental trials. Control trials were set up where no choice was offered. Females had to oviposit in the medium presented, or absorb their eggs. Control trials had an overall mean number of 7.0 $\pm$ 3.4 rafts laid in hay infusion (HI) per cage, and 6.0 $\pm$ 4.4 rafts laid in standing tap water (W) in each cage. The overall mean number of egg rafts laid in each infusion per experimental trial was 13.4 $\pm$ 9.0 (HI) and 40.8 $\pm$ 11.3 (W) (see Table 1).

In the lab trials, there were no differences between the hay infusion control (two HI filled petri dishes in same cage) and the water control (two water filled dishes in the same cage). The results are not significantly different from the null hypothesis (Ho = 50% of the rafts in each medium, $X^2 = .24$, $p>.05$).

The experimental results are significantly different from the null hypothesis (Ho = 50% of the rafts in each medium, $X^2 = 82.9$, $p<.05$). There seems to be a preference for the water over the hay infusion. There was some variation in the results, illustrated by the high standard deviations. However, the F statistic was calculated and there was no significant difference in the variability of the counts ($F_{4,4} = 1.57$, $p>.05$). The hay infusion medium was less attractive than standing tap water to ovipositing females.

Field Trials, 2000

In the field, during the summer of 2000, two egg rafts were collected from the McPhillips Sludge Beds, both of which were identified as *Cx. restuans*. No other rafts were collected. For a summary of total nights pools were operated and total rafts collected, see Table 2.

Field Trials, 2001

For the summer of 2001, a total of 98 egg rafts was collected from all pools (Figure 3). Thirty-seven rafts were collected from the large pools, 33 were collected from the medium pools, one was collected from the small pools and 27 were collected from the meter-square pools (Figures 5 and 6). It is important to note though, that the meter-square pools were only out for the month of August only. Seven point zero four per cent (5 / 71) of all rafts laid in experimental pools (excludes the meter square pools) were *Cs. inornata*, and 92.96% (66 / 71) were *Cx. restuans*. A summary of rafts of each species laid per night is available in Table 3.

The frequency of oviposition was calculated for each pool (Table 4). Frequency is defined as the number of nights the event *(i.e.* oviposition) occurred divided by the total nights observations were made. Small pools had a frequency of 0.03 ± 0.03, medium pools had a frequency of 0.10 ± 0.05, large pools had a frequency of 0.13 ± 0.06, and metre squared pools had a frequency of 0.167 ± 0.06. There was no difference between the frequencies seen for each pool size

(Ho = 25% of rafts in each pool size, X^2 = 3.0117, p= 0.3886). The standard error for each frequency was calculated and tested to determine whether it was within the 95% confidence interval. All of the frequencies were within this range, thus there were no significant differences among the frequencies of oviposition seen in each type of pool.

Walk-in Incubator Trials

Incubator trials yielded a total of 380 egg rafts, with 319 collected from tap water and 61 collected from the hay infusion (see Figure 7). A X^2 analysis shows that these counts are significantly different (Ho = 50% of the rafts in each medium, X^2 = 175.17, p< .05). There was a strong preference shown for the tap water over the hay infusion. These results support the findings of the lab trials. For a summary of rafts per day in each medium for each trial, see Table 5.

Section 4: Discussion

Over the last four decades, many new infectious diseases have emerged, and there has been a resurgence of various diseases thought to be under control. Many of these emerging and resurging diseases are spreading to new geographical areas either by natural means or by human introduction. In Gratz (1999), five of the twelve listed emerging vector-borne diseases are transmitted by mosquitoes (Table 6). Nine of the fifteen resurgent vector-borne pathogens are mosquito-borne (Table 7). Neither of these lists include West Nile virus, a flavivirus recently introduced to North America, which has been the focus of intense surveillance activity in Canada.

The resurgence of a mosquito-borne pathogen often can be attributed to an increase in vector abundance. It can also be spread by travellers from endemic regions to regions that have competent vector species. These introductions can result in successful transmission if the climate, immunity status of the human / animal host population, density of human and vector populations, and presence of a suitable reservoir host are all conducive to the pathogen's establishment. Of these factors, the most important (according to Gratz, 1999) is the presence of a suitable vector mosquito species at high enough densities for transmission to occur. Reeves (1971) found that increases in vector populations were frequently associated with arbovirus epidemics.

Ecological changes such as deforestation or urbanization may increase vector densities and /or result in increased human contact. Care should be taken

to minimize or prevent the introduction of new mosquito species that may act as a vector or compete with and possibly crowd out indigenous non-vector species. Weather is also an important factor in the outbreak or resurgence of mosquito-borne disease.

The current climactic trend towards increasing temperatures may pose a significant public health risk. It is estimated that in the next 100 years, global temperature will rise an average of 1-3.5 ^{0}C. This may cause a rise in sea level and a change in precipitation patterns, due to rising temperatures. These changes may significantly affect the distribution and interactions of arbovirus vectors and hosts.

Temperature influences the virus-vector-host interactions, vector survival and generation time. An increase in temperature may increase the period of time where autogeny is expressed (Reeves *et al.*, 1994). This could actually reduce transmission, since autogenous females are generally older when they take their first blood meal, therefore fewer will survive to acquire a pathogen with the first blood meal, and transmit that pathogen with subsequent feedings.

Conversely, an increase in temperature will also shorten the time required to complete the extrinsic incubation (EI) period, as viral replication will proceed faster at elevated temperatures. This shortening of the EI could compensate for the increased female mortality at higher temperatures, for some arboviruses (Hardy *et al.*, 1983).

As rising temperatures create changes in species compositions, new vectors and or new viruses will move to occupy these vacant ecological niches. Even a small increase in temperature may cause a decrease in vector survival, alter vector competence and modify the geographical distribution of viruses, vectors and human and animal populations. Reeves *et al.* (1994) clearly demonstrated this with their studies done on *Cx. tarsalis* in two regions of California where temperatures differed by 5 ^{0}C. Their study shows that if the temperature in the warmer region were to increase by 5 ^{0}C, transmission of WEE would virtually disappear, while SLE would persist and thrive.

It is logical to assume there will be a change in the prevalence of endemic arboviruses in many areas of the world. This means that public health officials should anticipate the control or disappearance of some endemic arboviruses, and the appearance of new arboviruses and mosquito-borne pathogens. For example, WEE was a common pathogen seen in the Northern United States and Canada in the 1970s. Reeves *et al.* found that for every 1 ^{0}C increase in daily temperature, there was a corresponding 1% increase in daily mortality for *Cx. tarsalis*. With that temperature increase, we should also expect a shortening of the extrinsic incubation period (EI) (time for viral replication) that would make up the difference in transmission seen caused by the increased daily mortality rate. However, *Cx. tarsalis* has an enhanced ability to modulate WEE replication and transmission at this higher EI (Kramer *et al.*, 1983; Reisen *et al.*, 1993). The net result would be a decrease in transmission rates seen for WEE in *Cx. tarsalis*

(Reeves *et al.*, 1994). Perhaps this is a factor in why no major outbreaks have occurred in Manitoba in the last 15 years.

Surveillance, immunization and vector control are the three factors necessary for outbreak prevention / management (Bres, 1988). Immunization and vector control fall outside the scope of this thesis and will not be discussed here.

Surveillance activities are critical in the preparation for and the control of mosquito-borne disease. The objective of arboviral surveillance is to provide early warning of the threat of epidemics / enzootics (Bres, 1988). Surveillance usually consists of sentinel animal surveillance to detect virus transmission and vector surveillance. A typical mosquito surveillance program consists of several components: New Jersey Light and CO_2 trap collections, landing / biting counts, larval surveys and ovipool collections.

A critical first step in any surveillance program is determining the species composition in an area, if it is not already known. A comprehensive search of the literature can be very useful but often in Canada there is very little data for a specific area, or the information collected and published many years ago is not an up-to-date reflection of what is found in the field today.

New Jersey Light traps and CO_2 traps are excellent tools to help identify which species are present in a particular area. New Jersey light traps will catch male mosquitoes, but both traps attract and capture biting females of most species. These females can then be identified in the lab. Setting these traps up

in both rural and urban locations, and collecting on a regular basis throughout the mosquito season can provide an accurate assessment of local species composition. Long-term surveillance of this type is invaluable, as data from past years and outbreak years can be compared to weather or other data to analyze trends in arboviral outbreaks and can provide warning of newly introduces mosquito species. Light trap data are also used to provide rough estimate of population density, but this practice should be approached with caution. Not all species, such as *Cx. restuans*, are accurately represented in light traps (Brust & Ellis, 1976), and only a small portion of a particular population (*e.g.* mated females which are seeking a blood meal) may be represented in CO^2 traps.

Landing and biting counts are conducted by field research assistants who voluntarily expose their skin at designated times in designated locations, and manually collect the females attempting to feed. These counts are useful for estimating which species are feeding on humans, and how many bites a human may receive, in that location, at a given time. These are important data for estimating possible transmission rates. Also, it can sometimes be surprising to see what species are currently feeding on humans. For example, during the West Nile virus surveillance operations conducted in Windsor, Ontario in 2001, a high proportion of mosquitoes collected during landing biting counts in some areas were *Ochlerotatus sollicitans* Walker. This species is generally expected to feed on small mammals (Wood *et al.*, 1979), not humans.

It has already been explained on pages 42-44 how sampling the blood-fed component of a mosquito population will increase the probability of detecting pathogens during a field surveillance program and that it is for this reason that ovipools are a part of arbovirus surveillance programs.

The ovipool collections can be used to determine which raft layer species are prevalent in an area and where in the virus transmission cycle a particular cohort is. Rafts collected in ovipools can be brought to the lab, where hatched larvae can be identified. These larvae can also be used for virus testing in cases where transovarial transmission is suspected, and where testing of males from light traps has been insufficient. The relative abundance of *Culex* egg rafts, as well as the timing of the first and final oviposition are important surveillance data (Brust, 1990), but ovipools are not an exact indicator of species density, and can only provide a rough sense of relative abundance. It was evident throughout this study that there are other limitations to ovipools surveillance as well. Baiting pools used for surveillance with an unattractive medium can result in very few egg rafts being collected. If the assumption is being made that those counts are representative of the target species population, researchers may be misled. Also, the amount of contradictory literature on the subject of infusions can make the selection of an effective oviposition medium difficult. Furthermore, even if a medium has been shown to be attractive to a species, the same results may not be obtained in a different geographical location.

Ovipools can also be less than effective if the ovisite preference for the species under surveillance has changed. Raft-layers who are unexpectedly exploiting container habitats will be missed or under-represented in ovipool surveillance. Again, this can lead to gross in accuracies in population estimates.

I focused on improving ovipool surveillance in Manitoba by examining a different oviposition medium and the effect of pool size. My first objective was to determine the minimum size ovipool that can be used for surveillance of *Cx. restuans*, *Cx. tarsalis* and *Cs. inornata* in Manitoba. My second objective was to determine whether gravid *Cx. tarsalis* are more likely to oviposit in a hay infusion medium (as described in Reiter, 1983) vs. a distilled / standing tap water medium.

Historically in Manitoba, large ovipools were filled with a high maintenance, labour-intensive sod infusion used as the oviposition medium. Media that increase a pool's attractiveness to ovipositing females were examined, in the hopes that gravid female mosquitoes could be attracted to small artificial ovipools instead of the natural oviposition sites available in the area. By finding a more attractive medium, it might be possible to reduce pool size and still collect meaningful data. The purpose of increasing the number of rafts laid in these pools is to collect numbers that are representative of the population being sampled, and in some cases to obtain more larvae for viral analysis (if required).

Several researchers have looked at the attractiveness of hay infusions and sod infusions compared to water. A medium was deemed more attractive if the majority of egg rafts were laid there in a simple choice experiment.

Weber and Horner (1992) conducted several field studies with *Cx. pipiens* and *Cx. restuans.* They found that aged tap water was not attractive to egg laying females, and their hay infusion was preferred. They found that females would travel up to 27.1 m from vegetative cover to oviposit in experimental pools baited with hay infusion.

Reisen and Meyer (1990) tested a number of infusions, including Reiter's hay infusion and Maw & Bracken's (1971) sod infusion. They found that gravid *Cx. tarsalis* were not attracted to any solution or traps under lab or field conditions. In all cases, females laid more rafts in tap water. These results conflict with those of Weber & Horner (1992). The reasons for this conflict will be examined later in this discussion.

Before beginning this project, there were a number of considerations. Historically, sod has functioned well as the medium in Manitoba (Brust, 1990). Maw and Bracken published the recipe for this infusion in the literature in 1971. Since then, there are reports in the literature advocating its use, and other reports which contradict these positive findings. This infusion can be very cumbersome and difficult to standardize. The sod itself has to be carried some distance into the field when setting up ovipools. It is heavy and the level of fertilization it has received varies. Over-fertilized sod tends to produce an algal

bloom in the ovipool after only 2-3 days, rendering the pool virtually useless for surveillance. Hay infusion is a popular oviposition medium for *Culex* spp. Weber and Horner (1992) and Reiter (1983) both found that this infusion worked very well to attract gravid ovipositing *Culex* spp. to their pools. Reisen's results with *Cx. tarsalis* and Reiter's medium in 1990 did not support these findings. The advantage of hay infusion was that it could be prepared before hand in the lab, and would not involve as much work to bring out to an experimental site (no large rolls of sod to carry). Also, the same bale (or bales) of hay could be used to prepare large amounts of the infusion at once, eliminating some of the problems of standardization encountered when using a sod infusion. When a test of the controls was performed, it was found that female mosquitoes will oviposit in the hay infusion medium rather than absorb their eggs.

After establishing that sod infusion was a reliable but cumbersome medium (some conflicting results in the literature [Brust, 1990; Reisen, 1990] but good anecdotal evidence) and the hay infusion showed significant promise, with some conflicting results in the literature (Weber & Horner, 1992; Reiter, 1983,1986; Reisen, 1990), I first sought to establish that a hay infusion was preferred by ovipositing females over tap water. Once that had been established, I planned to compare it to a sod infusion.

For this project, hay infusion proved to be less attractive to ovipositing females than tap water. The discrepancy between these results and those of Weber& Horner (1992) and Reiter (1983) could be due to geographical

differences in strain. In chapter two, I highlighted the variation seen even among species in terms of oviposition strategies. If this is the case, then it is not surprising that my results were negative, like those of Reisen (1990). The colony used for this project was obtained directly from Dr. Reisen's lab in Bakersfield, CA. Would indigenous *Cx. tarsalis* mosquitoes prefer the hay infusion? This appears to be unlikely. The summer of 2000 had a greater abundance of *Cx. tarsalis* and *Cx. restuans* than 2001, based on City of Winnipeg light trap data (Figure 9 & 10). Still, in 2000, I had no significant oviposition in my pools filled with hay infusion. In 2001, I had significant oviposition in sod infusion filled pools, even though the species abundance for Winnipeg and surrounding areas was lower.

It is difficult to say why my results do not support that of Weber & Horner. Again, this may be due to a geographical difference in strain. Weber and Horner's results were not tested in the lab. Perhaps there was another factor in the field (*i.e.* few natural oviposition sites) that influenced their results. It is interesting to note that in their paper, they indicated with some surprise that females were flying up to 27.1 m to reach their artificial oviposition sites. This may have been due to the attractiveness of their hay infusion, or it may have been due to a lack of any other oviposition sites.

It is believed that there was no edge effect seen in this experiment. To prove this, more pools would have been necessary to increase the replication at the centre of the layout. The three by three design chosen has only one pool that

is not on an edge. A four by four design would have been better. However, if the number of egg rafts are added up for each edge, the northern edge had 28 rafts laid, the eastern edge had 21, the southern edge had 23 and the western edge had 23. These numbers are all quite similar and do not imply any sort of bias. Also, the one egg raft that was laid in a small pool was laid in the center pool. If there was an edge effect, I would expect that raft to have been laid in one of the two smaller pools on the edges of the layout, but no conclusions should be drawn from this as the number of both pools and egg rafts is not sufficient to derive a meaningful interpretation through statistical analysis.

The second objective of this research was to determine if a smaller pool size could be used for surveillance. Several pool sizes have been used in previous studies: a one meter squared by 10 cm pool used by Brust (1990), a 30 cm squared by 15 cm pool used by Maw & Bracken (1971), and a 10cm diameter pool used by Weber & Horner (1992). The meter squared pool was currently being used in Manitoba but it is large, heavy and difficult to carry to remote field sites. These pools also require an enormous amount of water (75 L) to fill them to the appropriate depth. The transport of this water to an oviposition site often poses a problem. A smaller pool would allow for more pools to be established, for several to be carried out and set up at one location, for pools to be set up faster, and for the pools to cover a wider area. Also, smaller pools would save time and money.

I found that the medium or large sized pools used in this experiment may be as effective as the meter squared pool. The frequency of oviposition seen in medium and large sizes of pool was not significantly different from that seen in the meter-square pool. Definite recommendations cannot be made at this time, due to the small number of experimental units for these trials. To make recommendations, I would have liked to obtain at least 500 rafts in my field studies.

Low mosquito abundance posed a significant challenge to data collection in both field seasons. Typical species abundances for *Cx. restuans* and *Cx. tarsalis* (City of Winnipeg, unpublished data), based on light trap collections, are shown in Figure 8. The abundance for these species for the summers of 2000 and 2001, respectively, is shown in Figures 9 and 10. No significant oviposition was seen in my experimental pools in 2000. The overall nightly oviposition seen in 2001 is shown in Figure 3. Based on the light trap data for 2001, and assuming a three day gonotrophic cycle with a survivability rate of 0.7, I should have seen about 1.07 *Cx. tarsalis* rafts per night in July and August. I saw none. I should have seen 0.95 *Cx. restuans* rafts per night. I saw 1.11. While this may look as if 92.6% of the expected egg rafts were received, this is not the case. *Culex restuans* is typically much better represented in ovipools than in light traps (Brust & Ellis, 1976). We can assume the number of adults caught in light traps was a gross under representation of the adult population, which explains the excellent results for *Cx. restuans*. In June and July, I should have seen 0.421

Cs. inornata egg rafts per night. I saw .0885. This is only 21.04% of what I expected to see based on light trap data. These results were very disappointing, as they were much less than anticipated. It is important to note the above comparisons are rough estimates only and make the assumption that both types of traps sample equal proportions of available host-seeking and ovipositing females, and that the numbers and species composition obtained by the City of Winnipeg are representative of Glenlea Research Station.

It is interesting to note that in a recently started M.Sc. project by Scott McMahon at the University of Manitoba, there has been a relatively high level of oviposition in tire dumps in southern Manitoba. The overall nightly oviposition rate has not yet been calculated for this summer, and the corresponding light trap data will not be available for another two months. These data should be examined, as the oviposition rates seen in these tires may be higher than what is being seen in ovipools. This phenomenon could have two explanations. The first is that the abundance of our three target species is higher. This would quickly be confirmed by the light trap data. The second is that the three target species in Manitoba may prefer to oviposit in tires and other artificial containers, as opposed to traditional habitats such as ditches and ground pools. This may be an explanation if the light trap data for this summer are not significantly different from that of 2000 and 2001. This would be extremely important information when conducting arbovirus surveillance operations in Manitoba, and could explain why so little oviposition was seen in ovipools throughout this study.

The low species abundance and the surprising results of the hay infusion medium forced several changes to the experimental design. Still, my objectives concerning the attractiveness of the hay infusion and pool size remained the same. In regard to my first objective, the hay infusion results needed to be replicated to determine that it was not preferred over tap water by ovipositing mosquitoes. This was hampered in the lab by repeated lab colony crashes. For over a year, the number of females in the lab colony did not exceed 40. Larval mortality fluctuated from an estimated 75-95%. Several temperatures, humidities, diets and pan types were tried. Experts were consulted. It was hypothesized that the colony may have become infected with a pathogen, and while no identifications were made, larvae seemed to survive better when pans were treated with bleach between each generation. It was not until April, 2002 that the colony stabilized and became robust.

Field data were also difficult to obtain due to low species abundance. After the 2000 field season, based on preliminary lab results and lack of field data obtained, the hay infusion medium was abandoned. I sought to supplement the data to support my hypothesis on the hay infusion by running several lab-based chamber trials starting in April, 2002.

In the 2001 field season, I focused on obtaining data to meet my second objective regarding pool size. The sod infusion medium was chosen because it had worked before at the Glenlea Research Station. The change of medium did not affect my second objective.

The amount of data obtained in the second field season was good, given the very low mosquito abundance for my three target species. Still, it was lower that what was expected, based on light trap data. Results should be replicated to reinforce the conclusions drawn in this study. They were not replicated as part of this project due to time limitations. Overall, it is clear that there is no "universal attractant" for egg laying *Culex* spp. Oviposition preferences vary within a genus, and even within a species, as was shown with the discrepancy between this and Reisen's 1990 study, compared to Weber and Horner's 1992 study. Local vector species should be studied to determine ways to maximize ovitrap efficacy, based on the local population's behaviour and preferences. Sod infusion remains the better ovipool medium for *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata* in Manitoba compared to hay infusion.

Ovipools are a widely used tool for field surveillance of arboviruses, but their use is cumbersome and subject to certain limitations. The possibility of using smaller pools could make the setting and maintaining of these types of traps significantly easier, especially where time and resources are limited. The increased use of these pools would result in more data being collected from more locations, and may have a direct impact on our provinces ability to detect and control arboviral outbreaks.

SUMMARY

1. Ovipools, though cumbersome to use, are a widely used part of arbovirus surveillance programs. By increasing an ovipool's attractiveness to gravid females, pool size may be decreased and meaningful data may still be obtained.

2. Hay infusion used in this study was less attractive to gravid female *Culex tarsalis* than sod infusion or aged tap water. These results differ from other studies. Oviposition preferences seem to vary within a genus and even within a species. There is no universal attractant.

3. Smaller ovipools may be as effective as traditional metre square ovipools for surveillance. Preliminary results must be replicated.

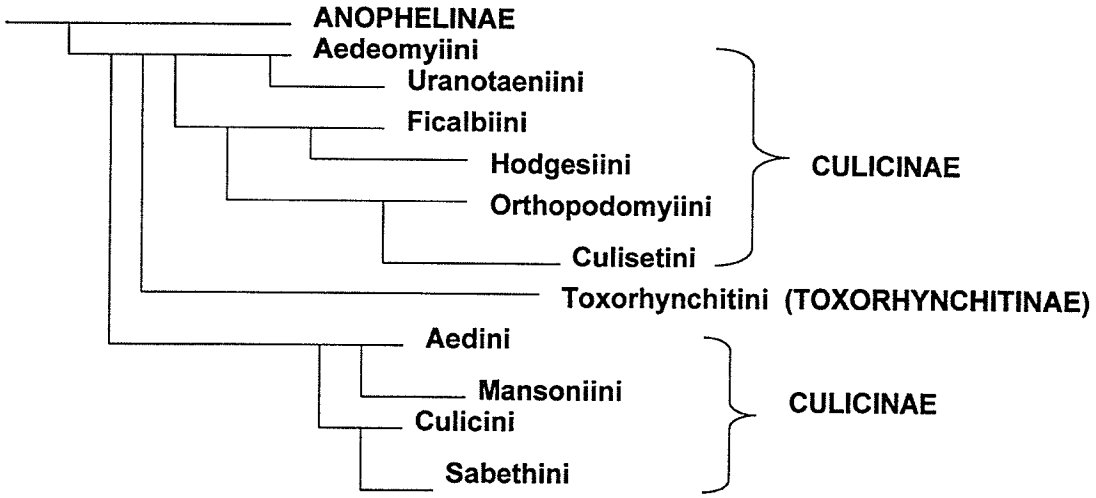

Figure 1. Phylogeny for the Culicidae, adapted from Harbach and Kitching (1998).

Sub-Family	Tribe	Genus	Distribution	Egg	Larval Habitat	Strategy
Anophelinae		*Chagasia*	Neotropical	single, floats	Ground water	egg dropper
		Anopheles	Cosmopolitan	single, floats	Ground water, phytotelmata	egg dropper
		Bironella	Australasian	single, floats	Ground water	egg dropper
Toxorhynchitinae		*Toxorhynchites*	Cosmotropical	single	Phytotelmata, containers	eggs drppoed or thrown
Culicinae	Sabethini	*Sabethes*	Neotropical	single	Phytotelmata	egg dropper
	Sabethini	*Wyeomia*	New World, mainly Neotropical	single	Phytotelmata	egg dropper
	Sabethini	*Phoniomyia*	Neotropical	single	Phytotelmata	egg dropper
	Sabethini	*Limatus*	Neotropical	single	Container	egg dropper
	Sabethini	*Trichoprosopon*	Neotropical	single	Fruit husks	egg dropper
	Sabethini	*Tripteroides*	Oriental, Australasian	single	Phytotelmata	egg dropper
	Sabethini	*Topomyia*	Oriental, Australasian	single	Phytotelmata, containers	egg dropper
	Sabethini	*Maorigoeldia*	New Zealand	single	Phytotelmata	egg dropper
	Sabethini	*Malaya*	Afrotropical, Oriental, Australasian	single	Phytotelmata	egg dropper
	Sabethini	*Johnbelkinia*	Neotropical	single	Phytotelmata	egg dropper
	Sabethini	*Runchomyia*	Neotropical	single	Phytotelmata	egg dropper
	Sabethini	*Shannoniana*	Neotropical	single	Phytotelmata	egg dropper
	Culicini	*Aedeomyia*	Cosmotropical, Australasian	single	Ground water with veg	egg dropper
	Culicini	*Aedes*	Cosmopolitan	single	Almost all	substrate layer, egg dropper
	Culicini	*Armigeres*	Oriental, Australasian	single, raft	Phytotelmata, containers	egg dropper, raft layer
	Culicini	*Eretmapodites*	Afrotopical	single	Phytotelmata, containers	egg dropper
	Culicini	*Haemagogus*	Neotropical	single	Phytotelmata, containers	egg dropper
	Culicini	*Heizmannia*	Oriental	single	Phytotelmata	egg dropper
	Culicini	*Opifex*	New Zealand	single	Saline rock pools	substrate layer
	Culicini	*Psorophora*	New World, mainly Neotropical	single	Permanent ground pools	substrate layer
	Culicini	*Udaya*	Oriental	single	Phytotelmata, containers	egg dropper
	Culicini	*Zeugnomyia*	Oriental	single	Phytotelmata, containers	egg dropper
	Culicini	*Culex*	Cosmopolitan	all	Ground pools, phytotelmata	egg dropper, layer, att veg
	Culicini	*Deinocerites*	New World, mainly Neotropical	mass	Crab holes	substrate layer
	Culicini	*Galindomyia*	Colombia	single	Ground waters	egg dropper
	Culicini	*Culiseta*	Cosmopolitan, mainly temperate Old World	raft or single	Ground pools	raft layer, substrate layer
	Culicini	*Ficalbia*	Afrotropical, Oriental, Australasian	mass	Permanent ground pools	att. to aquatic veg
	Culicini	*Mimomyia*	Afrotropical, Oriental, Australasian	single	Permanent ground pools	egg dropper
	Culicini	*Hodgesia*	Afrotropical, Oriental, Australasian	single	Permanent ground pools	egg dropper
	Culicini	*Coquillettidia*	Cosmopolitan	rafts	Ground waters	raft layer
	Culicini	*Mansonia*	mainly Cosmotropical	mass	Pools with vegetation	att. to aquatic veg
	Culicini	*Orthopodomyia*	Cosmopolitan	single	Phytotelmata	egg dropper
	Culicini	*Uranotaenia*	Cosmopolitan	rafts	Ground pools, phytotelmata	raft layer, egg dropper

Figure 2. Summary of genera, distribution and oviposition strategy for the Culicidae. Note the variation within a single genus.

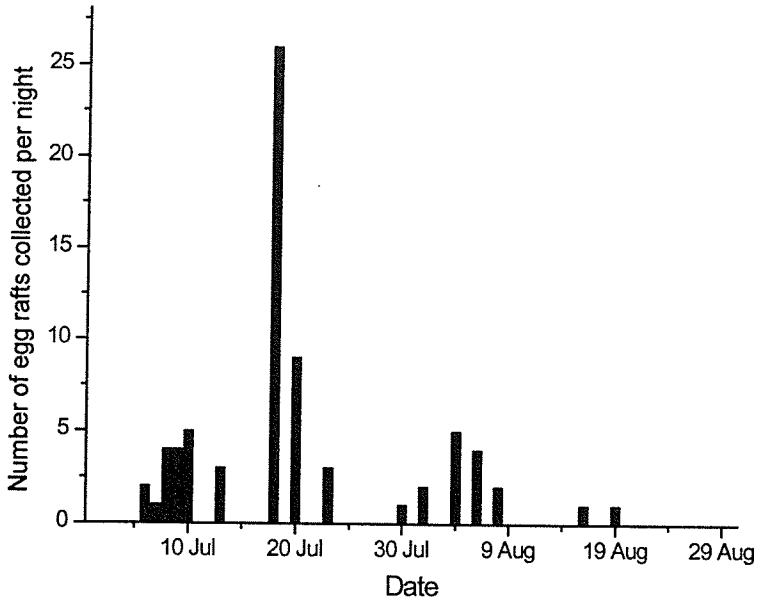

Figure 3. Nightly egg rafts collections from three sizes of experimental ovipools and two meter square pools at Glenlea Research Station, 1 July - 31 Aug 2001. Sixty-six *Cx. restuans* egg rafts and 5 *Cs. inornata* egg rafts were collected. Compare with nightly egg raft collections made by Brust at same location in 1981 (see Figure 4).

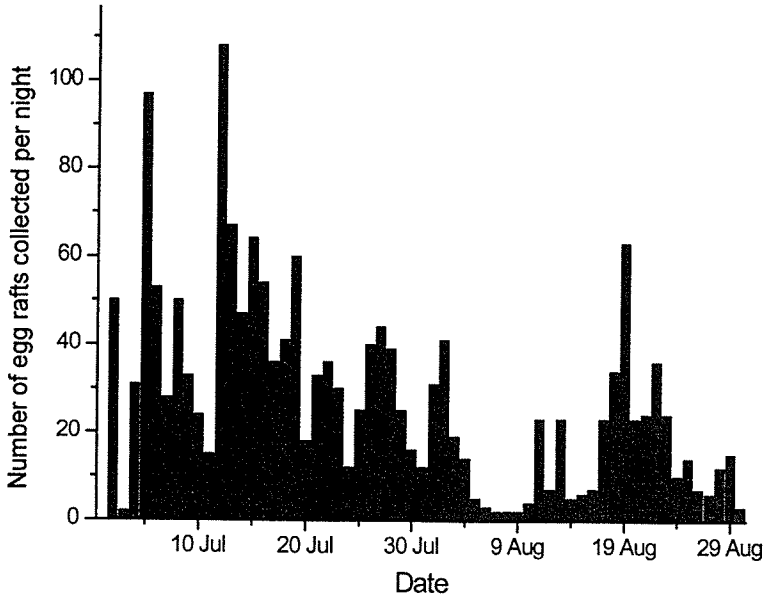

Figure 4. Total number of *Cx. restuans, Cx. tarsalis* and *Cs. inornata* egg rafts collected at Glenlea Research Station in meter square sod-lined pools by Brust in 1981 (Brust, 1990). These results are representative of an average year. Note the higher nightly counts when compared to Figure 3.

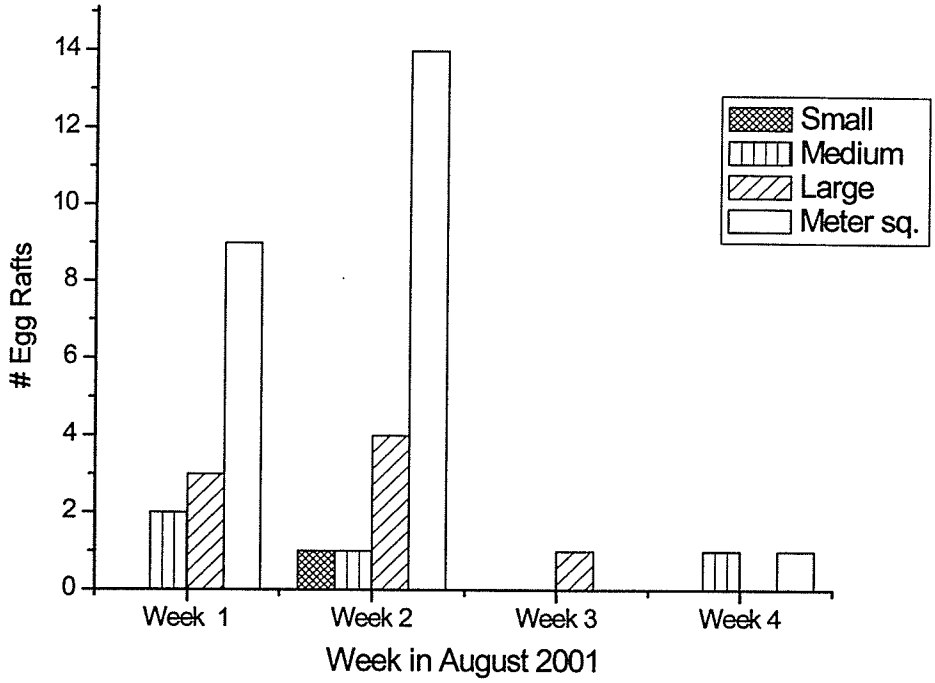

Figure 5. Number of egg rafts collected for each pool size in August, 2001 at Glenlea Research Station. August was the only month where all four sizes of pools were set up.

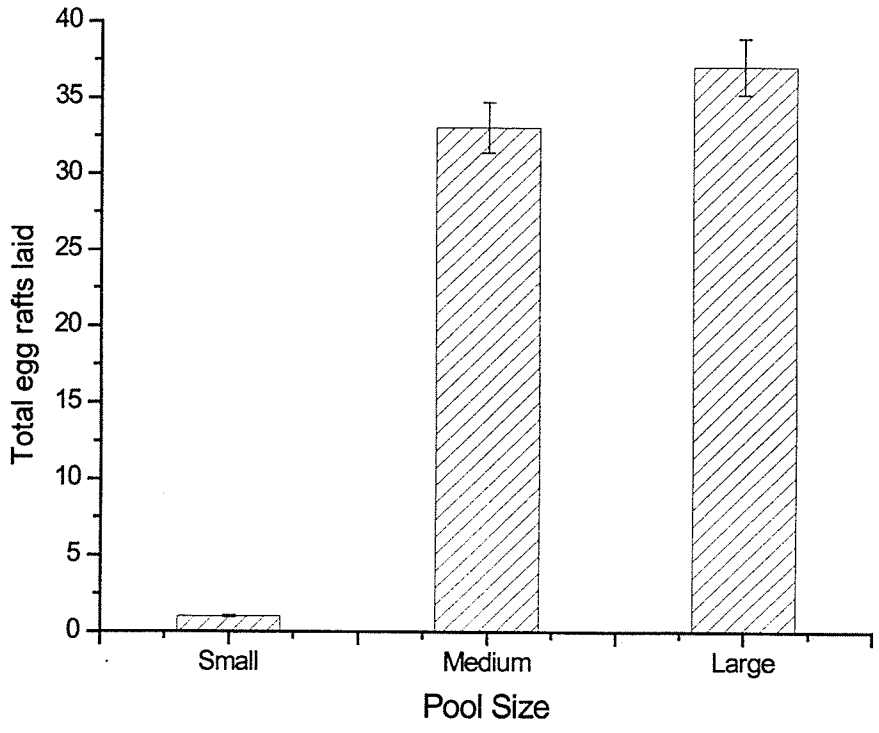

Figure 6. Number of egg rafts collected for each size of experimental pool Jul-Aug, 2001 at Glenlea Reseach Station.

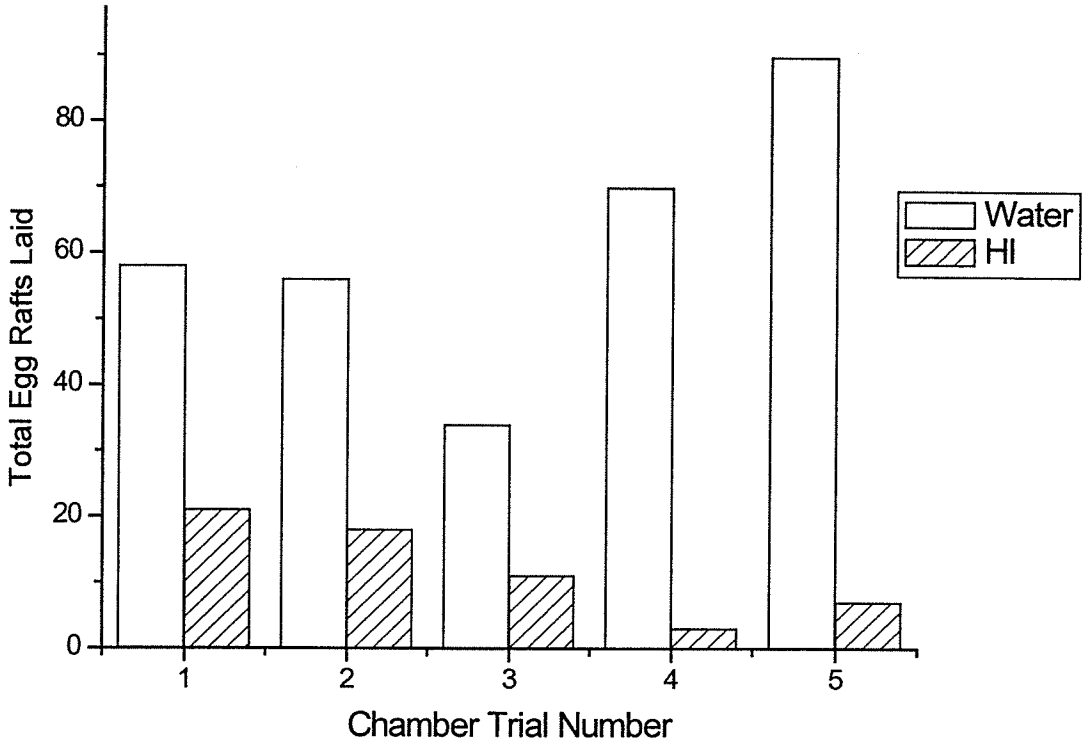

Figure 7. Number of *Culex tarsalis* (Bakersfield strain) egg rafts laid in Reiter's medium (Reiter, 1983) and aged tap water during five incubator trials.

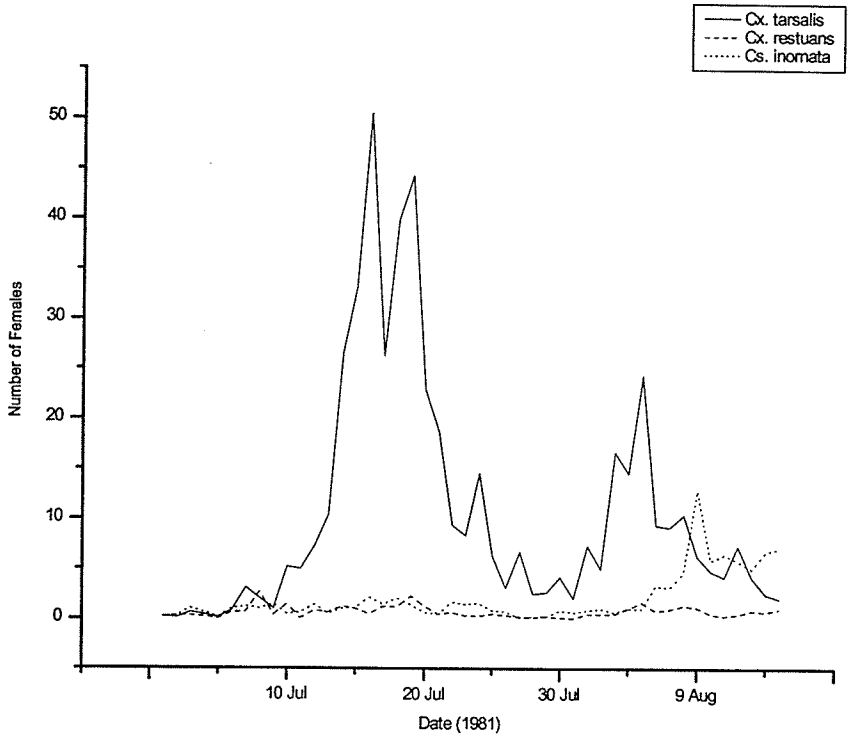

Figure 8. New Jersey Light trap data for *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata* for Jul - Aug 1981. Average number of females per night in thirteen traps placed throughout the city of Winnipeg. Data provided courtesy of the City of Winnipeg Insect Control Department.

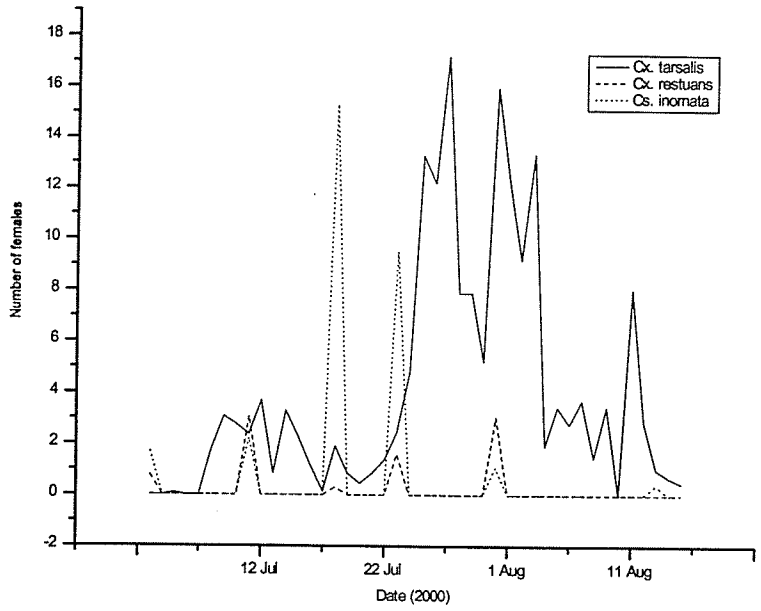

Figure 9. New Jersey Light trap data for *Cx. tarsalis, Cx. restuans* and *Cs. inornata* for Jul - Aug 2000. Avergae number of females per night in thirteen traps placed throughout the city of Winnipeg. Data provided courtesy of the City of Winnipeg Insect Control Department.

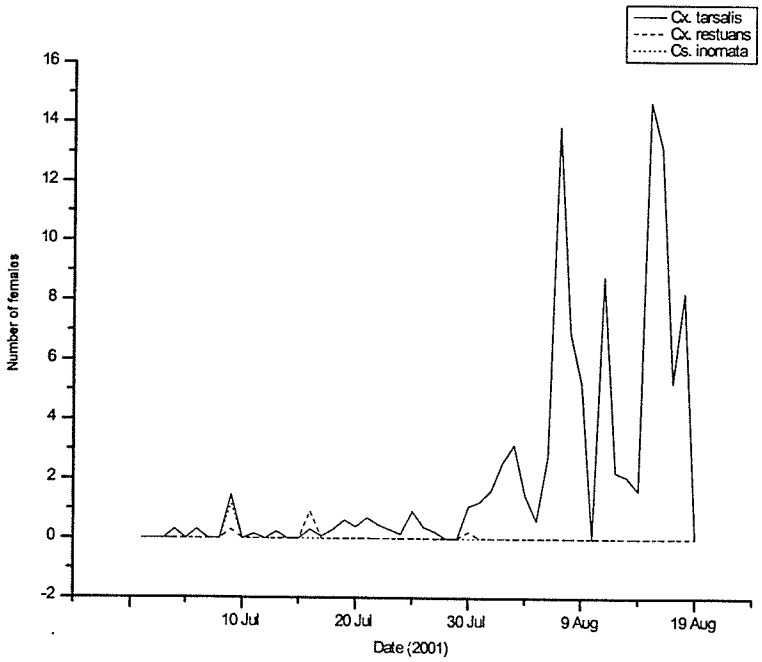

Figure 10. New Jersey Light trap data for *Cx. tarsalis, Cx. restuans* and *Cs. inornata* for Jul - Aug 2001. This summer had the lowest numbers of all three species. Data provided courtesy of the City of Winnipeg Insect Control Department.

	n		Mean	Std Dev	X^2	F-test
Control	20	HI	7.00	3.46		
		W	6.00	4.35	0.24, $p > 0.05$	
Exp	60	HI	13.40	8.99		
		W	40.80	11.28	82.90, $p < 0.05$	$F_{4,4} = 1.57$, $p > 0.05$

Table 1. Results of simple choice experiments (Reiter's medium = HI vs. tap water = W) with *Cx. tarsalis* conducted in the laboratory. The F statistic shows there is no significant difference in the variability of the counts.

Location	Collections	No. Rafts	Date rafts collected
Assiniboine Park	30 May - 1 Aug 00	0	NA
Apiary - U of M	15 Jun - 30 Aug 00	0	NA
Charleswood Sewage Lagoon	1 Aug - 30 Aug 00	0	NA
McPhillips Sludge Beds	1 Aug - 30 Aug 00	2	18-Aug-00

Table 2. Total egg rafts collected for the summer of 2000 in four experimental locations. All eggs collected were *Culex restuans*. Locations were chosen based on the levels of target species seen in City of Winnipeg light traps.

Date	No. rafts	*Cs. inornata*	*Cx. restuans*
6-Jul-01	4	3	1
7-Jul-01	1	0	1
10-Jul-01	12	0	12
13-Jul-01	3	0	3
18-Jul-01	26	2	24
20-Jul-01	9	0	9
24-Jul-01	3	0	3
4-Aug-01	5	0	5
6-Aug-01	4	0	4
8-Aug-01	2	0	2
16-Aug-01	1	0	1
19-Aug-01	1	0	1
TOTAL	**71**	**5**	**66**
% of Total		7.04%	92.96%

Table 3. Number of egg rafts of each species (*Cs. inornata* & *Cx. restuans*) collected in experimental pools 6 July - 19 Aug 2001.

Pool size	Nights eggs were obs	Freq	SE	95% CI	Within 95% CI
Small	1/30	0.033	+/- .0326	(.0309, .0969)	Yes
Med	3/30	0.100	+/- .0548	(.0074, .2074)	Yes
Large	4/30	0.133	+/- .0621	(.0113, .2547)	Yes
M^2	5/30	0.167	+/- .0680	(.0337, .2997)	Yes
X^2	**=**	**3.012**			
p	**=**	**0.389**			

Table 4. Frequency of occurrence of *Culex restuans* and *Culiseta inornata* oviposition in each of four sizes of experimental pools. Analysis shows there is no significant difference between frequencies in each pool. All frequencies and SE fall within a 95% confidence interval.

Days Oviposition Occurred

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9	
	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W
Trial 1	0	2	6	7	4	19	7	23	4	4	0	0	0	2	0	1	0	0
Total		**2**		**13**		**23**		**30**		**8**		**0**		**2**		**1**		**0**
Trial 2	3	2	5	17	2	6	2	15	2	13	2	2	2	1	0	0	0	0
Total		**5**		**22**		**8**		**17**		**15**		**4**		**3**		**0**		**0**
Trial 3	1	4	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total		**5**		**6**		**1**		**0**		**0**		**0**		**0**		**0**		**0**
Trial 4	1	1	0	5	0	10	9	11	0	7	0	0	1	0	0	0	0	0
Total		**2**		**5**		**10**		**20**		**7**		**0**		**1**		**0**		**0**
Trial 5	3	38	0	19	0	5	0	3	0	3	0	1	0	0	0	0	0	1
Total		**41**		**19**		**5**		**3**		**3**		**1**		**0**		**0**		**1**
Trial 6	0	2	1	12	1	20	1	37	0	14	2	3	2	2	0	0	0	0
Total		**2**		**13**		**21**		**38**		**14**		**5**		**4**		**0**		**0**
TOTAL		57		78		68		108		47		10		10		1		1
																		380

Table 5. Number of egg rafts laid each day during incubator trials. Trial 3 was not included in statistical analysis, due to low number of egg rafts obtained. H = hay infusion, W = aged tap water.

Infection	Distribution	Vector
Barmah Forest virus	Australia	Mosquitoes
Cat flea typhyus	United States	Fleas
Cat scratch disease	Global	Fleas, *Ctenocephalides felis*
Dengue hemorrhagic fever	Americas, Asia	*Aedes (Stegomyia)* mosquitoes
Human erlichiosis-monocytic	Americas, Asia, Europe	Ticks
Human erlichiosis-granulocytic	United States, Europe	Ticks
Kyasanur forest disease	India	Ticks
O'nyong-nyong fever	East Africa	*Anopheles* mosquitoes
Oriental spotted fever	Japan	Ticks?
Oropouche virus	South America, Panama	*Culicoides* midges
Potasi virus	United States	Mosquitoes
Rocio virus	Brazil	Mosquitoes

Table 6. Global emerging diseases with arthropod vectors (Gratz, 1999).

Infection	Distribution	Vector
Chikungunya	Africa, Asia	*Aedes (Stegomyia)* mosquitoes
Congo-Crimean hemorrhagic fever	Africa, Asia, Europe	Ticks
Dengue	Africa, Americas, Asia	*Aedes (Stegomyia)* mosquitoes
Filariasis - bancroftian	Africa, Americas, Asia	Mosquitoes
Japanese encephalitis	Asia	*Culex* mosquitoes
Leishmaniasis visceral	Africa, Americas, Asia	Sandflies
Leishmaniasis cutaneous	Global	Sandflies
Lyme disease	Global	Ticks
Malaria	Global	*Anopheles* mosquitoes
Plague	Africa, Americas, Asia	Fleas
Rift Valley fever	Africa	Mosquitoes
Ross River virus	Australia, Pacific Islands	Mosquitoes
Trench fever	United States, Europe	Body lice
Venezuelan equine encephalitis	Americas	Mosquitoes
Yellow fever	Africa, Americas	*Aedes (Stegomyia)* mosquitoes

Table 7. Global resurgent diseases with arthropod vectors (Gratz, 1999)

Literature Cited

Ahmadi, A. & G.A.H. McClelland. 1983. Oviposition attractants of the western tree-hole mosquito, *Aedes sierrensis*. *Mosquito News* 43: 343-345.

Allan, S.A. & D.L. Kline. 1995. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Chemical Ecology* 21: 1847-1860.

Anderson, R.A. & R.A. Brust. 1995. Field evidence for multiple host contacts during blood feeding by *Culex tarsalis, Cx. restuans*, and *Cx. nigripalpus* (Diptera: Culicidae). *Journal of Medical Entomology* 32: 705-710.

Artsob, H & L. Spence. 1979. Arboviruses in Canada pp. 39-66. *In*: E. Kurstack [ed.], *Arctic and Tropical Arboviruses*. Academic Press, New York.

Bates, M. 1940. Oviposition experiments with anopheline mosquitoes. *American Journal of Tropical Medicine* 20: 569-583.

Beaty B.J. & W.C. Marquardt. 1996. *The Biology of Disease Vectors*. University Press of Colorado, USA.

Beehler, J.W., J.G. Millar & M.S. Mulla. 1994. Protein hydrolysates and associated bacterial contaminants as oviposition attractants for the mosquito *Culex quinquefasciatus*. *Medical and Veterinary Entomology* 8: 381-385.

Beehler, J.W. & M.S. Mulla. 1993. The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes. *Proceedings of the California Mosquito Vector Control Association* 61: 121-124.

Beehler, J.W. & M.S. Mulla. 1995. Effects of organic enrichment on temporal distribution and abundance of culicine egg rafts. *Journal of the American Mosquito Control Association* 11: 167-171.

Beier, J.C., P.V. Perkins & R.A. Wirtz. 1983. Habitat segregation among larval mosquitoes (Diptera: Culicidae) in tire yards in Indiana, USA. *Journal of Medical Entomology* 25: 9-16.

Bellini, R., M. Carrieri, G. Burgio & M. Bacchi. 1996. Efficacy of different ovitraps and binomial sampling in *Aedes albopictus* surveillance activity. *Journal of the American Mosquito Control Association* 12: 632-636.

Belton, P. 1967. The effect of illumination and pool brightness on oviposition by *Culex restuans* (Theo.) in the field. *Mosquito News* 27: 66-68.

Bentley, M.D. & J.F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology* 34: 401-421.

Bentley, M.D., I.N. McDaniel & H.P. Lee. 1976. Studies of *Aedes triseriatus* oviposition attractants produced by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). *Journal of Medical Entomology* 13: 112-115.

Bentley, M.D., I.N. McDaniel & M.Yatagai. 1979. P-Cresol: an oviposition attractant of *Aedes triseriatus*. *Environmental Entomology* 8: 206-209.

Benzon, G.L. & C.S. Apperson. 1988. Re-examination of chemically mediated oviposition behavior in *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* 25: 158-64.

Blackwell, A., J.A. Mordue & B.S. Hansson. 1993. A behavioral and electrophysiological study of oviposition cues for *Culex quinquefasciatus*. *Physiological Entomology* 18: 343-348.

Blaustein, L. & B.P. Kotler. 1993. Oviposition habitat selection by the mosquito *Culiseta longiareolata*: effects of conspecifics, food and green toad tadpoles. *Ecological Entomology* 18: 104-108.

Bruno, D.W. & B.R. Laurence. 1979. The influence of the apical droplet of *Culex* egg rafts on oviposition of *Culex pipiens fatigans* (Diptera: Culicidae). *Journal of Medical Entomology* 16: 300-305.

Bres, P. 1988. Impact of arboviruses on human and animal health pp.1-18 *In:* Monath, T.P. [ed.], *The Arboviruses: Epidemiology and Ecology. Vol. I.* CRC Press, Florida.

Brust, R.A. 1976. Mosquito surveys in Manitoba during 1975. *Canadian Journal of Public Health* 67: 47-53.

Brust, R.A. 1982. Population dynamics of *Culex tarsalis* Coquillett in Manitoba, pp 21-30. *In:* L. Sekla [ed.], *Western equine encephalitis.* Manitoba Health Services Commission, Winnipeg.

Brust, R.A. 1990. Oviposition behavior of natural populations of *Culex tarsalis* and *Culex restuans* (Diptera: Culicidae) in artificial pools. *Journal of Medical Entomology* 27: 248-255.

Brust, R.A. 1991. Environmental regulation of autogeny in *Culex tarsalis* (Diptera: Culicidae) from Manitoba, Canada. *Journal of Medical Entomology* 28: 847-853.

Brust, R.A. & R.A. Ellis. 1976. Assessment of the emergency mosquito control operation in Manitoba, 1975. *Canadian Journal of Public Health* 67: 69-71.

Buth, J.L., R.A. Brust & R.A. Ellis. 1990. Development time, oviposition activity and onset of diapause in *Culex tarsalis*, *Culex restuans* and *Culiseta inornata* in southern Manitoba. *Journal of the American Mosquito Control Association* 6: 55-63.

Chadee, D.D., A. Lakhan, W.R. Ramdath & R.C. Persad. 1993. Oviposition response of *Aedes aegypti* mosquitoes to different concentrations of hay infusion in Trinidad, West Indies. *Journal of the American Mosquito Control Association* 9: 346-348.

Chesson, J. 1984. Effect of notonectids (Hemiptera: Notonectidae) on mosquitoes (Diptera: Culicidae): predation or selective oviposition? *Environmental Entomology* 13: 531-538.

Chow, C.Y. 1948. The bionomics of two important malaria vectors in China. *Proceedings of the 4th International Congress of Tropical Medicine and Malaria* 1: 681-685.

Clements, A.N. 1999. *The Biology of Mosquitoes Vol. 1.* CABI Publishing, New York.

Dadd, R.H. & J.E. Kleinjan. 1974. Autophagostimulant from *Culex pipiens* larvae: distinction from other mosquito larval factors. *Environmental Entomology* 3: 21-28.

Davis, E.E. & M.F. Bowen. 1994. Sensory physiological basis for attraction in mosquitoes. *Journal of the American Mosquito Control Association* 10: 316-325.

Dhileepan, K. 1997. Physical factors and chemical cues in the oviposition behavior of arboviral vectors *Culex annulirostris* and *Culex molestus* (Diptera:

Culicidae). *Environmental Entomology* 26: 318-326.

Dobrotworsky, N.V. 1965. *The Mosquitoes of Victoria (Diptera: Culicidae).* Melbourne University Press, Carleton, Victoria.

Dodge, H.D. 1966. Studies on mosquito larvae II: the first-stage larvae of North American Culicidae and of world Anophelinae. *The Canadian Entomologist* 98: 337-393.

Downs, W.G. & C.S. Pittendrigh. 1946. Bromeliad malaria in Trinidad, British West Indies. *American Journal of Tropical Medicine* 26: 47-66.

Du, Y. & J.G. Millar. 1999a. Oviposition responses of gravid *Culex quinquefasciatus* and *Culex tarsalis* to bulrush (*Schoenoplectus acutus*) infusions. *Journal of the American Mosquito Control Association* 15: 500-509.

Du, Y. & J.G. Millar. 1999b. Electroantennogram and oviposition bioassay responses of *Culex quinquefasciatus* and *Culex tarsalis* (Diptera: Culicidae) to chemicals in odors from Bermuda grass infusions. *Journal of Medical Entomology* 36: 158-166.

Foster, W.A. 1995. Mosquito sugar feeding and reproductive energetics. *Annual Review of Entomology* 40:443-474.

Fox, A.S. 1994. Autogenous-anautogenous oviposition in *Culiseta inornata* from Manitoba, Canada. *Journal of the American Mosquito Control Association* 10: 125-126.

Frank, J.H. 1986. Bromeliads as ovipositional sites for *Wyeomyia* mosquitoes: form and color influence behavior. *Florida Entomologist* 69: 7728-42.

Frank, J.H., G.A. Curtis & H.T. Evans. 1976. On the bionomics of bromeliad-inhabiting mosquitoes. I. Some factors influencing oviposition by *Wyeomyia vanduzeei. Mosquito News* 36: 25-30.

Fraser, H.M. & R.A. Brust. 1976. Weather conditions affecting mosquito populations in southern Manitoba during 1975. *Canadian Journal of Public Health* 67: 40-46.

Gary, R.E. & W.A. Foster. 2001. Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector *Anopheles gambiae* (Diptera:

Culicidae). *Journal of Medical Entomology* 38: 22-28.

Gillies, M.T. 1972. Some aspects of mosquito behaviour in relation to the transmission of parasites. *Zoological Journal of the Linnean Society* 51: 69-81.

Gjullin, C.M., J.O. Johnsen & F.W. Plapp. 1965. The effect of odors released by various waters on the oviposition sites selected by two species of *Culex*. *Mosquito News* 25: 268-271.

Gratz, N.G. 1999. Emerging and resurging vector-borne diseases. *Annual Review of Entomology* 44: 51-75.

Harbach, R. & I.J. Kitching. 1998. Phylogeny and classification of the Culicidae (Diptera). *Systematic Entomology* 23: 327-370.

Hardy, J.L., E.J. Houk, L.D. Kramer & W.C. Reeves. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. *Annual Review of Entomology* 28:229-262.

Hazard, E.I., M.S. Mayer & K.E. Savage. 1967. Attraction and oviposition stimulation of gravid female mosquitoes by bacteria isolated from hay infusions. *Mosquito News* 27: 133-136.

Helson, B.V., G.A. Surgeoner & R.E. White. 1979. Mosquitoes of southwestern Ontario, their seasonal distribution, prevalence and new records pp. 182-198. *In:* Mahdy, M.S., L. Spence & J.M. Joshua [ed.] *Arboviral Encephalitides in Ontario with Special Reference to St. Louis Encephalitis.* Ontario Ministry of Health, Ontario.

Horner, T.A. & R.G. Weber. 1992. Attractancy of oviposition sites containing conspecific larvae and rearing water to gravid *Culex pipiens* and *Culex restuans*. *Proceedings of the New Jersey Mosquito Control Association*. 79th Annual Meeting pp.104-111.

Ikeshoji, T. & M.S. Mulla. 1974. Attractancy and repellency of alkyl carbonyl compounds for mosquito oviposition. *Japanese Journal of Sanitary Zoology* 25: 89-94.

Ikeshoji, T., K. Saito & A. Yano. 1975. Bacterial production of the ovipositional attractants for mosquitoes on fatty acid substrates. *Applied Entomology and Zoology* 10: 239-242.

Isoe, J. & J.G. Millar. 1995. Characterization of factors mediating oviposition site choice by *Culex tarsalis*. *Journal of the American Mosquito Control Association* 11: 21-28.

Isoe, J., J.G. Millar & J.W. Beehler. 1995. Bioassays for *Culex* (Diptera: Culicidae) mosquito oviposition attractants and stimulants. *Journal of Medical Entomology* 32: 475-483.

Jones, C.J. & E.T. Schreiber. 1994. Color and height affect site preferences of *Toxorhynchites splendens* and *Toxorhynchites rutilus rutilus* (Diptera: Culicidae) in the laboratory. *Environmental Entomology* 23: 130-135.

Kay, B.H., P.A. Ryan, B.M. Russell, J.S. Holt, S.A. Lyons & P.N. Foley. 2000. The importance of subterranean mosquito habitat to arbovirus vector control strategies in North Queensland, Australia. *Journal of Medical Entomology* 37: 846-853.

Kennedy, J.S. 1942. On water finding and oviposition by captive mosquitoes. *Bulletin of Entomological Research* 32: 279-301.

Kettle, D.S. 1984. *Medical and Veterinary Entomology.* Croom Helm, Ltd., Sydney, Australia.

Kitron, U.D., D.W. Webb & R.J. Novak. 1989. Oviposition behavior of *Aedes triseriatus* (Diptera: Culicidae): prevalence, intensity, and aggregation of eggs in oviposition traps. *Journal of Medical Entomology* 26: 462-467.

Klowden, M.J. 1986. The effects of sugar deprivation on the host-seeking behaviour of *Aedes aegypti* mosquitoes. Journal of Insect Physiology 32: 479-483.

Klowden, M.J. 1999. The check is in the male: male mosquitoes affect female physiology and behaviour. *Journal of the American Mosquito Control Association* 15: 213-220.

Klowden, M.J. & J.L. Blackmer. 1987. Humoral control of pre-oviposition behavior in the mosquito, *Aedes aegypti*. *Journal of Insect Physiology* 33: 689-692.

Knight, J.C. & S.A. Corbet. 1991. Compounds affecting mosquito oviposition:

structure-activity relationships and concentration effects. *Journal of the American Mosquito Control Association* 7: 37-41.

Knight, K.L. & A. Stone. 1973. *A Catalog of the Mosquitoes of the World.* Entomological Society of America, USA.

Kramer, L.D., J.L. Hardy & S.B. Presser. 1983. Effect of temperature and extrinsic incubation period on the vector competence of *Culex tarsalis* for western equine encephalomyelitis virus. *American Journal of Tropical Medicine and Hygiene* 32: 130-139.

Kramer, W.L. & M.S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environmental Entomology* 8: 1111-1117.

Laird, M. 1988. *The Natural History of Larval Mosquito Habitats.* Academic Press, London.

Lampman, R.L. & R.J. Novak. 1996a. Oviposition preferences of *Culex pipiens* and *Culex restuans* for infusion-baited traps. *Journal of the American Mosquito Control Association* 12: 23-32.

Lampman, R.L. & R.J. Novak. 1996b. Attraction of *Aedes albopictus* adults to sod infusion. *Journal of the American Mosquito Control Association* 12: 119-124.

Lane, R.P. & R.W. Crosskey. 1993. *Medical Insects and Arachnids.* Chapman and Hall, London, UK.

Laurence, B.R. & J.A. Pickett. 1982. Erythro-6-acetoxy-hexadeconolide, the major component of a mosquito oviposition attractant pheromone. *Journal of the Chemical Society, Chemical Communications* 1: 59-62.

Laurence, B.R. & J.A. Pickett. 1985. An oviposition attractant pheromone in *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bulletin of Entomological* Research 75: 283-290.

Lee, J. & M.C. Klowden. 1999. A male accessory gland protein that modulates female mosquito (Diptera: Culicidae) host-seeking behavior. *Journal of the American Mosquito Control Association* 15: 4-7.

Linley, J.R. 1989. Laboratory tests of the effect of p-cresol and 4-

methylcyclohexanol on oviposition by three species of *Toxorhynchites* mosquitoes. *Medical and Veterinary Entomology* 3: 347-352.

Lothrop, H.D. & W.K. Reisen. 2001. Landscape affects the host-seeking patterns of *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. *Journal of Medical Entomology* 38: 325-332.

Lounibos, L.P. 1978. Mosquito breeding and oviposition stimulant in fruit husks. *Ecological Entomology* 3: 299-304.

Lowenberger, C.A. & M.E. Rau. 1994. Selective oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to a larval parasite, *Plagiorchis elegans* (Trematoda: Plagiorchiidae). *Environmental Entomology* 23: 1269-1276.

Madder, D.J., R.S. MacDonald, G.A. Surgeoner & B.V. Helson. 1980. The use of oviposition activity to monitor populations of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae). *The Canadian Entomologist* 112: 1013-1017.

Maw, M.G. & G.K. Bracken. 1971. The use of artificial pools in assessing populations of the mosquito *Culex restuans* Theobald. *Proceedings of the Entomological Society of Ontario* 102: 78-83.

Mboera, L.E. G., Mdira, K.Y. & F.M. Salum. 1999. The influence of synthetic oviposition pheromone and volatiles from soakage pits and grass infusions upon oviposition site selection of *Culex* mosquitoes in Tanzania. *Journal of Chemical Ecology* 26: 1193-1203.

Mboera, L.E.G., W. Takken, K.Y. Mdira & J.A. Pickett. 2000. Sampling gravid *Culex quinquefasciatus* (Diptera: Culicidae) in Tanzania with traps baited with synthetic oviposition pheromone and grass infusions. *Journal of Medical Entomology* 37: 172-176.

McArthur, J. 1947. The transmission of malaria in Borneo. *Transcripts of the Royal Society of Tropical Medicine and Hygiene* 40: 537-558.

Millar, J.G., J.D. Chaney, J.W. Beehler & M.S. Mulla. 1994. Interaction of the *Culex quinquefasciatus* egg raft pheromone with a natural chemical associated with oviposition sites. *Journal of the American Mosquito Control Association* 10: 374-379.

Mitchell, C.J. 1988. Occurrence, biology and physiology of diapause in

overwintering mosquitoes pp 191-218. *In*: T.P. Monath [ed.] *The Arboviruses: Epidemiology and Ecology* Vol. 1. CRC Press, Florida.

Murphey, F.J. & P.P. Burbutis. 1967. Straw infusion attractiveness to gravid female *Culex salinarius*. *Journal of Economic Entomology* 60: 156-161.

Murphy, M.W., R.F. Dunton, M.J. Perich & W. A. Rowley. 2001. Attraction of *Anopheles* (Diptera: Culicidae) to volatile chemicals in western Kenya. *Journal of Medical Entomology* 38: 242-244.

Nayer, J.K. & D.M. Sauerman. 1975. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 1. Utilization of sugar for survival. *Journal of Medical Entomology* 12: 92-98.

Petersen, J.J. & D.M. Rees. 1967. Comparative oviposition and selection preference by *Aedes dorsalis* and *Aedes nigromaculis* for three inorganic salts in the laboratory. *Mosquito News* 27: 136-133.

Petranka, J.W. & K. Fakhoury. 1991. Evidence of a chemically mediated avoidance response of ovipositing insects to bluegills and green frog tadpoles. *Copeia* (1991): 234-239.

Reeves, W.C. 1971. Mosquito vector and vertebrate host interaction: the key to maintenance of certain arbovirses, pp. 223-231. *In:* A.M. Fallis [ed.], *Ecology and Physiology of Parasites.* University of Toronto Press, Toronto.

Reeves, W.C, R.E. Bellamy & R.P. Scrivani. 1961. Differentiation of encephalitis virus infection rates from transmission rates in mosquito vector populations. *American Journal of Hygiene* 73: 303-315.

Reeves W.C, Hardy, J.L., Reisen, W.K. & M.M. Milby. 1994. Potential effect of global warming on mosquito borne arboviruses. *Journal of Medical Entomology* 31: 323-332.

Reisen, W.K. & R.P. Meyer. 1990. Attractiveness of selected oviposition substrates for gravid *Culex tarsalis* and *Culex quinquefasciatus* in California. *Journal of the American Mosquito Control Association* 6: 244-250.

Reisen, W.K. & A.R. Pfuntner. 1983. Effectiveness of five methods for sampling *Culex* mosquitoes in rural and urban habitats in San Bernardino County, California. *Journal of the American Mosquito Control Association* 3: 601-606.

Reisen, W.K. & T.F. Siddiqui. 1978. The influence of conspecific immatures on the oviposition preferences of the mosquitoes *Anopheles stephensi* and *Culex tritaeniorhynchus* (Diptera: Culicidae) in Pakistan. *Pakistan Journal of Zoology* 10: 31-40.

Reisen, W.K., H.D. Lothrop & R.P. Meyer. 1997. Time of host seeking by *Culex tarsalis* (Diptera; Culicidae) in California. *Journal of Medical Entomology* 34: 430-437.

Reisen ,W.K., R.P. Meyer, S.B. Presser & J.L. Hardy. 1993. Effect of temperature on the transmission of western equine encephalomyelitis and St. Louis encephalitis viruses by *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology* 30: 151-160.

Reiter, P. 1983. A portable, battery powered trap for collecting gravid *Culex* mosquitoes. *Mosquito News* 43: 496-498.

Reiter, P. 1986. A standardized procedure for the quantitative surveillance of certain *Culex* mosquitoes by egg raft collection. *Journal of the American Mosquito Control Association* 2: 219-221.

Reiter, P., M.A. Amador & N. Colon. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *Journal of the American Mosquito Control Association* 7: 52-55.

Rejmankova, E., D. Roberts, S. Manguin, K. Pope, J. Komarek & R.A. Post. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. *Environmental Entomology* 25: 1058-1067.

Ritchie, S.A. 1984. Hay infusion and isopropyl alcohol-baited CDC light trap: a simple, effective trap for gravid *Culex* mosquitoes. *Mosquito News* 44: 404-407.

Ritchie, S.A. & C. Laidlaw-Bell. 1994. Do fish repel oviposition by *Aedes taeniorhynchus*? *Journal of the American Mosquito Control Association* 10: 380-384.

Roberts, D. 1996. Mosquitoes (Diptera: Culicidae) breeding in brackish water: female oviposition preferences or larval survival? *Journal of Medical Entomology* 33: 525-530.

Roberts, L.S. & J. Janovy. 1996. *Foundations of Parasitology*, 5th Ed. Wm. C. Brown Publishers, Dubuque, USA.

Russel P.F. & T.R. Rao. 1942. On relation of mechanical obstruction and shade to ovipositing *Anopheles culicifacies*. *Journal of Experimental Zoology* 91: 303-329.

Steinly, B.A., R.J. Novak & D.W. Weber. 1991. A new method for monitoring mosquito oviposition in artificial and natural containers. *Journal of the American Mosquito Control Association* 7: 649-650.

Strickman, D. 1980. Stimuli affecting selection of oviposition sites by *Aedes vexans*: conditioning of the soil. *Mosquito News* 40: 413-417.

Torres-Estrada, J.L., M.H. Rodriguez, L. Cruz-Lopez & J.I. Arredondo-Jimenez. 2001. Selective oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to *Mesocyclops longisetus* (Copepoda: Cyclopoidea) under laboratory and field conditions. *Journal of Medical Entomology* 38: 188-192.

Turell, M.J. 1988. Horizontal and vertical transmission of viruses by insect and tick vectors. pp.127-152 *In:* Monath, T.P. [ed.], *The Arboviruses: Epidemiology and Ecology. Vol. I.* CRC Press, Florida.

Wallace, J.R. & R.W. Merritt. 1999. Influence of microclimate, food, and predation on *Anopheles quadrimaculatus* (Diptera: Culicidae) growth and development rates, survivorship, and adult size in a Michigan pond. *Environmental Entomology* 28: 233-239.

Weber, R.G. & T.A. Horner. 1992. The ability of *Culex pipiens* and *Culex restuans* to locate small ovisites in the field. *Proceedings of the New Jersey Mosquito Control Association.* 79th Annual Meeting. pp. 96-103.

Weber, R.G. & C. Tipping. 1990. Drinking as a pre-oviposition behavior of wild *Culex pipiens* (Diptera: Culicidae). *Entomological News* 101: 257-265.

Weber, R.G. & C. Tipping. 1992. Oviposition by naturally impaired, wild *Culex pipiens* and *Culex restuans* females. *Proceedings of the New Jersey Mosquito Control Association.* 77th Annual Meeting pp. 96-103.

Wilton, D.P. 1968. Oviposition site selection by the tree-hole mosquito, *Aedes triseriatus. Journal of Medical Entomology* 5: 189-194.

Wood, D.M., P.T. Dang & R.A. Ellis. *The Insects and Arachnids of Canada Part 6: The Mosquitoes of Canada*. Canadian Government Publishing Centre, Hull.

World Health Organization. 1997. World malaria situation in 1994. Part I. Population at risk. *Weekly Epidemiology Record*. 70: 347-48.

Xue, R., J. Edman & T.W. Scott. 1995. Age and body size effects on blood meal size and multiple blood feeding by *Aedes aegypti*. *Journal of Medical Entomology* 32: 471-474.

Yap, H.H., C.Y. Lee, N.L. Chong, A.E.S. Foo & M.P. Lim. 1995. Oviposition site preference of *Aedes albopictus* in the laboratory. *Journal of the American Mosquito Control Association* 11: 128-132.

Zahiri, N., M.E. Rau, D.J. Lewis & S. Khanizadeh. 1997. Intensity and site of *Plagiorchus elegans* (Trematoda: Plagiorchiidae) infections in *Aedes aegypti* (Diptera: Culicidae) larvae affect the attractiveness of their waters to ovipositing, conspecific females. *Environmental Entomology* 26: 920-923.

Zulueta, J. 1950. Comparative oviposition experiments with caged mosquitoes. *American Journal of Hygiene* 52: 133-42.