THE ROLE OF TIRES IN PROVIDING SUITABLE OVIPOSITION SITES AND LARVAL HABITAT FOR MOSQUITOES IN MANITOBA

A Thesis

Submitted to the Faculty

of

Graduate Studies

University of Manitoba

by

Thomas John Scott McMahon

In partial fulfillment of the requirements for the degree

of

Master of Science

Department of Entomology

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

THE ROLE OF TIRES IN PROVIDING SUITABLE OVIPOSITION SITES AND LARVAL HABITAT FOR MOSQUITOES IN MANITOBA

BY

Thomas John Scott McMahon

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

OF

MASTER OF SCIENCE

ACKNOWLEDGMENTS

The Manitoba Tire Stewardship Board deserves many thanks for providing funding to Dr. Anderson at the University of Winnipeg, without which, this research would not have been possible. Bill Harper at the Tire Stewardship Board assisted in helping me locate the provincial tire dealers, and facilitated the transport of over 500 tires to my farm in Roland.

Wendy Ralley at Manitoba Conservation helped me by giving me locations, maps, and contact information of the waste management grounds located province-wide.

I sincerely thank my co-advisors Dr. Terry Galloway and Dr. Robert Anderson, for their patience and advice to help me become a better writer (hopefully). They also lent a hand in the field on several occasions. I also thank my external committee member Dr. Darren Gillis for giving that important point-of-view from outside entomology.

My parents gave me, not only the land on which to run an experiment but lots of support and encouragement to get me through this project. Another thank-you goes out to all the friends who supported and helped as well: Lisa Baspaly, Mike Fuentespina, Nadya Baspaly, Scott Derham, Rochelle Gnanapragasm, Rob Senkiw, Chandra Chambers, Anita Stjernberg, and Dave Holder.

Last, but not least, thanks to all the tire dealers and rural municipalities who allowed me to sample their tires. Reliable Tire in Winnipeg, the Tire Recycling Corporation, and Sturgeon Tire on Sturgeon Road also helped by lending me tires for experiments.

ABSTRACT

This research was conducted to determine the species composition and prevalence of immature mosquitoes in waste tires throughout Manitoba. Further research was done to determine if there were temporal factors, environmental factors, or tire characteristics that could predict the likelihood of finding mosquito larvae in tires. Over 95% of the larvae and egg rafts collected from tires in the two years of this study were *Culex restuans*. An egg raft was discovered in a tire within 24 hours of it being filled with water. Prevalence and abundance of immature mosquitoes in tires increased as the summer progressed. Tire size, and the amount of sun exposure had a significant effect on abundance and prevalence of immature mosquitoes in tires. Larger tires had significantly more immature mosquitoes and significantly more often than smaller tires. (F value 47.14, df = 3, $p<0.0001$; χ value 68.21, df = 3, $p<0.0001$). Exposed tires contained more egg rafts than the shaded tires ($p = 0.042$). Stacking method affected mosquito prevalence and abundance when sampling was conducted in August. Horizontally stacked tires contained significantly fewer immature mosquitoes than either random or vertically stacked tires ($p< 0.05$).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I: INTRODUCTION

Mosquitoes may pose a significant risk to public health because they transmit human pathogens. Exotic pathogens, such as West Nile virus, which was documented in Manitoba in 2002, increase the importance of research on the biology of local species of mosquitoes to understand their role in transmission. Such studies would also be expected to help inform decisions about potential control strategies. One of the most effective means of controlling mosquito populations is to reduce larval habitat, but this strategy depends on good information about where vector species of mosquitoes lay eggs. In some instances, oviposition sites are made available to mosquitoes as a result of human activities. Because oviposition success is a requirement for larval success, which in turn, directly affects the initial size of the adult population, which then influences the magnitude of the public health risk, it is essential to know more about the aquatic ecology of immature mosquitoes and the factors that influence oviposition site selection.

Mosquitoes lay eggs in sites with at least two basic characteristics: water, or the high probability of becoming inundated, and organic material that supports aquatic bacteria on which mosquito larvae feed. However, some species are very site specific with respect to other ecological characteristics of oviposition sites. For example, some species have been documented to lay eggs only in rock pools, while others only lay eggs in snowmelt pools (Darsie and Ward 2005; Wood *et al.* 1979). Some species use containers as oviposition sites. Container habitats are those that have a limited amount of water, and are not contiguous with ground water sources. Natural containers include treeholes, rock pools, bromeliads and other phytotelmata (water filled containers in or on plants) (Laird 1988; Clements 1999). Artificial containers are objects made by humans that can retain water and organic debris, the two basic requirements for oviposition by mosquitoes. Rain barrels, water troughs, and tires are all examples of artificial containers in which mosquitoes have been recorded (Gomes *et al.* 1995; Baumgartner 1987; Andreadis 1988; Hedberg *et al.* 1985).

The focus of this thesis is the use of tires as oviposition sites by mosquitoes. Tires collect rainwater and organic debris, resulting in a bacteria-rich pool that can be attractive to gravid females and provide a suitable habitat in which mosquito larvae can complete their development. Also, tires are frequently stored outside, often in proximity to human populations, and can accumulate in very large piles. Tires may thus pose a significant health risk if they contribute substantially to local vector mosquito populations.

The accumulation of used tires in the environment increases annually. Approximately 250 million used tires are stored outside in the United States each year (Holst *et al.* 1998). This number is a 25% increase over the ten years since Baumgartner (1988) reported 200 million used tires generated in the United States. Moore *et al.* (1990) and Romi *et al.* (1997a) classified truck and large equipment dealers, tire retreaders, tire recyclers, used tire stockpiles, salvage yards, and waste transfer stations as "high-risk premises" for the presence of mosquitoes because of the large accumulations of used tires usually found at these locations.

Tire recycling in Manitoba is administered by the Manitoba Tire Stewardship Board and more than one million tires are recycled each year. Even with this program, stockpiles of used tires do accumulate for various periods in the environment. A preliminary phone survey of tire dealerships and waste management grounds in Manitoba showed that in many instances, tires remained outside for an entire summer. Therefore, some tires may remain in the environment long enough to accumulate water and organic debris and to be colonized by mosquitoes. Research described herein was carried out to improve the understanding of the role of tires in mosquito ecology in Manitoba, and to evaluate potential management techniques for reducing populations of mosquitoes emerging from tires.

A preliminary study of the geographic distribution of mosquito-infested tires at waste management grounds and tire dealerships was conducted in the summer of 2003. The objectives of the study were: 1) to determine the species diversity and community structure of mosquito larvae in tires in southern Manitoba, 2) to study the phenology of oviposition activity, and 3) to examine the correlation of mosquito abundance with attributes of the tire microhabitat, including tire orientation, size and water volume.

In North America, *Culex tarsalis* Coquillett, *Culex restuans* (Theobald), *Culex pipiens pipiens* (L.), *Culiseta inornata* (Williston), *Ochlerotatus hendersoni* Cockerell, *Ochlerotatus triseriatus* (Say), *Ochlerotatus atropalpus* (Coquillett), *Aedes aegypti* (L.), *Ochlerotatus albopictus* (Skuse), and *Ochlerotatus japonicus* (Theobald) are some of the species known to lay eggs in tires and develop successfully as larvae (Joy *et al.* 2003; Rueger *et al.* 1964; Barker *et al.* 2003; Rupp 1977; Andreadis *et al.* 2001; Laird 1988; Sprenger and Wuithiranyagool 1986). Of these, only five (*Cx. tarsalis, Cx. restuans, Cs. inornata, Oc. hendersoni*, and *Oc. triseriatus*) have been documented in Manitoba, and were expected in the survey in 2003.

In 2004, tire pile attributes identified as important for mosquitoes during the survey of 2003 were experimentally manipulated to test the hypotheses developed during the field survey phase of this study. The objectives of the experiments were: 1) to explore the effect of environmental variables, such as exposure to sunlight and proximity to trees, in determining frequency of mosquito oviposition in tires, 2) to determine if tire stacking methods influence mosquito abundance, and 3) to combine the results from the first two objectives to devise a system of tire storage to minimize oviposition by mosquitoes in tires. These objectives were accomplished by placing tires piled vertically, horizontally, and randomly, as well as in shade, and direct sunlight, throughout the summer, and monitoring them regularly to see if there were differences in the prevalence, species composition, and number of mosquito larvae among any of the treatments.

This thesis is written in a paper style. Chapter II is a review of the literature that pertains to the topic of mosquitoes and tires. Chapter III focuses on the survey carried out in 2003. Chapters IV and V focus on experiments performed in 2004. Chapter VI is a discussion of the whole thesis, combining results from both years.

CHAPTER II: LITERATURE REVIEW

With growing concern for public health threats such as West Nile virus, many jurisdictions with no previously existing mosquito control programs now control mosquito larvae and, in some cases, adults. Many *Culex* spp. of mosquitoes are susceptible to West Nile virus infection, are capable of transmission and feed to greater or lesser extent on wild bird species that develop high levels of viraemia, and so are considered important vectors (Brinton 2002; Turell *et al.* 2005). Consequently, *Culex* spp. are usually the targets of most West Nile virus larviciding programs. One component of an effective larviciding program is identification and cataloguing of larval habitats, and for West Nile virus, targeting *Culex* larval habitats specifically. For example, several counties, rural municipalities and cities have a catch-basin treatment program, as they have been found to be frequently populated by *Culex* spp. mosquitoes (Geery and Holub 1989; Thomson 2004) and are in close proximity to human populations. Rimless tires also can be near human populations, and often contain mosquito larvae in countries across the world (Joy *et al.* 2003; Baumgartner 1988; Beier *et al.* 1983a; Cornel and Hunt 1991; Savage *et al.* 1992; Laird *et al.* 1994; Romi *et al.* 1997a). There is no published research from Manitoba that documents the species composition of mosquito larvae in tires. The goals of this research project were to ascertain which species of mosquitoes commonly are found in tires in Manitoba, and to determine what factors related to tire distribution in the environment influence the presence of mosquito larvae. A review of previous research about mosquitoes, oviposition and tires is necessary to set the context for the research described in this thesis.

Mosquito Oviposition:

Female mosquitoes determine the success of their progeny by selection of appropriate habitats, which in turn, can lead to the build up of mosquito populations, which can increase transmission of certain pathogens. It then follows that although mosquitoes are vectors in the adult stage only, the biology of preimaginal stages is just as important as adult biology.

In the early 1900's, it was thought that female mosquitoes scattered their eggs indiscriminately among any aquatic sites available, and that larvae of only certain species were able to survive in a particular habitats (Wallis 1954). Kennedy (1942) cited studies in which *Anopheles quadrimaculatus* Say and *Anopheles punctipennis* (Say) larvae never co-occurred in nature, but when eggs of both species were placed in the same pool, both species developed equally well. This provided evidence that females of some mosquito species likely oviposit selectively.

Because oviposition sites are not uniformly distributed and are highly variable in quality and quantity, they must be located, assessed and then either accepted or rejected. Pre-oviposition events usually occur in the following sequence: motivation to take flight, ranging flight (a random flying pattern) until long range specific cues are detected, orientation, encounter with a potential oviposition medium, and acceptance or rejection of that medium (Clements 1999). Visual, olfactory, thermal, and humidity cues are most important when the female is in flight (orientation). Post-landing stages (encounter and acceptance) involve physical cues such as water and substrate, and chemical cues in the various aspects of the water chemistry.

Wallis (1954) cited research where two types of factors that influence egg laying were recognized. Site-extrinsic factors including light levels, rain, and wind probably affect flying behaviour. Known site-intrinsic factors include water vapour, temperature, ammonia, pH, and microorganisms. He also cited research supporting the hypothesis that some mosquitoes exhibit a preference for oviposition in darker areas rather than brighter areas.

Kennedy (1942) tested brightness and conspicuity, i.e. the measure of contrast between the aquatic habitat and its surroundings, of potential ovisites to see what levels became most attractive to gravid mosquitoes. He found the most effective target was one that was contrastingly dark against its surroundings. He also found that water vapour emanating from the water surface was an important oviposition stimulus. By placing a screen over a dish of water to prevent the laying of eggs, he found that the mosquitoes produced "vigorous responses" (hovering, numerous landings, crawling and probing the screen with proboscis) in comparison to empty dishes. Reflection was also determined to be an oviposition stimulus when glass, which reflects some light, was placed over black paper, with no water present. It produced more responses than black paper alone, which reflects ultraviolet light. Kennedy (1942) concluded that the presence of a reflecting surface induces oviposition behaviour whether the target is lighter or darker than its surroundings.

Laird (1988) described only two main categories of oviposition sites: above ground sites, which includes containers, and subterranean sites. Artificial containers are, by definition, of human origin. Each type of container may be characterized by different environmental factors and associated cues, which may deter, stimulate, or be neutral to oviposition by any given species of mosquito. This selectivity may be important for the survival of the resulting larvae.

Important container-breeding mosquitoes:

The consequences to humans of mosquitoes being capable of using artificial containers as oviposition sites are numerous. The existence of containers in an environment augments the number of oviposition sites available, and as a result, mosquito populations can become larger. Artificial containers, by definition, are created by humans, and are often in close proximity to human populations. These two factors can have a direct effect on human public health, because a greater number of mosquitoes in an area around human populations increase the probability of pathogen transmission. There have been documented cases where the presence of artificial containers was linked to subsequent human disease outbreaks. Hedberg *et al.* (1985) showed a significant relationship between the presence of artificial containers and the number of LaCrosse encephalitis cases in humans.

In addition to population amplification, containers may serve as vehicles in which mosquitoes can be transported internationally. Containers colonized in one area, can be transported via ships or trucks to regions where the mosquito species does not exist. Depending on many factors including but not limited to, the species success, vector competence, vectorial capacity, pathogen availability, and feeding behaviour, public health could be negatively affected.

Species indigenous to North America:

Tree holes are the most common habitat of *Oc. triseriatus*, the eastern tree hole mosquito, and its sibling species, *Oc. hendersoni*, and are commonly encountered throughout the forested areas of eastern and midwestern United States (Barker *et al.* 2003). *Ochlerotatus triseriatus* is the most abundant and widely distributed tree hole mosquito in North America, and is the primary vector for La Crosse virus (LACV) (Paulson and Grimstad 1989). *Ochlerotatus hendersoni* is believed to have a mechanism that prevents this virus from being present in the mosquito's saliva when it takes its next blood meal. Therefore *Oc. hendersoni* is precluded from being an efficient vector of LACV (Paulson and Grimstad 1989). Transovarial (vertical) transmission, or the transmission of pathogen from female to egg, can occur and the virus is capable of overwintering along with the mosquito in the egg stage (Barker *et al.* 2003). Contact between *Oc. triseriatus* and humans has increased recently due to humans populating forested areas and the mosquito's ability to colonize artificial containers near homes (Barker *et al.* 2003). If the number of blood meals taken from humans is increasing because of increased contact, the risk of pathogen transmission to human populations can increase (Hedberg *et al.* 1985).

Another native mosquito species, *Oc. atropalpus,* normally occurs in rock pools near fast flowing streams and along the rocky shores of lakes (Covell and Brownell 1979). Until 1979, female *Oc. atropalpus* were only known to oviposit in rock pools. Prior to 1979, its distribution was limited to eastern Canada, south to Alabama, and as far west as Minnesota (Berry and Craig 1984). Subsequently, *Oc. atropalpus* was first identified and reported from tires by Covell and Brownell (1979). Now, it is much more common and widespread due to its acceptance of tires for egg deposition (Nawrocki and Craig 1989). *Ochlerotatus atropalpus* is now found in Kentucky, Ohio, and Indiana (Berry and Craig 1984). Romi *et al.* (1997a) reported the introduction of *Oc. atropalpus* into Italy from used tires originating in North America.

Other species native to North America, important in terms of their vectorial capacity, and that have been found in tires are *Cx. tarsalis*, *Cx. restuans*, *Cs. inornata*, and *Cx. pipiens pipiens* (Joy *et al.* 2003; Rueger *et al.* 1964). These females lay egg rafts, producing as many as three hundred eggs in a single oviposition event. Natural ovisites for these species include marshes, hoof prints, and small stagnant creeks. *Culex* spp. are generally susceptible to West Nile virus infection, and feed on wild bird species that could have high levels of viraemia, making them important vectors of this pathogen (Brinton 2002). *Culex tarsalis* is also an important vector of WE virus, SLE virus and is believed to be important in the epidemiology of West Nile virus. *Culex restuans* and *Cs. inornata* are potential vectors of both viruses although their biology differs substantially (Turell *et al.* 2005).

Culex restuans has been found in a wide variety of aquatic habitats, suggesting that it is opportunistic, that is, there is little evidence of oviposition site selectivity, and lays eggs when it finds water. The geographic range of *Cx. restuans*, which mainly takes blood meals from birds, includes much of Canada, and the United States (Wood *et al.* 1979; Darsie and Ward 2005). Because of its feeding habits, it may not be an important bridge vector of West Nile virus, but may serve to amplify the virus among birds (Turell *et al.* 2005).

Culex tarsalis is found in western Canada, across the United States and into Mexico, excluding the northeastern states (Darsie and Ward 2005). Mammals and birds are potential blood sources for this species, although birds are the primary hosts (Anderson and Brust 1995). However, mammals are a minor host, which means *Cx. tarsalis* can serve as a bridge vector (transfer from bird populations to mammal populations) for arboviruses that have bird reservoir hosts, including West Nile virus.

Culiseta inornata is widely distributed and abundant in western North America, but in eastern Canada, it is found only in southern Ontario. *Culiseta inornata* feeds primarily on mammals (Anderson and Gallaway 1987) and probably is of less importance in West Nile virus epidemiology than the other species listed above because it rarely feeds on birds. Theoretically, it does not come into contact with the virus as often as *Cx. tarsalis* and *Cx. restuans* because it mostly feeds on incidental hosts, which have not been documented to be infectious to mosquitoes; however, some virus has been identified from this species.

Species Introduced to North America:

Aedes aegypti, also known as the yellow fever mosquito, was introduced into North America, from East Africa, over a century ago (Laird 1988). It is believed that it first came to the New World aboard ships in water tanks (Laird 1988). This species has also been introduced into Pakistan by tire importation (Suleman *et al.* 1996). Hopkins (1936 in Laird 1988) stated that its probable original breeding sites were tree holes. The primary larval habitats of the presently cosmotropical *Ae. aegypti* are now artificial containers of many kinds (Laird 1988). This species is the main vector of urban yellow fever virus and dengue (Laird 1988).

Haverfield and Hoffman (1966) suggested tires were the primary means of long-range dispersal for this species in Texas. Tires have been considered the "most prolific *Ae. aegypti* producing units" (Bond and Fay 1969).

Aedes albopictus, or the Asian tiger mosquito, is indigenous to Taiwan, Japan, Hong Kong, and Korea (Reiter and Sprenger 1987). It has been introduced into North America via importation of used tires, and can oviposit in containers and inhabits areas near human dwellings, but not to the same extent as *Ae. aegypti* (Reiter and Sprenger 1987; Craven *et al.* 1988). In August, 1985, *Ae. albopictus* larvae were discovered in used tires in Harris County, Texas (Sprenger and Wuithiranyagool 1986). This species had been introduced to the United States at least twice before via used military tires from Asia after World War II, and the Vietnam War, but an established population was not detected until what was at least the third introduction (Craven *et al.* 1988). *Aedes albopictus* has a flight range of less than 1 km (Bonnet and Worchester 1946). Niebylski and Craig (1994), using a mark-recapture method, found that the greatest dispersal distance they could detect was 525 meters. The rapid spread of the species to many locations in the United States implies an efficient human-caused dispersal mechanism, specifically, the used tire trade (Reiter and Sprenger 1987; Reiter and Darsie 1984; Hawley *et al.* 1987).

Aedes albopictus has also been introduced into Italy; interestingly, tires from the United States, not from Asia, were implicated in the introduction (Dalla Pozza and Majori 1992). Savage *et al.* (1992) discovered *Ae. albopictus* in continental Africa and suggested that used tires shipped from Japan to Nigeria were responsible. Jupp and Kemp (1992) also found *Ae. albopictus* larvae in tires in Durban, South Africa, while Cornel and Hunt (1991) found *Ae. albopictus* in Cape Town, South Africa. Inspection of imported cargo is rare in Africa, and Savage *et al.* (1992) suggested that there may be other populations of *Ae. albopictus* and other introduced species undiscovered in Africa.

Aedes albopictus has been extensively studied and there is much concern about its introduction because it is known to be physiologically capable of transmitting several viruses. *Aedes albopictus* is a competent experimental vector of LACV, with higher infection and oral transmission rates, but lower transovarial transmission than *Oc. triseriatus* (Barker *et al.* 2003). Simmons *et al.* (1930) demonstrated that this species of mosquito could transmit dengue virus to humans by allowing female mosquitoes to feed on human volunteers diagnosed with dengue fever, then later allowing the same mosquitoes to feed on healthy human volunteers. The volunteers showed symptoms of dengue fever within a short period of time. *Aedes albopictus* is also considered by Turell *et al.* (2001) to be a bridge vector for West Nile virus between the avian cycle and humans because it will feed on a variety of hosts. *Aedes albopictus* has a higher cold tolerance, and is a more successful colonizer of disturbed habitats such as scrap yards, tires, and discarded containers than *Ae. aegypti* (Rai 1986). These characteristics are cause for concern because as a result, this species (and possibly any pathogens it may carry) will be able to go farther north, become established faster, and out-compete *Ae. aegypti* (Rai 1986). However, no literature could be found implicating *Ae. albopictus* as important in any disease outbreaks in North America.

Ochlerotatus japonicus, another Asian mosquito, was discovered in North America in August and September of 1998 in New York and New Jersey (Peyton *et al.* 1999). It has also been found in Quebec (Savignac *et al.* 2002). *Ochlerotatus japonicus* is common throughout Japan and is also known from Korea (Andreadis *et al.* 2001). Larvae occur in a variety of natural and artificial containers, including used bamboo stumps, tree holes, tire casings, concrete barrels, and stone vessels, but rock holes are the favoured habitat (Andreadis *et al.* 2001). Peyton *et al.* (1999) suggested that *Oc. japonicus* was introduced to North America in used tires. The tendency of this mosquito to feed on birds and humans makes it a possible bridge vector for West Nile virus, but more information is needed about the feeding behaviour before its role can be determined (Turell *et al.* 2001).

Mosquitoes and Tires

Production of tires is increasing (Baumgartner 1988; Holst *et al.* 1998). They are transported across the world, often between places with different mosquito faunas. Tires can hold water in the environment for a sufficient amount of time to allow development and emergence of adult mosquitoes. Tire ecosystems are attractive to a number of tree hole-utilizing or pollution-tolerant mosquitoes (Laird 1988). Lampman *et al.* (1997) stated that tire dumps are foci for the proliferation and dispersal of mosquitoes, potentially causing public health problems that range from an increase in nuisance complaints to an increase in the transmission of mosquito-borne pathogens.

Novak (1995) suggested that the distribution of mosquito larvae in a tire yard is primarily influenced by the age, size, and physical structure of the tire dump, the heterogeneity of the environment, weather conditions, availability of blood hosts, and the bionomics and behaviour of the associated mosquito species. Most researchers have focused primarily on surveys of local tire piles, to determine which species of mosquitoes use tires locally, or to determine if certain introduced species have been successful in colonizing a certain area. *Ochlerotatus triseriatus*, *Oc. japonicus*, and *Ae. albopictus* are the potential vector species that are usually the focus of tire studies in the United States, where most of this type of research is being done. Very few people have dealt with environmental factors and the role they can play in mosquito oviposition in tires. Tire yards are heterogeneous, and many factors can influence mosquito production. For example, there are several methods of storing tires, the availability of other ovisites differs from yard to yard, the abundance of tires varies, and the amount of sunlight is not always uniform across a tire yard or among tire yards. These factors need to be examined further.

The most commonly examined environmental factor is the presence or absence of shade. Beier *et al.* (1983b) defined exposed tires as those in the open and exposed to direct sunlight for the diurnal period. Shaded tires were defined as those that were shaded for the entire day. Shaded tires contain water with darker colour, turbidity, and alkalinity than exposed tires, but exposed tires contain water with higher temperatures and more algae (Beier *et al.* 1983a). These characteristics could have an effect on the selectivity of mosquitoes to use certain tires as oviposition sites. Andreadis (1988) surveyed used tire dumps and tire dealerships in Connecticut, and found *Oc. triseriatus* occurred significantly more often in shaded tires (91%) compared to exposed tires (46%). There was no significant difference for *Cx. restuans*, being found in 91% of the shaded and 83% of the exposed tires, the same result as found by Beier *et al.* (1983a, b). The most abundant species found in their study were *Oc. atropalpus*, *Oc. triseriatus*, *Cx. restuans*, and *Cx. pipiens*. Beier *et al.* (1983a) suggested that the species composition of mosquitoes in tires depends on their local abundance as well as their ability to colonize and develop in tires.

Baumgartner (1987) looked at stacking method, and tire size in Illinois, and found that tires vertically stacked were less likely to contain water than scattered tires, but when wet, had mosquito larvae more often. He also showed that species in his study were generally indiscriminate and did not preferentially oviposit in tires of different size classes. Additionally, the mean number of larvae per dip decreased slightly with increasing tire size, suggesting that the number of oviposition events did not greatly increase with increasing water volume or surface area. Most common species found in this study were *Cx. restuans*, *Cx. pipiens*, and *Oc. atropalpus*. Only two *Cx. tarsalis* larvae were found.

Lampman *et al.* (1997) also examined used tire piles in Illinois, and found *Ae. albopictus*, *Oc. atropalpus*, *Oc. triseriatus*, and *Cx. restuans* larvae. Mosquito larvae were found in approximately one-quarter of the tires they sampled. Again, *Oc. triseriatus*, the eastern tree hole mosquito, was more common in tire piles at the edge of the woods, while *Cx. restuans* was found more often in the open-field tire piles.

Berry and Craig (1984) surveyed scrap tires in Indiana specifically for *Oc. atropalpus*, but found ninety per cent of the larvae collected were *Cx. restuans*. They also measured the time between placing the tire in the environment, and subsequent colonization. They observed that *Cx. restuans* was the fastest colonizer, appearing in seven days, whereas they found *Oc. triseriatus* after thirty-one days. They only looked at colonization time once during the year, specifically, the tires were placed in the environment on 24 May, and examined each day until eggs were discovered. My hypothesis is that the time required for tires to become colonized by mosquitoes varies over the breeding season. As mosquito abundance increases over the summer, generations begin to overlap, and adult densities increase, decreasing the amount of time an ovisite will remain uncolonized because on any one day there will likely be a large number of females ready to deposit eggs. With a greater number of females in the environment, the probability of discovery will also be higher. Berry and Craig (1984) also suggested that females, after locating a host, find a suitable oviposition site where they remain until they have deposited their eggs so tires could be acting as resting sites for the females as well as oviposition sites. This suggestion is borne out by my own observations of adult mosquitoes flying out of tires when disturbed.

Joy *et al.* (2003) reported that there were surprisingly few quantitative data on monthly frequencies of mosquito species colonizing tire habitats, and carried out a study to investigate the topic in West Virginia. The three most common species in peridomestic sites were *Oc. triseriatus*, *Cx. restuans*, and *Culex territans* Walker. These species were present in 60, 47, and 31 per cent of the observations, respectively but no densities were recorded. In studies prior to Joy *et al.* (2003), *Cx. territans* was a rare species in tires. Jamieson *et al.* (1994) gave *Cx. territans* a cursory mention, and the species was unreported by Lee and Rowley (2000). Joy et al. (2005) recorded only two *Aedes vexans* (Meigen) larvae, and these were considered accidental colonizers of the tire habitat. These two larvae are possibly a result of damp soil being present in the tires, providing a female *Ae. vexans* with an oviposition site. *Ochlerotatus triseriatus* was found most frequently in forested sites, while *Cx. restuans* was more common in peridomestic sites. *Culex restuans* was not found in any of the tires sampled in August.

In 1992, Morris and Robinson (1994) thoroughly examined a tire pile in Florida. They looked at the greatest number of factors of any study in the literature, however they only examined one randomly stacked pile, and their study is therefore unreplicated. They measured height above ground, distance from one side of the pile, orientation (to the nearest ten degrees), configuration (the gap between the beads where the tire would meet the wheel), contents (water volume and larvae), and size (rim diameter). It took twelve people three days to examine 450 tires. Prevalence and density were not correlated to the amount of water in a tire, nor the degree of tilt. Larger tires were more frequently wet, but the density of mosquitoes did not differ significantly between different sized tires. Wet tires were found more often on the edges and at the bottom of the pile. Mosquito prevalence was also highest at the edges of the pile and lowest in the centre. The tires at the top and the bottom of the pile had mosquitoes more often than tires in the middle.

Logically, if a species has a geographic range that includes Manitoba, and has been found in tires elsewhere in North America, it may be found in tires in Manitoba. If tires provide the same habitat for mosquitoes in Manitoba as they do in the other studies, similar mosquito distributions within a pile should be seen as were reported in the previous studies.

Manitoba and Tires:

In 1992, under the Waste Reduction and Prevention Act, the Government of Manitoba instituted a tire levy of three dollars for every new tire sold to be used on a licensed highway vehicle or trailer in Manitoba. In April of 1995, the Tire Stewardship Board (TSB) was established as a corporation independent from the provincial government. Their mandate is to operate the scrap tire waste reduction and prevention program, and administer the fund. The fund pays for collection, transportation, storage, processing, and disposal of scrap tires. The Board may also contract research and development on behalf of registered scrap tire processors.

Even with this comprehensive program, there are still stockpiles of used tires in the environment. It is not possible for the recyclers to remove all the tires from the environment within a length of time that would preclude colonization by mosquitoes, because of the number of tires involved and distances the recyclers have to travel. Therefore, some tires will remain in the environment long enough to accumulate enough water and the appropriate cues to attract gravid mosquitoes. One aim of the TSB is to minimize the risk that tires and their associated mosquitoes in Manitoba could pose to public health by funding research to examine what, if any, threats exist and how they may be mitigated.

Summary and Questions Arising:

There have been few studies to examine in detail the large number of potential factors affecting oviposition by mosquitoes associated with tires. There have been no studies in Manitoba, and therefore there are no published records of mosquito species which use tires as oviposition sites in Manitoba. The following species have been found in tires elsewhere, and are recorded from Manitoba: *Cx. restuans*, *Cx. tarsalis*, *Cx. territans, Cs. inornata Ae. vexans*, *Oc. hendersoni*, and *Oc. triseriatus*.

The critical gaps in the literature include: 1) what is the time required by tires to become colonized by mosquitoes, and does it change throughout the summer? 2) Does the surface area of the water in tires play a role in determining the frequency of oviposition? 3) Does the amount of sunlight tires receive affect the species composition or abundance found in tires? 4) Can tires be placed in the environment in such a way as to reduce the amount of oviposition by mosquitoes? 5) What are the characteristics of tire piles in Manitoba (mosquito species/abundance, water volume variation)? These questions were the basis for the research conducted in the field in 2003 and 2004.

By studying tires in different spatial arrangements and in piles of different sizes, we can begin to learn about what may influence the role of tire aggregations as sources of mosquitoes in a given area, and then apply that knowledge to vector control strategies. It is possible that mosquitoes seeking to lay eggs may do so on the outer tires of a pile because of the tendency to lay eggs in the first available suitable site. Also, tire age, time stored outside, and position within a pile may affect the amount of organic debris, and determine whether a tire will contain the water necessary for mosquito oviposition. The position within a pile also affects the probability that a female mosquito will encounter any one tire. If it can be determined experimentally that not all tires are equally likely to be chosen by mosquitoes seeking to lay eggs, control measures can be focused on the tires most likely to be suitable sites, thereby increasing efficiency of control actions.

An understanding of mosquito oviposition behaviour can be applied in many ways. Knowledge of what is attractive to female mosquitoes can be used to control mosquitoes effectively through bait traps or through habitat reduction and habitat modification. Habitat reduction has been proven to be an effective method of reducing potential vector and pest mosquito populations (Lardeux *et al.* 2000). A system of tire storage to make the tires unattractive or unavailable to females searching for an oviposition site would minimize the number of mosquitoes arising from tires, and would be useful in controlling mosquito populations that could spread pathogens, resulting in diseases such as West Nile neurological syndrome and West Nile fever, both of which were documented extensively with a large number of cases in western Canada during the summer of 2003.

CHAPTER III: THE MOSQUITOES INHABITING TIRES IN MANITOBA: AN INITIAL SURVEY

ABSTRACT

In 2003, a survey of tires in 40 southern Manitoba waste management grounds and tire dealerships was conducted to examine the relationships between tire microhabitats and the associated mosquito fauna. Over 1200 tires were sampled in the three months of the study, of which over 25% contained immature mosquitoes. *Culex restuans* (Theobald) comprised 95% of total immatures collected. Two and a half per cent of the immatures were *Culex tarsalis* Coquillett, while *Culiseta inornata* (Williston), and *Ochlerotatus triseriatus* (Say) comprised the remaining two and a half per cent. Mosquito prevalence was three times greater in August than in June. *Culex restuans* remained at or above 95% of the larvae collected for each month of the summer, while *Cs. inornata* peaked in July (3%), and *Cx. tarsalis* reached its greatest numbers in August (3%). Orientation affected mosquito abundance: vertical tires (tires standing on their treads) contained mosquitoes more often (32%) than horizontal tires (tires parallel to ground) (19%). The eastern region of Manitoba had tires containing mosquitoes more often irrespective of date (48%) than Winnipeg (25%), the central region (24%) or the western region (15%). Mosquito prevalence was significantly greater in larger tires than in smaller tires.

INTRODUCTION

Research done on waste tires as an oviposition site for mosquitoes has increased since Sprenger and Wuithiranyagool (1986) discovered an established population of the exotic *Ae. albopictus* at a roadside tire dump in Houston, Texas in 1985. Tires have been implicated in the spread and introduction of mosquitoes and their associated pathogens in many studies (Hedberg *et al.* 1985; McLintock and Iversen 1975; Lounibos 2002).

In Canada, the scrap tire industry is worth 2.3 billion dollars annually (D. Lamb, personal communication). Due to the large number of used tires and limited recycling capacity, not all tires can be processed and recycled in sufficient time to eliminate stockpiles. As a result, tires are frequently kept outdoors, and marketable stock is often kept beside permanent piles of tires that serve as reservoirs for mosquitoes, creating the possibility of rapid infestation of the new stock as it arrives (Reiter and Sprenger 1987). Moore *et al.* (1990) classified truck and large equipment dealers, retreaders and used tire stockpiles, as well as salvage yards and waste transfer stations as "high-risk premises" for the production of vector mosquitoes.

In Manitoba, there are 306 waste transfer stations that could have used-tire piles at any given time (Manitoba Conservation). In addition, there are 868 tire dealerships that may keep used or marketable tire stock outdoors (Manitoba Tire Stewardship Board data). Under the Waste Reduction and Prevention Act (WRAP), the Manitoba Tire Board was created to start a recycling program in 1995. The Board collects levies from the sale of new tires and uses that money to run a tire-recycling program. Collectively, the tire-recycling corporations in Manitoba, Reliable Tire Recycling and the Tire

Recycling Corporation, have processed over 9.5 million tires since the program's inception (Manitoba Tire Stewardship Board 2005)

Even with this program, there are still stockpiles of used tires in the environment. It is not possible for the recyclers to remove all the tires from the environment within a length of time that would preclude colonization by mosquitoes. Therefore, some tires will remain in the environment long enough to accumulate enough water and the appropriate cues to attract gravid mosquitoes. One aim of the TSB is to minimize the risk that tires and their associated mosquitoes in Manitoba could pose to public health by funding research to examine what, if any, threats exist and how they may be mitigated. To achieve this aim, a survey was carried out to investigate the relationship between tires and mosquitoes in Manitoba. The objectives of the survey were: 1) to determine which species of mosquitoes are using tires as oviposition sites in Manitoba, 2) to determine if geographical regions of Manitoba affect mosquito prevalence or abundance in tires, 3) to see what times of the year oviposition activity is highest, and 4) to correlate mosquito numbers to tire attributes such as orientation, water volume, and tire size.

MATERIALS AND METHODS

Site Selection

Lists of rural municipalities containing any of the 306 waste transfer stations, or waste management grounds in Manitoba were obtained from Manitoba Conservation. The Tire Stewardship Board provided the list of names and addresses of the 868 registered tire dealers in Manitoba. Using the West Nile Response Boundaries created by Manitoba Health, Manitoba was stratified into five regions: Northern, Eastern, Western, Central, and Winnipeg (Figure 1). The Northern region was not included in the survey as the distribution of *Culex* species is limited in the north (Wood *et al.* 1979; Darsie and Ward 2005). The potential sites were assigned numbers, and five of each type were randomly selected from three of the remaining four regions. Winnipeg does not contain any waste management grounds; therefore, ten tire dealers were selected within the city limits.

Phone calls were then made to each of the selected sites, to determine the number of tires and the length of time the tires would spend outside at each location. Sites with fewer than thirty tires or a two-week exposure period were discarded, and new sites were randomly selected to replace them. The minimum exposure period of two weeks was arbitrarily chosen as an estimate of immature development time of *Culex* species mosquitoes in Manitoba (Buth *et al.* 1990). The lower limit of thirty tires was arbitrarily chosen because the desired sample size was thirty from each site. After random assignment, a large portion of southwestern Manitoba was not represented in the sampling plan. As the objective of the survey was to determine the extent of mosquito presence in tires in all regions of Manitoba, two sites that were in close proximity to another site were discarded, and Brandon and Portage La Prairie were included. These cities have a large number of residents, and it is important to know something about the ecology of mosquitoes near humans.

Reliable Tire and the Tire Recycling Corporation, both tire recyclers, were considered under the category of waste disposal grounds in the central region, due to the accumulation of tires from a wide region around the central location, much the same as a waste transfer station would. Each site was visited at least once during the course of the summer.

Sampling

Upon arriving at a site, a straight stick was randomly thrown to obtain transect lines, along which, tires were sampled. If there were fewer than thirty wet tires on one transect, the stick was thrown again to obtain a new transect, until 30 tires were sampled. Because time was limited, only easily accessible tires were sampled. Thirty tires were sampled at each site unless there was fewer than thirty wet tires, or in a few instances, hazardous conditions (e.g. broken glass, sharp metal). No tire was sampled more than once. Sampling involved: recording orientation of the tire (all tires were put into one of two categories, horizontal or vertical, depending on which they were closer to) and one of four tire sizes. The four categories in increasing size were car, truck, semi-trailer, and tractor with outside tire diameters around 63.5, 76.2, 101.6, and 177.8cm, respectively. The entire contents of each tire were run through a 212μm mesh size sieve into a graduated pail, and volume was recorded (to the nearest 0.5 L). This was repeated until thirty tires were sampled. A note of the percentage of wet tires was made. In June and

July, all egg rafts and immature mosquitoes collected were brought back to the lab and stored at room temperature until they could be counted. Egg rafts were allowed to hatch; larvae in the first instar were reared to fourth instars, and then identified using the keys in Wood *et al.* (1979). If a larva died in the first instar, the key in Dodge (1969) was used to make the identification. In August, due to potential larval mortality and the decomposition of the organic matter accumulated, collections were stored in a cold room (5°C) until they could be processed. When pupae were collected, or larvae pupated before they were counted and identified, they were allowed to emerge in clear acrylic cages (16.5cm x 16.5cm x 16.5cm), and identified as adults using the key in Wood *et al.* (1979). Individuals were counted, and recorded, keeping note of number of each species. Voucher specimens were deposited in the J. B. Wallis Museum, Department of Entomology, University of Manitoba. Where variation is reported, it is the 95% confidence interval.

RESULTS

One thousand one hundred and forty-two tires at different locations were sampled during June, July, and August of 2003 (Figure 1). Tires that contained immature mosquitoes contained 100.3 on average (mean intensity), and over one quarter of the tires contained larvae (26.8%). *Culex restuans* accounted for 95.3% of the total larvae found. The remaining 4.7% was comprised of *Cx. tarsalis* (2.5%), *Cs. inornata* (1.8%), and *Oc. triseriatus* (0.4%). Across the entire summer, there was an average (±95% C.I.) of 26.77 ± 4.75, 0.67 ± 0.34, 0.51 ± 0.49, and 0.08 ± 0.07 *Cx. restuans*, *Cx. tarsalis*, Cs. *inornata*, and *Oc. triseriatus* per tire, respectively. Because *Cx. restuans* dominated the collection, further statistical analyses were done using the total number of immature mosquitoes collected. More tires were sampled in the vertical orientation (755) than horizontal (387). The number of tires sampled per month varied: June (179), July (673), and August (290). The most tires were sampled in the central region (383) followed by Winnipeg region (364), western region (230), and eastern region (165). Most of the tires sampled were car tires (410), followed by semi-trailer tires (300), tractor tires (245), and truck tires (187).

Factors Affecting Prevalence

As a total, prevalence increased from June (11.7%) to July (28.7%) to August (36.1%). Logistic regression showed that mosquito prevalence increased significantly as summer progressed (χ^2 value = 3.98, df = 1, 1136, p = 0.046). Sampling region significantly affected prevalence, (χ^2 value = 28.8, df = 3, 1138, p < 0.0001) and the interaction between sampling region and sampling date was significant (χ^2 value = 9.64, df = 3, 1138, p = 0.022). The prevalence of immature mosquitoes decreased as summer progressed in Winnipeg, in contrast to the other three regions.

Mosquito prevalence was significantly affected by tire size (χ^2 value 68.21, df = 3, 1138, p < 0.0001). Prevalence increased with increasing tire size, 18.8% of car tires contained immature mosquitoes, with 19.8%, 26.7%, and 47.8% of truck, semi-trailer and tractor tires containing immatures respectively. The interaction of tire type and region was also significant (χ^2 value = 20.62, df = 9, p = 0.014). The interaction of tire size and sampling date was also significant (F value = 2.64, df = 3, p = 0.048). The prevalence of immature mosquitoes collected from semi-trailer tires decreased across the summer, while the prevalence in the other three tire types increased.

Tire orientation also significantly affected mosquito prevalence ($\chi^2 = 20.93$, df = 1, 1139, p < 0.0001), while the interaction between tire size and orientation was not significant ($\chi^2 = 5.37$, df = 3, p = 0.15). Immature mosquitoes were found in 18.9% of horizontally stacked tires, while 31.4% of vertically stacked tires contained immatures. The interaction between region and orientation was non-significant, (F value = 1.63, df = 3, p = 0.18). The interaction between sampling date and orientation was not significant (F value = 1.06, df = 1, p = 0.303).

Factors Affecting Abundance

Mosquito abundance increased significantly with increasing Julian date (F value = 8.5, df = 1, 1136 p = 0.004). Sampling region significantly affected abundance, (F value = 6.4, df = 3, 1134 p = 0.0003) and the interaction between sampling region and sampling date was not significant (F value = 0.733, df = 3, 1134 p = 0.532).

Mosquito abundance was significantly affected by tire size (F value 47.14, df = 3, 1134, p < 0.0001). Abundance increased with increasing tire size. On average, car tires contained 12.1 ± 3.6 immature mosquitoes, with 16.7 ± 8.0, 21.5 ± 6.3, and 71.1 ± 19.0 mosquitoes per tire in truck, semi-trailer and tractor tires respectively. Using Tukey-Kramer's honestly significant difference test, only tractor tires had a significantly different mean abundance than the other tire types (p < 0.0001). The interaction of tire type and region was also significant (F value = 6.94, df = 15, 1122, p < 0.0001). Tractor tires in the eastern region had a large increase in abundance in comparison to the abundance of mosquitoes in the other tire sizes in the east. The interaction of tire size and sampling date was also significant (F value = 5.84, df = 7, 1130, p = 0.0006). The increase in abundance of immature mosquitoes in tractor tires later in the season was greater than the increase in the other three sizes.

A mean of 18.2 ± 8.4 immature mosquitoes was found in horizontally stacked tires, while a mean of 33.0 ± 6.0 immatures were found in vertically stacked tires. Tire orientation did not significantly affect mosquito abundance (F value 2.24, df = 1, 1135, p = 0.135), and the interaction between tire size and orientation was not significant (F value = 0.22, df = 3, 1132, p = 0.88). The interaction between region and orientation was not statistically significant, (F value = 1.36, df = 7, 1129, p = 0.25). The interaction between sampling date and orientation was significant (F value = 4.28, df =3, 1133, p = 0.0387). More immature mosquitoes were found in horizontal tires earlier in the summer. Later in the summer more immature mosquitoes were found in vertical tires. The combination of all four factors explained over 24% of the variation seen in the abundances (R^2 = 0.241) and the interaction was significant (F value = 3.42, df = 63, 1073, p = 0.0004). The interactions of tire size, orientation and date was significant (F value = 4.46, df = 63, 1073, p = 0.0040), as well as the interactions of region, orientation and date (F value = 2.86, df = 63, 1073, p = 0.0361).

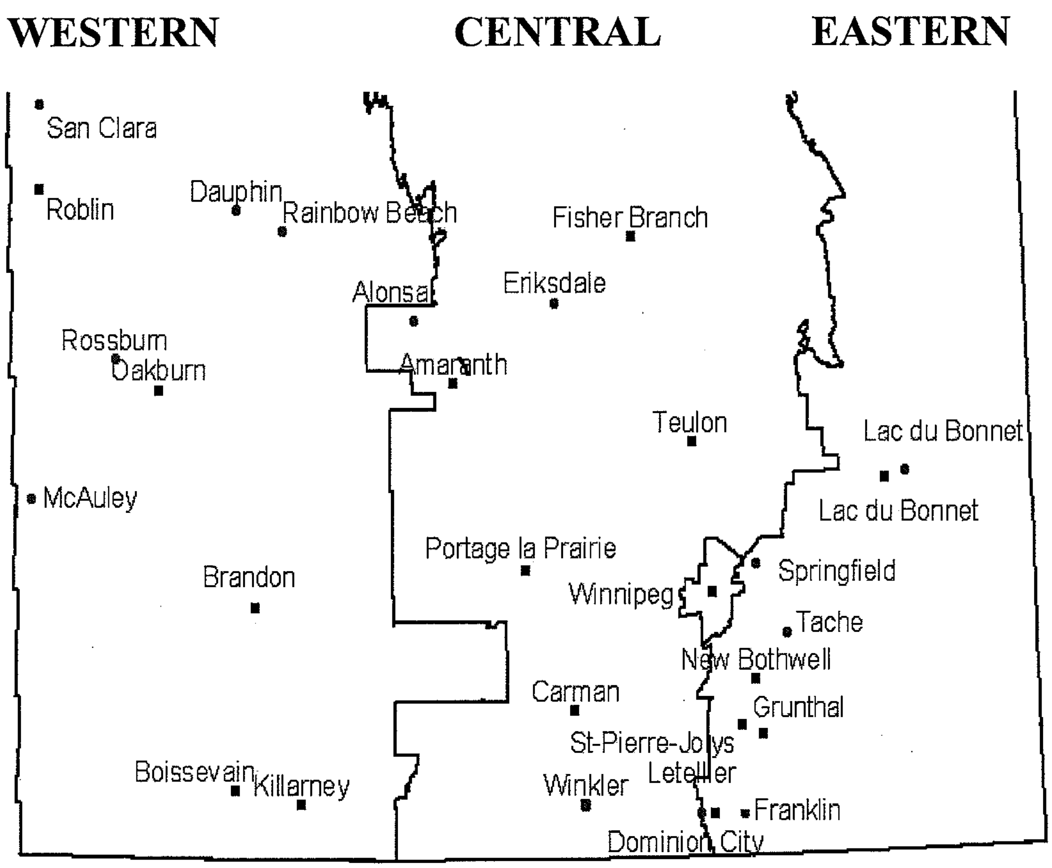


Figure 1. A map of southern Manitoba showing four of the five regions, and sites visited during the survey. Square = tire dealership; circle = waste transfer station.

Table I: Abundance and prevalence (percentage of tires containing each species in that month) of four species of mosquitoes found in tires in Manitoba in the summer of 2003.

	Month	Abundance (% of Monthly Total)	Prevalence (%)
Culex restuans	June	3,332 (97)	12.23
	July	14,551 (95)	28.20
	August	13,032 (95)	34.95
Culex tarsalis	June	68 (2)	1.06
	July	278 (2)	1.45
	August	487 (4)	5.88
Culiseta inornata	June	21 (1)	0.53
	July	475 (3)	1.60
	August	82 (1)	3.48
Ochlerotatus triseriatus	June	0 (0)	0.00
	July	27 (0)	0.29
	August	121 (1)	3.11

Table II. Mean abundance (± 95% C.I.) and prevalence found in four different sizes of tires in Manitoba in the summer of 2003 (values followed by different letters differ significantly at the $p = 0.05$ level using Tukey-Kramer's HSD).

	Car	Truck	Semi-trailer	Tractor
Mean Abundance	12.1 ± 3.6^{a}	16.7 ± 8.0^{a}	21.45 ± 6.3^{a}	71.1 ± 19.0^{b}
Prevalence (%)	18.78^{a}	19.79^{a}	26.67^{a}	47.76^{b}

Table III. Mean abundance (± 95% C. I.) and prevalence found in tires in four different regions in Manitoba in the summer of 2003 (values followed by different letters differ significantly at the $p = 0.05$ level using Tukey-Kramer's HSD).

	West	Central	Winnipeg	East
Mean Abundance	13.8 ± 6.0^{a}	27.0 ± 7.4^{a}	25.2 ± 8.6^{a}	56.4 ± 20.6^{b}
Prevalence (%)	16.09^{a}	27.67^{a}	25.27^{a}	46.06^{b}

DISCUSSION

Based on the literature, it was hypothesized that the mosquito fauna of tires in Manitoba would be comprised of species previously found in tires that have habitat ranges that include Manitoba. *Culex restuans* dominated the larval collection from tires in Manitoba. *Culex tarsalis*, *Cs. inornata*, and *Oc. triseriatus* were present, but in low numbers. *Ochlerotatus hendersoni*, *Cx. territans* and *Ae. vexans* were not found, but these species were not commonly found in other studies (Joy *et al.* 2003; Baumgartner 1988; Beier *et al.* 1983a, b). *Culex restuans* appears to be less discriminating than other mosquito species in terms of accepting oviposition sites, as they have been found in tree holes, rock pools, ditches, temporary pools, catch basins, and many artificial containers (Gallaway and Brust 1982; Wood *et al.* 1979). The other species, which have been found from a much more limited array of locations, are either finding tires unsuitable, or not recognizing the oviposition cues presented by tires. The prevalence of mosquitoes in tires probably depends to a large extent on the availability of natural oviposition sites. For example, Beck (1961) found that *Culex tarsalis* would begin oviposition in containers when the populations peaked in August when other habitats dried up.

It is not surprising that the numbers of immature *Cx. tarsalis* in tires peaked in August as it is a late-summer species in Manitoba, and populations build up in August (Buth *et al.* 1990). Immature *Cx. restuans* comprised similar proportions of the total larvae collected throughout the summer, but it is difficult to relate this to the adult populations, as they are not easily caught with light or CDC traps. In other studies, the proportion of *Cx. restuans* larvae in tires and ovipools declined over the summer as other species proportions increased. (Brust 1990; Berry and Craig 1984; Beier *et al.* 1983a, b).

Culiseta inornata intensity was highest in July, but prevalence was highest in August. This species is usually thought of as a cool-weather mosquito, with an optimum rearing temperature of 21°C with increased mortality at higher temperatures (Buth *et al.* 1990; Hanec and Brust 1967). In 2003, August was quite warm, reaching highs of more than four degrees above normal (http://www.climate.weatheroffice.ec.gc.ca). If a greater number of *Cs. inornata* emerged in July, a higher prevalence would occur in August, but due to warm weather, the mortality of the larvae could have increased, possibly resulting in lower intensity.

Overall, mosquito prevalence increased each month from June to August. Nearly 27% of the tires surveyed contained mosquito larvae, similar to Baumgartner (1988) where he found approximately 30% of tires surveyed in Illinois to contain mosquito larvae. He also found 82% of the larvae to be *Cx. restuans*. Our result of 95% *Cx. restuans* larvae is slightly higher, but the result of *Cx. restuans* dominating the collection is the same. Berry and Craig (1984) surveyed tire stations in Indiana, and found that *Cx. restuans* accounted for 90% of the total larvae collected. Without looking at variability, this agrees with our findings in that *Cx. restuans* is the most frequently collected mosquito species in tires. The average number of mosquitoes per tire also increased significantly with date, probably driven by the increase in prevalence, as the intensity (or the average number of mosquitoes per positive tire) did not significantly increase.

However, our study is in contrast to Beier *et al.* (1983a, b) who found that *Oc. triseriatus* was more common than *Cx. restuans* in tires in Indiana. However, *Oc. triseriatus* is uncommonly collected in Manitoba. They also found a much higher prevalence, reporting 60 to 100% of the tires they examined contained immature mosquitoes. However, they only examined tires that had at least 800 mL of water present, and then sub-sampled 300 mL of the total. Combined with the presence of *Ae. atropalpus*, which was the most common species in tires in Indiana, these geographic and sampling factors could account for the different results.

The interaction of region and date was not significant. The mosquito abundance and prevalence in tires in all four regions increased similarly as the summer progressed. The number of tires present would definitely influence the number of mosquitoes present, however; no estimate of tire numbers were made. The increase in abundance and prevalence is expected due to increasing mosquito populations from other sources. The probability of an individual tire being found increases with the higher density of mosquitoes, more tires would be found, and a greater number of oviposition events would take place with the increasing number of females present.

Tire size was a significant factor in determining both abundance and prevalence. Tractor tires (the largest) had more immature mosquitoes and more often than the three other smaller tire sizes. The interaction between region and tire size was also significant, for both prevalence and abundance. Car tires had the lowest prevalence in three regions, but were second highest in the Winnipeg area. Tractor tires had a large increase in mosquito abundance in the eastern region. In fact, the eastern region of Manitoba had tires with significantly greater abundance and prevalence of mosquitoes. Most of the sites in the eastern region were located further south than the ones in the western or central regions. The temperature difference could have had an impact on the prevalence and abundance of immature mosquitoes in tires. For example, the western region included sites around Russell (average temperature in June, 15.5°C, average temp in

August 16.8°C). The most northerly site in the east was Lac du Bonnet, near Beausejour (average temperature in June, 16.7°C, average temperature in August 18.0°C) (http://www.climate.weatheroffice.ec.gc.ca). This two-degree increase would shorten larval development, amplifying populations earlier, and result in an earlier second generation. Plus, the province was divided into regions using politically defined boundaries that have no ecological bearing. These factors could have confounded observations of both prevalence and abundance of immature mosquitoes in tires in eastern Manitoba.

Tires stored in different orientations significantly influenced the prevalence of immature mosquitoes, affected by the proportion of tires in each orientation. For example, a site with many vertical tires should have a higher prevalence than a site with mostly horizontally stacked tires later in the season. The average number of mosquitoes in wet tires was not significantly different between the two orientations. None of the other interactions between factors affected either prevalence or abundance except date and orientation. Horizontal tires contained more mosquitoes on average earlier in the season than vertical. Vertical tires had a higher average later in the season than vertical. Baumgartner (1988) found that tires stacked horizontally were less likely to contain water than scattered tires, but did not report anything about vertically stacked tires. I examined wet tires only, so the prevalence of water in tires cannot be calculated; however, I observed horizontally stacked tires to be dry more often than vertical, but no data were recorded. Because the presence of water is required for *Culex* oviposition, the tendency of vertical tires to contain water more often could explain the higher prevalence of mosquitoes.

Bidlingmayer and Hem (1980) found that *Cx. quinquefasciatus* Say had a visual range of less than 7.5 metres, and *Culex nigripalpus* Theobald and *Culiseta melanura* (Coquillett) 15.5 to 19.0 metres. Therefore, the presence of water may not play a role in locating a tire pile, but rather provide close-range cues to locating individual tires, once within 7.5 meters of a pile.

If the intensity, or the average number of immature mosquitoes when mosquitoes are found, is 100, but the average across all tires is 28, that suggests that many tires had zero, or few immature mosquitoes, while few tires had many mosquitoes. The consequence of mosquito larvae showing this type of distribution is that, if there are few tires with many immature mosquitoes, control efforts need not focus on all tires, but just the tires that are heavily infested. If it could be determined which tires will become heavily infested based on position, orientation, or environmental variables, control efforts would become more time, cost, and labour-efficient.

With the recent discovery of West Nile virus in Manitoba, there is new incentive to revisit mosquito biology. There is evidence that at least some female mosquitoes actively select oviposition sites (Wilton 1968; Reisen and Siddiqui 1978; Trexler *et al.* 2003; Dhileepan 1997). This selection is based on specific cues to evaluate the characteristics of oviposition media. Mosquito larvae have been found in tires; therefore tires can act as discrete pools of water, which are suitable for certain species of mosquitoes to lay eggs. The mosquitoes that are found in tires include more than just the species that are thought of colloquially as "artificial container breeders" (e.g. *Ae. aegypti*); in fact, species that naturally lay eggs on semi-permanent and permanent aquatic habitats are also found (e.g. *Cx. tarsalis*, *Cx. restuans*)

There is a high degree of variation in the microhabitat characteristics within and even among tire yards, and a dearth of literature concerning the effect of these factors on mosquito oviposition. Work needs to be done to determine what makes tires attractive to female mosquitoes, and how large an impact tires have on naturally occurring mosquito populations. Also, we need to know what may influence the role of tire aggregations as sources of female mosquitoes in a given area, and then apply that knowledge to control strategies for vector species. It is possible that mosquitoes seeking to lay eggs may do so on the outer tires of a pile due to a tendency to lay eggs in the first available suitable site. Also, tire age, time stored outside, and position within a pile may also be expected to affect the amount of organic debris and determine whether a tire will contain the water necessary for mosquito egg laying. Further research into these areas should shed more light on the complex picture presented here.

CHAPTER IV: THE EFFECTS OF EXPOSURE TIME, SUN EXPOSURE, AND TIRE SIZE ON THE FREQUENCY OF MOSQUITO OVIPOSITION IN TIRES IN MANITOBA

ABSTRACT

The length of time a tire is in the environment affects several characteristics of the tire microhabitat, including amounts of leaf litter, water, and bacteria. These factors also vary with tire size, and location, and whether in shaded, or exposed sun-lit areas. Gravid female mosquitoes that choose oviposition sites that have characteristics such that successful larval development is possible should be favoured. In three different experiments, exposure time, sun exposure and tire size were tested to determine the effects of each variable on mosquito oviposition.

The mean interval between setting up a tire and an oviposition event was 19.5 ± 3.4 days (95% C. I., range: 1-50). This interval decreased as the summer progressed.

Forty-five egg rafts were collected during the two months of the experiment on sun-exposure. Thirty-one (69%) of the egg rafts were collected from the exposed tires, while 14 (31%) were collected from the shaded tires. Thirty-three (77%) of the egg rafts were *Cx. restuans*. Four (9%) were *Culiseta inornata* (Williston), while three (7%) were *Cx. tarsalis*. Five (11%) could not be identified because they did not hatch. A greater proportion of *Cx. restuans* egg rafts were found in sun-exposed tires, as 26 (79%) egg rafts were found in the sun-exposed tires, however, the means were not significantly different. The numbers of other species' egg rafts were too small to detect a trend.

Tire size was hypothesized to be most important in determining the number of mosquito oviposition events in a tire. Eleven (85%) of the egg rafts collected came from large tires, significantly more than smaller tires.

INTRODUCTION

With the introduction of West Nile virus to Manitoba, there is increased concern about the availability of larval habitats used by the species of mosquitoes in the genus *Culex* in close proximity to human populations. *Culex* spp. mosquitoes have been documented to be the main vectors of this pathogen among birds and to humans (Turell *et al.* 2005). Immature mosquitoes are exclusively aquatic, and require sufficient water to be present throughout their development. The larval stages feed on organic matter and microorganisms found in the water.

In Manitoba, automotive tires can act as artificial containers in which rainwater and organic debris can be caught and stored for periods of time ranging from a couple of days to several years (personal research). In the every scientific article examined, at least one species of *Culex* was present in tires (Baumgartner 1983; Joy *et al.* 2003; Kaufman *et al.* 2005). In Manitoba, *Cx. restuans* accounted for greater than 95% of the immatures collected from tires in 2003.

However, sun exposure, duration of tire presence outside, and tire size were thought to affect variation in mosquito oviposition among tires. These three factors were tested in separate experiments in the summer of 2004. If experiments support the hypothesis that certain tires are more likely to contain mosquito eggs, then control measures, if deemed necessary, can focus on those tires with characteristics that make them more likely to have mosquito larvae.

METHODS

Tires:

Tires were obtained from waste piles maintained by tire dealers. Tires were separated into four categories based on size. The four categories in increasing size were car, truck, semi-trailer, and tractor with tire diameters around 63.5, 76.2, 101.6, and 177.8cm, respectively. Before being placed outdoors, all tires were washed with hot water, cleaned, and dried with a cloth to ensure absence of organic debris. Tap water that had been allowed to sit for 24 hours to eliminate chlorine and thus simulate fresh rainfall was placed in each tire. Each tire received 3L of water to ensure similar initial depths in all tires. A hoof knife was used to mark the waterline inside each tire. Tires were examined for egg rafts every other day, and refilled to the mark as required. Actual amounts of water added to tires were not recorded. In the event of rainfall, water was scooped out of the tire, until 3L remained. For the tire size experiment, depth, instead of volume, was maintained constant at five centimeters, as outlined above. Tires were monitored until 29 August, 2004. In several tires, temperature was recorded using battery-powered HOBO brand data loggers. Due to the unreliability of most data collected, only one comparison is made to 2003 data. To determine differences in weather between years, I accessed Environment Canada's website (http://www.climate.weatheroffice.ec.gc.ca).

Egg rafts:

Each egg raft was put in a separate plastic container, half-filled with water and transported to the University of Manitoba, where they were allowed to hatch. Larvae were fed on a diet of bovine liver powder, reared to fourth instar, and identified using Wood *et al.* (1979). If the larvae died in earlier stages, the key in Dodge (1969) was used for larval identification.

<u>Adult Collection:</u>

Three Center for Disease Control (CDC) traps with dry ice (CO_2) bait were used to monitor presence of adult mosquitoes. One trap was located at each of the three experimental locations: the Faculty of Agricultural and Food Sciences Glenlea Field Research Station (Glenlea), Sturgeon Tire (Sturgeon), and the eastern point of the University of Manitoba grounds (Point). Beginning 13 July, a second CDC trap was run each trap night at Glenlea, along with the first, approximately 800 meters from the first one to sample at one additional location. The results were pooled in one table, as there was no detectable difference in collections. The traps at Glenlea were placed in a tree line composed of primarily Siberian elm (*Ulmus pumila* L.), American elm (*Ulmus americana* L.), and green ash (*Fraxinus pennsylvanica* Marsh), though some willows (*Salix* spp.) are also present. There were no trees in close proximity to the traps at Sturgeon Tire, and the trees at the Point were mostly oak (*Quercus* spp.) and elm (*Ulmus* spp.)

Mosquitoes were brought to the laboratory at the University of Manitoba and identified to species, to a maximum of 250 individuals per collection and the remaining mosquitoes were counted and recorded.

Time to Colonization Experiment:

At each experiment location, a metal rod affixed with brightly coloured ribbon (pinflag) was placed at thirty-meter intervals. Starting 24 May, 2004, and then repeated each week, one of the pinflags at each location was chosen at random as a location to place three tires tied to stakes in the vertical (tire perpendicular to ground) position. A sod-lined, water-filled ovipool measuring 30x15x15 cm was set up at one of the designated pinflags at each location to detect any species that may not lay eggs in tires. Because the period of time between setting up tires and colonization was much longer than anticipated, space became a limiting factor. When all the pinflagged locations contained tires, the tires that had been out the longest were removed. The smallest area was Sturgeon, and as a result tires had to be removed if they did not contain mosquito eggs within 21 days. Similarly, tires had to be removed after 28 days for the Point, and 35 days for Glenlea if no oviposition had occurred.

Shade Experiment:

On 8 July, twenty-four tires were placed in eight different locations, each at least thirty meters apart at the Glenlea site. These eight sites were broken up into four blocks. Within a block, one group of three tires was placed on the shaded side of a tree line and the other group of three was placed on the non-shaded side of the treeline. A coin was tossed to determine the treatment for each site in the block. A wooden stake was hammered into the ground to anchor the three tires.

Tire Size Experiment:

Nine passenger tires (outside tire diameter 63.5cm) and nine tractor tires (outside tire diameter 177.8cm) were moved to Glenlea on 3 August. Each replicate consisted of one tractor tire and one passenger tire, located a maximum of one meter from each other, propped against a tree so they remained standing on their treads. Each replicate was 30m apart. The length and width of the pool of water within each tire was measured, to give a rough estimate of surface area.

Statistics:

Throughout the thesis, various statistical analyses were used to draw conclusions from the data. In all instances the standard alpha value of 0.05 was used to determine significance. Where applicable, analysis of variance (ANOVA) was used to determine if there was a significant difference between means. When there were more than two means being compared, Tukey-Kramer's honestly significant difference (HSD) procedure was used to tell which of the means was significantly different. χ^2 values were obtained when the variable response was either a one or a zero (e.g. presence/absence) to determine whether the observed values were different from expected values for categorical variables. Logistic regression was used where continuous variables were compared to categorical (nominal or ordinal) variables.

RESULTS

Adult Collection:

The majority of the mosquitoes collected in the CDC trap were *Ae. vexans*, *Oc. sticticus*, (Meigen)and *Oc. dorsalis* (Meigen). They comprised 56.8%, 9.7%, and 5.7% of the identified collection respectively. *Culex tarsalis* made up 4.5% of the total identified collection. However, *Cx. tarsalis* comprised 38.2% of the collection in the first week in August. Given the species composition of immature mosquitoes in the survey of 2003, *Cx. restuans* and *Cs. inornata* were expected to be present, but they never made up more than 0.3% of a weekly collection. Table I is a list of the four most common species and how they compared to the total number of mosquitoes identified. The numbers of the infrequently collected species are in Table II with the location at which they were collected.

Time to Colonization Experiment:

The average number of days between setting up a tire and subsequent mosquito oviposition at each of the three sites was 23.2 ± 7.1, 17.8 ± 3.2 and 16.7 ± 4.23 days for Glenlea, Sturgeon and the Point, respectively (95% C.I.). On average, the tires that were colonized were exposed for 19.5 ± 3.4 (95% C. I.) days before becoming colonized but the interval ranged from 1 to 50 days. This interval decreased significantly across the summer (F value = 12.67, df = 1, 30, p = 0.001) (Appendix 1). Thirty-two of the seventy-five tire sets (43%) eventually contained an egg raft in one of the three tires that were set up. The first egg raft was collected on 14 July, in a tire that had been outdoors for 28 days. Temperatures recorded from tire water are shown in Figure 2, compared with the temperature recorded from one tire during the same three days in 2003. Daily mean temperatures and precipitation during 2003 and 2004 are compared with the 29-year average (1971-2000) in Table III.

Shade Experiment:

Forty-five egg rafts from three species were collected in the two months that the study was carried out. Thirty-three (73%) of the total number of egg rafts were deposited by *Cx. restuans*. Four (9%) were from *Cs. inornata*, while three (7%) were from *Cx. tarsalis*. Five (11%) could not be identified because they did not hatch. Thirty-one (69%) of the egg rafts were collected from the non-shaded tires, while 14 (31%) were collected from the shaded tires. Twenty-six (79%) of *Cx. restuans* egg rafts tires were found in the sun-exposed tires. One *Cx. tarsalis* egg raft was collected from shaded tires, while two were collected from sun-exposed tires. Three *Cs. inornata* egg rafts were collected from shaded tires, and one was collected from a sun-exposed tire (Table IV).

The difference in the mean number of egg rafts between sun-exposed and shaded tires was not significant (F value = 0.400, df = 1, 6 p = 0.550). The interaction between treatment and site location was not significant (F value = 0.655, df = 1, p = 0.464). The difference between number of *Cx. restuans* egg rafts in sun-exposed and shaded tires was not significantly different (F value = 0.511, df = 1, 6 p = 0.502).

Tire Size Experiment:

Thirteen egg rafts were collected from the eighteen tires from 3 to 27 August, 2004. Eleven egg rafts (85%) were collected in large tires, and two (15%) from the small tires. All egg rafts collected were from *Cx. restuans*. Two large tires contained three egg rafts each, five large tires contained one egg raft each, and two large tires contained no egg rafts. One egg raft was collected from each of two small tires, while seven of these contained no egg rafts. Tire size had a significant effect on the number of oviposition events (F value = 5.818, df = 1, p = 0.030). The number of egg rafts in a tire was positively correlated with surface area ($R^2 = 0.322$). Replicate number and the interaction between size and replicate number were non-significant (F value = 0.269, df = 1, 14 p = 0.612) and (F value = 0.097, df = 1, p = 0.760), respectively. Oviposition occurred in large tires on five nights out of the twenty-four nights of observation, while oviposition occurred in small tires on two nights. No oviposition was observed after 18 August in either treatment (Table V).

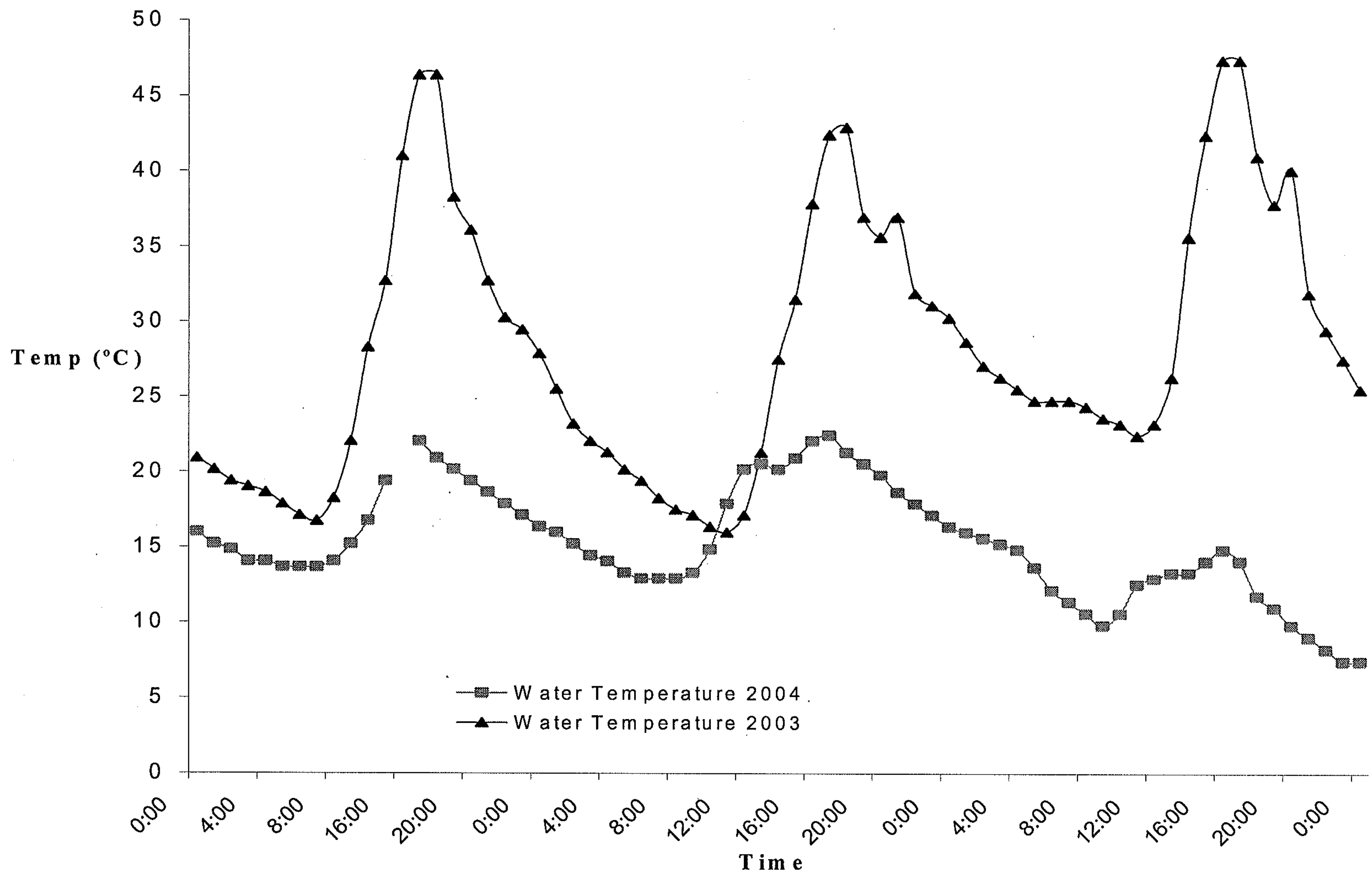


Figure 1: Comparison of the water temperatures found in tires in sun-exposed areas from 16 August to 19 August of 2003 and 2004. The tire in 2003 was located at the apiary at the University of Manitoba. The tire in 2004 was located at the University of Manitoba Faculty of Agricultural and Food Sciences Glenlea Research Station.

Table I: Abundance of the four most common mosquito species caught in 4 CDC traps operated from 21 June (Week 23) to 17 August 2004 (Week 32) and the total number of mosquitoes identified in each week (# MOSQ ID'D). (VEX = *Ae. vexans*, STI = *Oc. sticticus*, DOR = *Oc. dorsalis*, CXT = *Cx. tarsalis*).

Week #	VEX	STI	DOR	CXT	# MOSQ ID'D
23	566	37	53	0	695
24	82	7	8	2	100
25	544	130	41	0	754
26	328	107	34	0	578
27	655	119	13	70	924
28	646	46	131	58	973
29	281	26	11	9	351
30	24	9	23	52	136
32	49	3	2	18	96
TOTAL	**2615**	**448**	**263**	**209**	**4607**

Table II: The number of the less commonly collected mosquito species in the four CDC traps and the locations in which they were found (P = Point, S = Sturgeon, G = Glenlea).

Species	Number Collected	Locations Collected
Ochlerotatus flavescens (Müller)	56	P,S,G
Coquillettidia perturbans (Walker)	39	P,S,G
Culex restuans	17	P,G
Ochlerotatus canadensis (Theobald)	15	P,G
Ochlerotatus spencerii (Theobald)	7	S,G
Ochlerotatus cinereus Meigen	6	P,S,G
Culiseta inornata	5	P,S,G
Ochlerotatus euedes Howard, Dyar & Knab	4	P,S
Ochlerotatus triseriatus	2	P
Anopheles walkeri Theobald	2	P
Culiseta incidens (Thomson)	2	P
Ochlerotatus fitchii (Felt & Young)	1	P
Ochlerotatus excrucians (Walker)	1	G

Table III. Differences in precipitation and mean daily temperature in Winnipeg between 2003, 2004, and average values from 1971 to 2000. (Actual – Average) (data from Environment Canada http://www.climate.weatheroffice.ec.gc.ca) (Accessed February 20, 2006).

	2003		2004	
	Precipitation (mm)	**Daily Mean Temp**	**Precipitation (mm)**	**Daily Mean Temp**
May	+19.7	-0.6	+76.0	-4.6
June	-47.0	+4.6	-54.5	-2.9
July	-26.1	-0.1	-3.6	-1.3
August	-3.1	-1.8	+52.4	-4.2

Table IV: Numbers of egg rafts of each species collected from each replicate in exposed and shaded treatments at Glenlea Research Station in 2004. Totals are the total numbers of egg rafts from each treatment with the percentage of species total egg rafts across treatments in brackets (unidentified egg rafts are rafts that did not hatch).

	Replicate	***Culex restuans***	***Culex tarsalis***	***Culiseta inornata***	**Unidentified**
Exposed (69%)	1	13	1	0	0
	2	4	0	0	1
	3	6	0	1	0
	4	3	0	0	2
	Total (%)	**26 (79)**	**2 (67)**	**1 (25)**	**3 (60)**
Shaded (31%)	1	5	0	1	0
	2	2	1	0	0
	3	0	1	1	1
	4	0	0	1	1
	Total (%)	**7 (21)**	**1(33)**	**3 (75)**	**2 (40)**

Table V: Numbers of egg rafts collected from large and small tires placed at Glenlea Research Station from 13-18 August 2004.

Date	Large	Small
13-Aug	1	0
15-Aug	3	0
16-Aug	1	1
17-Aug	3	0
18-Aug	3	1
Total	**11**	**2**

DISCUSSION

Tire recyclers could exploit any expected interval between the outdoor discarding of tires and oviposition by mosquitoes as a period during which tires could be left outside without contributing to the mosquito population. Of the tires that were colonized, in the experiments described herein, the average time from placement outside to colonization was nineteen days. The presence of an egg raft within one day indicates that colonization can occur very quickly. Therefore, it is possible that tires can begin producing adult mosquitoes in as few as twelve days after being flooded, depending on temperature (Buth *et al.* 1990). However, higher temperatures could decrease development time, but increase mortality for *Cx. restuans* (Buth *et al.* 1990). Rueda *et al.* (1990) found that *Cx. quinquefasciatus* could develop in as little as seven days when held at a constant temperature of 27°C. In the field, however, temperatures are not constant, but fluctuate daily. These daily temperature fluctuations were reflected in the temperature of the tires (Figure 2). In 2003, the water temperature exceeded 40°C for a short period of time. Hanec and Brust (1967) found that *Cs. inornata* would not successfully complete development at 29°C. This suggests that if temperatures were high enough, tires could act as sinks, rather than sources, of mosquitoes, at least for some species.

The first egg raft was found in a tire on 14 July, in a tire that had been outside for 28 days. In 2003, egg rafts were found in tires approximately one month earlier in Manitoba (see Chapter 3). An explanation for this delay in mosquito presence is the difference in daily mean temperatures and precipitation recorded in Manitoba between 2004 and a 29-year average (Table III). Lower temperatures delay mosquito development, and the subsequent build-up of populations (Hanec and Brust 1967; Brust 1990).

When tires were colonized, the mean interval of 19.5 (± 3.4) days exposed could reflect a number of things. *Culex restuans* has been found in a variety of oviposition sites, suggesting that it is a relatively indiscriminant egg-layer (Gallaway and Brust 1979; Brust 1990; Buth *et al.* 1990). The high degree of variability in colonization time, ranging from one to over fifty days, could be due to the possibility that a gravid mosquito simply did not encounter those tires, and hence, no oviposition occurred within them.

Culex restuans will accept oviposition media that are much fresher than *Cx. tarsalis* will, as demonstrated by Brust (1990). He found that in sod-lined ovipools that were changed every week, much larger numbers of *Cx. restuans* egg rafts were found than *Cx. tarsalis*. Only one other study could be found where the time delay between tire exposure and mosquito oviposition in tires was examined (Berry and Craig 1984). They found that *Cx. restuans* was the fastest colonizer, detected 10 days after they set up tires (24 May – 3 June), while *Oc. triseriatus* was the last to be found, taking 31 days (24 May – June 24). They stated that rainfall was the only source of water, but did not report the dates of rainfall. They only set up clean tires once, at the start of the summer, and did not look at trends in colonization time across the season.

Ochlerotatus triseriatus has a very limited distribution in southern Manitoba (Kalpage and Brust 1967, Gallaway and Brust 1982). However, this species was found in tires in the current study, albeit rarely (see chapter 3). The lack of *Oc. triseriatus* in these experiments is probably due the removal of tires after the collection of an egg raft. If *Oc. triseriatus* eggs were present, they would not have been detected, but in the event some eggs hatched, as in 2003, the larvae would have been detected. However, only one individual of *Oc. triseriatus* was collected with the CDC traps (Table II), suggesting that there is a very small population of *Oc. triseriatus*, and their absence in tires is not surprising. *Culex restuans* was abundant in the egg rafts collected from tires, but only 17 adults were caught in the CDC trap. It has been demonstrated that *Cx. restuans* is not appropriately represented in CDC trap catches and is much sampled with ovipools in Manitoba (Brust and Ellis 1976; Brust 1990). Only five adult *Cs. inornata* were caught during the trapping period, and there were also low numbers of *Cs. inornata* egg rafts. *Culex tarsalis* was the fourth most frequently caught in the CDC trap, particularly later in the season, but no egg rafts of this species were collected. In the survey in 2003, *Cx. tarsalis* were infrequently found in tires, and this species is not typically thought of as a container mosquito. The three most common species collected were *Ae. vexans, Oc. sticticus*, and *Oc. dorsalis* (Table I). Although *Ae. vexans* has been found in tires (Joy *et al.* 2003) it was very infrequent in their study, and not found in either year of my research. The other two species have not been reported from tires in the literature. These mosquitoes, like most *Aedes/Ochlerotatus* mosquitoes prefer to oviposit in moist soil and need to be flooded to hatch, and are not thought of as container-inhabiting species (Wood *et al.* 1979).

Tire recyclers must travel considerable distances in Manitoba, as they collect tires from the entire province. Therefore, it is unlikely that all tires could be picked up and recycled before mosquito oviposition can occur, even though there is a mean interval of 19.3 days between tires becoming wet and mosquito oviposition. Tires often are left outside for over a year before they are picked up for recycling (phone survey 2003).

For applied practices, it would be useful to know whether storing tires in shaded or exposed areas would produce fewer mosquitoes. The concept of mosquito production is central to the thesis and perhaps the most important applied aspect of the research. Unfortunately, variation was high, the replicates were few, and the number of egg rafts low. The numbers of egg rafts in a sun-exposed replicate ranged from 5 to 14, and in a shaded replicate, ranged from 2 to 6, with an overall greater number of egg rafts in sun-exposed than shaded tires (31 to 14). Another factor influencing mosquito production is survivorship, which I did not address. If there is insufficient organic material (food) for the larvae in sun-exposed tires, or if the temperature of the water rises to lethal levels for a prolonged period in sun-exposed tires, production may be higher in shaded tires despite the lower number of egg rafts. In 2003, I put a temperature logger in tires to measure water temperature, and found that a high of 47°C was reached. More research would be required, looking at survivorship to determine whether this difference in egg rafts results in a difference in production.

Most of the variation in egg raft numbers across the treatments is attributed to the number of *Cx. restuans* egg rafts collected. They comprised 73% of the total number of egg rafts, and over three-quarters of them (78%) were found in sun-exposed tires. This result is in contrast to all previous literature mentioned. *Culex restuans* has been reported in the literature as either showing a preference for shaded oviposition sites (Brust 1990), or showing no preference at all (Andreadis 1988; Beier *et al.* 1983). In the latter two studies, they sampled larvae, rather than egg rafts as I did. Perhaps differential survivorship played a role here, in decreasing the number of larvae in the sun-exposed tires, although there were originally more oviposition events, which could create equal numbers of larvae in both treatments, or greater numbers in the shaded tires. Brust (1990) used sod-lined oviposition pools, which could provide slightly different cues to mosquitoes than tires with a more open and accessible pool of water than tires.

There are possible confounding factors within this study; chemical factors, organic material, and temperature, all can affect mosquito oviposition (Beier *et al.* 1983a; Trexler *et al.* 2003; Bentley and Day 1983) and may have been different between the two treatments as a result of the treatment. The trees planted at the experimental site are designed to be a windbreak; so presumably, wind velocity is different inside and out of the tree belt. However, these experiments represent a typical setting in which tires are often stored. Another variable in tires that could affect productivity is the size of each individual tire.

From the survey in 2003, I found larger tires, contained more mosquitoes and more frequently than in smaller tires. In 2004, I wanted to test the hypothesis that larger tires would be more productive than smaller tires in the same location. Unfortunately, only thirteen egg rafts were collected in the whole month of the study. Eleven were collected from large tires and two from small tires, which represents a significant difference. This difference probably arises primarily due to the size of the tire, and secondarily due to the surface area of the water within the tire. Mosquitoes, can detect a 1 cm black stripe at a maximum length of 9 cm, and can see a distance approximately seven meters, with dichromatic vision, which leads to good contrast sensitivity, but poor resolution of colour (Allan 1994). Tires, which are black, provide a good contrast against surrounding vegetation, or against the sky, and the greater the contrast, the greater the distance at which the object can be seen (Allan 1994). This contrast would be reduced in the crepuscular period in which gravid *Culex* mosquitoes are most active (MacDonald *et al.* 1981). However, they could still provide contrast to the surrounding environment. In addition, the larger the object, the greater the distance at which it can be seen (Allan 1994). Therefore, I reason that larger tires have a higher probability of being detected by a mosquito. If the mosquito orients to, and flies towards a tire with water inside, mosquitoes can detect polarized light, which is reflected off water, which is important for recognition of water (Allan 1994).

In this experiment, large tires sat immediately next to small tires. There is a chance that once a mosquito is close enough to detect reflected polarized light, the mosquito could fly into the small tire, especially in instances where the difference in surface area is not as great between the two sizes. However, I believe the majority will fly to the large tire, due to the large surface area of water, providing a greater area of reflectance than the smaller tires. If surface area of the water is the primary factor that is responsible for the differences, and not the size of the tires, it is still the larger tires that must be of primary concern. Large tires had triple the density (egg rafts/mm^2), but only double the surface area of the smaller tires. Plus, for the same amount of rainfall, large tires will generate a larger surface area. This is a result of two characteristics: a larger opening that would allow more rain in the tire, and a flatter bottom of the tire, spreading the water over a greater area. Water in larger tires will also be shallower, for the same volume of water as in small tires. Dhileepan (1997) found that several *Culex* species preferentially oviposited on deeper water. In this experiment, depth was kept constant, but surface area varied, possibly causing the difference seen in oviposition between the two tire sizes.

There are a number of variations to this experiment that would test the attractiveness of larger tires more fully. Control groups of two similar sized tires with similar surface areas would be expected to have equal numbers of oviposition events. Another treatment with similar sized tires, but varied surface areas would test the effects of larger surface areas. However due to time and space constrains, tires were set up in this manner because I wanted to test not only whether tractor tire piles have more oviposition events, but whether mosquitoes will oviposit significantly more often in the larger tire in the presence of a smaller tire. In effect, I wanted to know whether larger tires would be selected over smaller tires. This cannot be called a true choice experiment, in that we do not know if the mosquito detected tires that remained without egg rafts and the tires with egg rafts simultaneously although they probably did, given the capability to see for seven metres (Allan 1994). However, the fact remains that most (85%) of the egg rafts were collected from the larger tires. This experiment could have been improved with the addition of controls, where two large tires and two small tires formed a pair and no difference between the similar tires should have been seen.

More research is needed, as numbers of egg rafts were low for this experiment, and no other studies concerning the size of a tire could be found. Another interesting variation of this experiment would be to vary the size of tire piles, to determine if larger piles would have more oviposition events than smaller piles. This would show that it is, in fact, the size of the dark object that is initially attracting gravid mosquitoes.

More research is required to discover the optimum arrangement for minimizing mosquito oviposition, but according to the trends discovered in these experiments, placing tires in shaded areas could lower the frequency of mosquito oviposition. It also appears that larger tires could be a focus for control efforts to maximize control while minimizing time and cost depending on the frequency of each tire size. However, many small tires could produce as many mosquitoes as a few large tires. Because tires were removed when space became limited without oviposition, and once oviposition occurred tires were removed, a quantitative estimate of oviposition reduction based on the number of days outdoors can not be calculated. However, it can be concluded that oviposition can occur within one day, and the interval becomes significantly smaller late in the season.

CHAPTER V: THE EFFECT OF STACKING METHODS ON MOSQUITO ABUNDANCE IN TIRE PILES IN SOUTHERN MANITOBA

ABSTRACT

Ten thousand and eight mosquito larvae were collected from 405 tires that were stacked in nine groups of 45. Three groups were randomly assigned to each of three stacking methods, vertical, (tire radius perpendicular to ground), horizontal (tire radius parallel to ground), or random. Tires were placed in the field on 12 May 2004, and sampled 91 and 93 days later on 11 and 13 August 2004. All tires from each pile were examined and all larvae observed were collected, retained, and volume of water in each tire recorded. Larvae were identified to species, and counted. The greatest prevalence of infestation was observed in random and vertically stacked tires: 53/136 and 66/135 respectively, whereas only 19 of 135 horizontally stacked tires contained mosquitoes. Most mosquito larvae (98%) were *Cx. restuans* in comparison to very few *Cx. tarsalis* larvae (n = 45; 0.4%) and *Cs. inornata* larvae (n = 211; 1.6%). Mosquito abundance was not correlated with water volume. There were significantly more mosquitoes and they occurred more often, at the bottom of a horizontal stack ($p<0.05$). This has implications for the ease of application of control measures. These differences observed between stacking methods could influence the way tires are managed so as to reduce numbers of mosquitoes that select tires as oviposition sites.

INTRODUCTION

Tires can potentially augment the natural populations of nuisance or vector mosquitoes in close proximity to human populations (Niebylski and Craig 1994), and as a result, they can pose a public health risk (Hedberg *et al.* 1979). Tires holding water are attractive to a number of tree hole-using or pollution-tolerant mosquitoes (Laird 1988). Lampman *et al.* (1997) considered tire dumps to be foci for the proliferation and dispersal of mosquitoes, potentially causing public health problems that range from an increase in nuisance complaints to an increase in the transmission of mosquito-borne pathogens.

Novak (1995) outlined several factors that could influence the abundance as well as diversity of mosquitoes that colonize tires. They suggested that the distribution of mosquito larvae in a tire yard is primarily influenced by the age, size, and physical structure of the tire dump, the heterogeneity of the environment, weather conditions, availability of blood hosts, and the bionomics and behaviour of the associated mosquito species. In Manitoba, there are 306 waste transfer stations that may hold tires, as well as many other undocumented tire piles (Manitoba Conservation data).

This experiment was designed to examine the physical structure of tire piles and how that may influence the degree to which mosquitoes use tires as oviposition sites. Differently stacked piles might be expected to appear different to a gravid female searching for an oviposition site. If it could be shown that there is a significant difference in the number of mosquito larvae found in tires stacked differently, tire dealers could use this information to guide them in setting up their tire yards and minimize the potential risk their tires pose in producing vector or nuisance mosquitoes, and the subsequent transport of these mosquitoes to other geographic areas.

METHODS

Four hundred and six used passenger tires (outside tire diameter ranging from 63.5-76.2cm) were obtained from a tire recycler in Winnipeg, and transported out to a farm located 17-4-4NW, about 7.4 kilometers south of Roland, Manitoba. They were all washed out with a garden hose, and wiped out with rags to ensure that there was no organic material in any of the tires prior to the start of the experiment. They were then arranged in the field on 11 May, in nine groups of 45. One replicate of the randomly stacked treatment unintentionally contained 46 tires. Each group was assigned one of three treatments: random, vertical or horizontal. Randomly stacked tires were just thrown from the back of the truck into a roughly pyramidal-shaped pile. Vertically stacked tires were in five rows of nine tires, perpendicular to the ground, leaning on the next tire and held up by a wooden stake hammered into the ground at the back of each row. Horizontally stacked tires were in a 3x3 square, each five tires in height, lying parallel to the ground.

Each group was less than five meters from a tree line and at least thirty meters from the next group. Nine sites were selected, then stratified into three blocks of three, and then each site was randomly designated one of three treatments. Six sites (two of each treatment) were on the south side of a tree line running east/west while the remaining three sites were on the west side of a tree line running north/south.

Data were collected on the 11 and 13 August, after 91 and 93 days outside. Each tire was given a code that indicated which treatment, replicate, and region the tire (and its contents) came from. In randomly stacked tires, six regions were identified. Any tire touching the ground was considered bottom (B), while tires on top of the pile were labeled top (T), and those in between were middle (M). Any tire on the outside of a pile was considered outer (O), while the others would be inner (I).

Each column was numbered in the horizontal stacks, and a second number indicated height in each column. Vertical rows were numbered, and a second number indicated position in the row. Later, to compare with random stacks, horizontally and vertically stacks were labeled either inner or outer and top, middle, or bottom using the same criteria as for randomly stacked tires. Thus, all vertically stacked tires were considered bottom, with the outer ring of 24 tires labeled outer, and the rest inner.

A 250mL cup was used to scoop the entire contents of each tire out. The water, debris, and if present, immature mosquitoes, were passed through a 0.2 micron sieve into a 22L pail. If immature mosquitoes were present, they were rinsed into a specimen cup for transport back to the laboratory for identification and enumeration. Tires containing only pupal skins were considered negative, as an estimate of productivity of the tires at the point of data collection was desired. The water was then poured into a 2L graduated cylinder to measure volume. If the volume of water in a tire exceeded 2L, the cylinder was emptied and filled as many times as necessary.

In the lab, specimen cups containing larvae were placed in a 5°C room until identifications could be completed. Second to fourth instar larvae were identified using the key in Wood *et al.* (1979), while first instars were identified using Dodge (1969). Any pupae that were collected were allowed to emerge in clear acrylic cages, and identified as adults using Wood *et al.* (1979).

In all instances the standard alpha value of 0.05 was used to determine significance. Where applicable (*i.e.* when the dependent variable was continuous), analysis of variance (ANOVA) was used to determine if there was a significant difference between means. When ANOVA revealed the presence of a significantly different mean in a group of 3 or more, Tukey-Kramer's honestly significant difference (HSD) procedure was used to tell which of the means were significantly different. Chi-Square values were obtained when the variable response was either a one or a zero (e.g. presence/absence) to determine whether the observed values were different from expected values for categorical variables. Logistic regression was used where continuous variables were compared to nominal variables.

RESULTS

There were 10,008 immature mosquitoes collected in 138 of the 406 tires (33%), an average of 24.6 mosquitoes per tire, and an intensity (average number of immature mosquitoes in tires containing mosquitoes) of 72.5. The number of immature mosquitoes found in a individual tires ranged from zero to 306. *Culex restuans* comprised 97.4% (9752 individuals) of the total collection. Forty-five immature mosquitoes (0.4%) were *Cx. tarsalis*, while *Cs. inornata* comprised the remaining 2.2% (211). Although the slope of the relationship between mosquito abundance and water volume was significantly different from zero, there was a lot of variation not explained by the model ($r^2 = 0.025$, F-ratio 10.53, df = 1, 408 $p = 0.0013$). The same results occurred when logistic regression was performed on the relationship between prevalence and water volume ($r^2 = 0.017$, $\chi^2 = 9.19$, df = 1, $p = 0.0024$). Vertically stacked tire piles had the greatest abundance and prevalence (percentage of tires containing mosquitoes) with 66 tires containing mosquitoes (48.89%) and a total of 4550 immature mosquitoes. Randomly stacked tires had intermediate prevalence with 53 tires containing immatures (38.97%) and an intermediate abundance with a total of 4276 mosquito immatures. The lowest prevalence and abundance were the horizontally stacked tires with 19 tires containing immatures (14.07%) and a total of 1166 mosquito immatures (Table I). Using the Tukey-Kramer HSD to compare means, each replicate of a treatment was not significantly different from the other replicates in the same treatment, either in abundance ($p > 0.05$), or prevalence ($p > 0.05$). The average number of mosquitoes per tire followed the same trend as prevalence: vertical, random and horizontal (33.5, 30.9, and 8.6 mosquitoes per tire, respectively).

Position within a pile was also analyzed to determine if prevalence or abundance differed significantly at varying depths or heights. Neither prevalence nor abundance was significantly affected by depth (inner versus outer) (prevalence F ratio = 0.143, df = 1, 408, $p = 0.706$), (abundance F ratio = 0.064, df = 1, 408, $p = 0.801$), but both were significantly affected by height (top, middle, bottom) (prevalence F ratio = 16.84, df = 2, 407, $p < 0.0001$), (abundance F ratio = 14.78, df = 2, 407, $p < 0.0001$). The interaction between height and depth was not significant for both abundance (F ratio = 0.101, df = 2, 404, $p = 0.904$) and prevalence (F ratio = 0.958, df = 2, 404, $p = 0.384$). Forty-nine per cent of the tires on the bottom of a pile, 19% of the middle tires, and 4% of the top tires contained immature mosquitoes.

Because three tire piles were placed along a north-south tree line, while six replicates were placed behind a tree line running east-west, both abundance and prevalence were tested against tree line orientation to look for an effect of tree line.

There was no significant difference in the mean abundance of mosquitoes (F ratio = 0.042, df = 1, 408, $p = 0.838$) or prevalence (F ratio = 2.563, df = 1, 408, $p = 0.110$).

Table I: Summary of the three treatments and each of the three replicates of 45 tires stacked in three treatments near Roland, Manitoba, showing abundance, number of infested tires, and average abundance per treatment with 95% confidence interval. (different letters within columns denote significantly different means ($p < 0.05$).

Treatment	Replicate	Abundance	Infested Tires (%)
Random	1	1160	35.56
	2	1896	41.30
	3	1220	40.00
Mean		1425 ± 1015[a]	38.97[a]
Vertical	1	1530	46.67
	2	1423	37.78
	3	1597	62.22
Mean		1517 ± 218[a]	48.89[a]
Horizontal	1	252	8.89
	2	536	15.56
	3	378	17.78
Mean		389 ± 353[b]	14.07[b]

DISCUSSION

The goal of this research was to investigate methods by which people that need to store tires can minimize the number of mosquitoes arising from tire piles in a natural setting. Many of the results of this experiment corresponded with the results of the survey of tire piles in 2003, which would suggest that this experiment simulated natural tire pile settings in Manitoba.

The average number of mosquitoes per tire is an estimate of the productivity of a given tire pile. Interestingly, the average number of mosquitoes per wet tire in 2003 was 25.7, while the average number of mosquitoes per wet tire in 2004 (97% were wet) was 25.4. The sampling in 2003 combined vertical, horizontal, and randomly oriented tires, while the 2004 experiment had equal numbers of each type. In 2003, all tires (even from random piles) were placed into either horizontal or vertical categories so it is impossible to tell which type of pile each tire came from. Due to heavy rainfall during the two days prior to data collection, we were able to determine that at least 97% of tires stacked in all three methods are capable of holding water, and thus become potential mosquito oviposition sites. It is impossible to determine, from these data, the productivity over an entire season; however, it can be assumed that it would be greater than what was observed in this experiment given that *Culex* mosquitoes are multivoltine in southern Manitoba (Wood *et al.* 1979). Pupal skins were found in 15 of the tires, suggesting that successful development was completed in those tires; however, they were not included in the counts presented here.

The prevalence of mosquitoes was slightly higher in 2004 (33%) than 2003 (27%), but this may be because the bottom inner tires (with presumed higher prevalence) in randomly stacked piles, and the bottom tires in horizontal stacks (with presumed higher prevalence) were not sampled in 2003. However, all the tires in vertical rows (with presumed lower prevalence) were accessible to survey. These three sampling biases would alter the observed prevalence of mosquitoes from the actual prevalence. The actual prevalence was known in 2004, because all tires were sampled. The high prevalence in inner bottom tires is contradictory to the findings by Novak *et al.* (1990) and Morris and Robinson (1994) who found that tires in the inner half of a stack contained significantly fewer mosquitoes than the outer half of the stack. However, their piles were much larger than the piles in this experiment. They looked at tire piles that were at least three metres in height. Our piles were less than 1.5 meters tall. In Quebec, the prevalence of larvae in wet tires was 25.1%, of which most tires had 100 or more immatures, thereby making the average abundance approximately 25 mosquitoes per wet tire, which is in close agreement with my findings depending on the amount of variation observed (M. Boisvert, personal communication)

As in 2003, *Cx. restuans* dominated the collection in 2004. *Culex tarsalis* abundance was low in 2003, and much lower in 2004. *Culiseta inornata* was found in similar numbers to 2003. Therefore, tires may either be poor mimics of natural oviposition sites of *Cx. tarsalis*, or they could be repellant in some way. *Culex restuans*, which can be found almost anywhere water collects, will accept tires. If it is determined that *Cx. restuans* is a major element of West Nile virus transmission cycles, then perhaps treatment of tires may become necessary. However, they are not significant in transmission to humans, and in fact, their role as a primary enzootic vector is not completely understood on the prairies. It is impossible to tell, from these data, how much tires augment natural populations of *Cx. restuans* because this species is grossly under-represented in light trap catches in Manitoba (Brust and Ellis 1976; Brust 1990), making it difficult to estimate natural populations. Another reason that the significance of tires to natural populations is unknown is because tire productivity over a season is unknown and the populations emerging from other habitats are impossible to quantify.

Vertically stacked tires contained the most larvae and those tires contained larvae most frequently. This is in agreement with the survey in 2003. Randomly stacked tires were intermediate with respect to abundance and prevalence, while horizontal tires had the lowest values in both categories. Baumgartner (1988) looked at three storage methods: vertical (horizontal in this paper's context), scattered singly, and shingle stacked (treads in the space of other tires above or below in a lattice-like pile). He found a prevalence of 25% in the horizontally stacked tires, whereas 14% of the horizontally stacked tires in my study contained immature mosquitoes. However, Baumgartner (1988) randomly selected three to five tires, and did not sample the entire contents, merely a dip of 300mL. Baumgartner (1988) noted that larvae, especially when present at low densities, could have avoided detection by swimming to the bottom.

Mosquito abundance was only weakly correlated to water volume in this study. However, interpretation of correlation between abundance and water volume is difficult, because time elapsed between oviposition and sampling, so there could have been a different volume of water at each event via rainfall or evaporation. In addition, I only measured abundance at the time of sampling in this experiment. Survivorship and oviposition frequency are impossible to determine with the methods used. A tire with many oviposition events, but a high mortality rate would appear to have the same abundance as a tire with fewer oviposition events, but a lower mortality rate. In addition, the heavy rainfall for the two days prior to sampling may have affected the data collected. The volume recorded would not necessarily have reflected the volume when the mosquito laid its eggs. Therefore, there are a number of factors that could explain why abundance and volume were only weakly correlated, or, perhaps, they truly aren't correlated. In fact, the highest prevalence and abundance values were recorded from some of the smallest volumes of water. The goal of this experiment was to determine if there was a difference in mosquito abundance among three different stacking methods. Whereas oviposition frequency and survivorship are important factors, ultimately what matters to public health and to tire dealers is how many mosquitoes actually emerge from tires: horizontal tires contained significantly fewer mosquitoes.

Each stacking method poses different advantages and disadvantages to a potential control operation. Although vertical tires had the greatest number of larvae, they are aligned in a row, and the greatest prevalence was on the outer tires, where it would be easiest to reach with a larvicide. Horizontal tires had the fewest larvae but the greatest prevalence was on the bottom of the stack where it would be most difficult to introduce a larvicide for effective control. If tire dealers were not going to use control measures, perhaps stacking horizontally would minimize mosquito production. If control measures were planned, vertically stacked tires would be the best configuration. The difference in mosquito abundance could be due to the area that each pile occupied. Horizontal stacks were the most space-efficient method of stacking tires. Vertically stacked tires were the most space inefficient method.

In conclusion, tire-stacking methods influenced the abundance of mosquitoes within tires, which then could influence adult populations, particularly of *Cx. restuans*, which may be an important species in the transmission cycle of West Nile virus, a serious public health threat in Manitoba andelsewh *p+1Xere.

CHAPTER VI: DISCUSSION

Biological invasions consist of three characteristics: arrival, establishment, and spread (Lounibos 2002). The rate of arrival (introduction) of invasive species has increased in the last century with the increase in international business and travel (Sutherst 2004). One of the international businesses that are contributing to this increase is the tire trade. In Manitoba and throughout the world, several industries are based on tires. As a result of these industries, tires are transported across the world for recycling or re-use. This aspect of the tire trade makes it capable of becoming important in the first stage of a biological invasion.

It has been known for sixty years that tires have the potential to provide suitable oviposition sites and larval habitat for mosquitoes (Pratt *et al.* 1946). This aspect of tires would enable the second stage of a biological invasion, establishment. The focus of this study was on the suitability of tires as oviposition sites and larval habitats in Manitoba. The survey in 2003 was necessary to determine what species of mosquitoes occur in tires in Manitoba specifically, as no research had been published about mosquitoes and tires in Manitoba.

Surveys of tire piles are designed to determine which species are using tires as oviposition sites. *Culex restuans* was found in all of the studies that examined the mosquito fauna found in tires in North America (Andreadis 1988; Baumgartner 1988; Beier *et al.* 1983a; b; Jamieson *et al.* 1994; Joy *et al.* 2003; Lampman *et al.* 1997; Lee and Rowley 2000; Kaufman *et al.* 2005). It seems that tires are good mimics of the natural oviposition sites of *Cx. restuans*. References to *Cx. tarsalis* in tires were found infrequently, while *Cs. inornata* was not documented in the literature examined.

Culex restuans seems to accept oviposition sites with less organic matter than *Cx. tarsalis* accepts (Brust 1990). I recorded the presence of an egg raft from *Cx. restuans* found in a clean tire (virtually no organic matter) less than 24 hours after becoming wet. More evidence that *Cx. restuans* is less choosy in oviposition sites is that this species has been found from a wide variety of aquatic habitats in Manitoba: tree holes, tires, catch basins, ditches, curbside puddles, and rain barrels (Gallaway and Brust 1979; Thomson 2004). As twelve different species of adult mosquitoes were captured in CDC traps at Glenlea Research Station, and in much higher numbers than *Cx. restuans*, it is probable that these other mosquitoes encountered tires, but did not lay eggs. It should be noted that CDC traps are known to underestimate population sizes for *Cx. restuans* in particular (Brust and Ellis 1976; Brust 1990). Only one egg raft from one species other than *Cx. restuans* was found in tires at the Research Station in 2004 (*Cs. inornata*), further evidence that tires are less suitable for oviposition to most mosquitoes.

During the second year of research, trends noted in the initial survey were experimentally assessed to determine if all tires are equally likely to be chosen by mosquitoes. Following from such evidence, control measures can be concentrated on the tires most likely to be suitable sites, thereby increasing efficiency.

During the survey, I found more larvae and found them more often when sampling a larger tire when compared to smaller tires. The same results were obtained when large and small tires were set up experimentally. Mosquitoes can see a distance of approximately seven meters, and have dichromatic vision, which leads to good contrast sensitivity (Allan 1994). Tires, which are black, provide a high degree of contrast against surrounding vegetation, or against the sky, and the greater the contrast, the greater the distance at which the object can be seen (Allan 1994). It is possible that once mosquitoes are within visual range of the water inside the tires, the larger surface area in large tires in comparison to smaller tires has a higher probability of being detected by mosquitoes than the small tires. A variation of this experiment could use orientation as a factor in which surface areas between the two orientations could be examined.

The second trend discovered in the survey was the effect of tire orientation on the presence of immature mosquitoes. Again, the trend found in the survey was backed up by the experiment, in that horizontally stacked tires had approximately one-quarter the number of mosquitoes that either randomly or vertically stacked tires had. Unfortunately, a horizontal stack is the most difficult configuration to achieve in tires and due to the presence of steel belts in some tires, punching a hole in them is difficult. Even with my stacks of five, they began to wobble due to the fact tires were slightly different sizes. Therefore, it is much more time and cost-efficient to stack vertically. In addition, if control was deemed necessary, it is recommended to stack tires in vertical rows, despite the increased number of larvae, treatment would be easiest. A modified leaf-blower could apply granular larvicide through the tunnel created by the tires.

Another aspect of the environment surrounding tires that was tested experimentally was the degree of sun exposure. More egg rafts were collected in exposed tires than from shaded tires, opposite to what Brust (1990) found in his exposed and shaded ovipools. One difference between ovipools and tires is the presence of a protected area where mosquitoes may rest, increasing the probability of contact between mosquitoes and tires. This would explain why more egg rafts were collected in ovipools located in a tall dense tree canopy in the study by Brust (1990). The close proximity of an oviposition site to the adult resting sites increases the probability that a mosquito will discover the pool of water. Because the tires were located along a tree line, mosquitoes returning to the resting places in the tree line would encounter tires first, and possibly accept them as a resting site.

Overall, given that one-quarter of the tires examined in 2003 contained *Cx. restuans*, which could play a major role in virus epidemiology, tires may play an important role in providing additional oviposition sites to natural habitats for mosquitoes. Without an accurate assessment of the natural population of *Cx. restuans*, no reliable prediction can be made of the role of tires in providing oviposition sites and contribution to natural mosquito populations. However, in areas where large numbers of tires accumulate, tires may supplement local populations of mosquitoes. Tires may be colonized within 24 hours of becoming wet (at least for *Cx. restuans*), and on average, approximately one-quarter of them harbour immature mosquitoes at any given time.

Further research needs to be done to determine the significance of tires on naturally occurring populations. An exhaustive list of oviposition sites other than tires needs to be compiled for an area that could be considered a local mosquito population (e.g. town, municipality). Placing some sort of marker (fluorescence, radioactive) on the adult mosquitoes emerging from the tires within a town, and then extensively sampling (for *Cx. restuans*, bird-baited traps) would begin to give an estimate of the proportion of the total mosquito population arising from tires.

An understanding of mosquito oviposition behaviour can be applied in many ways. Knowledge of what is attractive to female mosquitoes can be used to effectively control mosquitoes through reduction of suitable habitats. Habitat reduction is the most effective method of reducing potential vector and pest mosquito populations. A system of tire storage to make the tires unattractive to females searching for an oviposition site would minimize the number of mosquitoes arising from tires. This would be useful in controlling mosquito populations that could spread pathogens, resulting in disease such as West Nile neurological syndrome and West Nile fever, both of which were documented extensively in western Canada since 2002.

SUMMARY

- The importance of tires as they relate to West Nile virus epidemiology is directly dependent on the importance of *Culex restuans* as a vector. More than 95% of the immature mosquitoes collected from tires were *Cx. restuans*.
- Stacking tires horizontally minimized the abundance and prevalence of immature mosquitoes in tires.
- Larger tires contained immature mosquitoes more often than smaller tires. Control efforts could focus on these as foci of immature mosquito production. Assuming that my random sampling accurately represented the population, tractor tires comprise roughly 21% of the total number of tires in Manitoba.
- The mean interval between a tire becoming wet and being colonized by mosquitoes decreases as the summer progresses. This has implications for tire management.

References Cited

Allan, S. A. 1994. Physics of mosquito vision: an overview. *Journal of the American Mosquito Control Association* **10:** 266-271.

Anderson, R. A. and Brust, R. A. 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.

Anderson, R. A. and Gallaway, W. J. 1987. The host preferences of *Culiseta inornata* in southwestern Manitoba. *The Journal of the American Mosquito Control Association* **3:** 219-221.

Andreadis, T. G. 1988. A survey of mosquitoes breeding in used tire stockpiles in Connecticut. *Journal of the American Mosquito Control Association* **4:** 256-260.

Andreadis, T. G., Anderson, J. F., Munstermann, L. E., Wolfe, R. J., and Florin, D. A. 2001. Discovery, distribution and abundance of the newly introduced mosquito *Ochlerotatus japonicus* (Diptera: Culicidae) in Connecticut, USA. *Journal of Medical Entomology* **38:** 774-779.

Barker, C. M., Paulson, S. L., Cantrell, S., and Davis, B. S. 2003. Habitat preferences and phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in southwestern Virginia. *Journal of Medical Entomology* **40:** 403-410.

Baspaly, L. M 2003. An evaluation of Reiter's medium and three different pool sizes for ovipool surveillance of *Culex tarsalis*, *Culex restuans*, and *Culiseta inornata* in Manitoba. M.Sc. Thesis, University of Manitoba.

Baumgartner, D. L. 1987. Importance of construction sites as foci for urban *Culex* in northern Illinois. *Journal of the American Mosquito Control Association* **3:** 26-34.

Baumgartner, D. L. 1988. Suburban accumulations of discarded tires in northeastern Illinois and their associated mosquitoes. *Journal of the American Mosquito Control Association* **4:** 500-508.

Beck, D. E. 1961. Central Utah county, Utah, mosquito survey studies. *Mosquito News* **21:** 6-11.

Beier, J. C., Patricoski, C., Travis, M., and Kranzfelder, J. 1983a. Influence of water chemical and environmental parameters on larval mosquito dynamics in tires. *Environmental Entomology* **12:** 434-438.

Beier, J. C., Travis, M., Patricoski, C., and Kranzfelder, J. 1983b. Habitat segregation among larval mosquitoes (Diptera: Culicidae) in tire yards in Indiana, USA. *Journal of Medical Entomology* **20:** 76-80.

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

Berry, W. J. and Craig, Jr, G. B. 1984. Bionomics of *Aedes atropalpus* breeding in scrap tires in northern Indiana. *Mosquito News* **44:** 476-484.

Bidlingmayer, W. L. and Hem, D. G. 1980. The range of visual attraction and the effect of competitive visual attractants upon mosquito (Diptera: Culicidae) flight. *Bulletin of Entomological Research* **70:** 321-342.

Bond, H. A. and Fay, R. W. 1969. Factors influencing *Aedes aegypti* occurrence in containers. *Mosquito News* **29:** 113-121.

Bonnet, D. D. and Worchester, D. J. 1946. The dispersal of *Aedes albopictus* in the territory of Hawaii. *American Journal of Tropical and Medical Hygiene* **26:** 465-476.

Brinton, M. A. 2002. The molecular biology of West Nile virus: a new invader of the western hemisphere. *Annual Review of Microbiology* **56:** 371-402.

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

Buth, J. L., Brust, R. A., and Ellis, R. A. 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.

Clements, A. N. 1999. The biology of mosquitoes Vol. 2: Sensory reception and behaviour. New York, New York: CABI Publishing.

Cornel, A. J. and Hunt, R. H. 1991. *Aedes albopictus* in Africa? First records of live specimens in imported tires in Cape Town. *Journal of the American Mosquito Control Association* **7:** 107-108.

Covell, C. V., Jr. and Brownell, A. J. 1979. *Aedes atropalpus* in abandoned tires in Jefferson County, Kentucky. *Mosquito News* **39:** 142.

Craven, R. B., Eliason, D. A., Francy, D. B., Reiter, P., Campos, E. G., Jakob, W. L., Smith, G. C., Bozzi, C. J., Moore, C. G., Maupin, G. O., and Monath, T. P. 1988. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. *Journal of the American Mosquito Control Association* **4:** 138-142.

Dalla Pozza, G. and Majori, G. 1992. First record of *Aedes albopictus* established in Italy. *Journal of the American Mosquito Control Association* **8:** 318-320.

Darsie, R. F. and Ward, R. A. 2005. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Gainesville, Florida: The University Press of Florida.

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.

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

Fonseca, D. M., Campbell, S., Crans, W. J., Mogi, M., Miyagi, I., Toma, T., Bullians, M., Andreadis, T. G., Berry, R. L., Pagac, B., Sardelis, M. R., and Wilkerson, R. C. 2001. *Aedes* (*Finlaya*) *japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: analyses of genetic variation in the United States and putative source populations. *Journal of Medical Entomology* **38:** 135-146.

Gallaway, W. J. and Brust, R. A. 1982. The occurrence of *Aedes hendersoni* Cockerell and *Aedes triseriatus* (Say) in Manitoba. *Mosquito Systematics* **14:** 262-264.

Geery, P. R. and Holub, R. E. 1989. Seasonal abundance and control of *Culex* spp. in catch basins in Illinois. *Journal of the American Mosquito Control Association* **5:** 537-540.

Gomes, A., Gotlieb, S. L. D., Marques, C., Paula, M. B., and Marques, G. 1995. Duration of larval and pupal development stages of *Aedes albopictus* in natural and artificial containers. *Revista de Saude Publica* **29:** 15-19.

Hanec, W. and Brust, R. A. 1967. The effect of temperature on the immature stages of Culiseta inornata (Diptera: Culicidae) in the laboratory. *The Canadian Entomologist* **99:** 59-64.

Haverfield, L. E. and Hoffmann, B. L. 1966. Used tires as a means of dispersal of *Aedes aegypti* in Texas. *Mosquito News* **26:** 433-435.

Hawley, W. A., Reiter, A. P., Copeland, R. S., Pumpuni, D. B., and Craig, G. B., Jr. 1987. *Aedes albopictus* in North America: probable introduction from northern Asia. *Science* **236:** 1114-1116.

Hedberg, C. W., Washburn, J. W., and Sjogren, R. D. 1985. The association of artificial containers and La Crosse encephalitis cases in Minnesota, 1979. *Journal of the American Mosquito Control Association* **1:** 89-90.

Holst, O., Stenberg, B., and Christiansson, M. 1998. Biotechnological possibilities for waste tyre-rubber treatment. *Biodegradation* **9:** 301-310.

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

Jamieson, D. H., Olson, L. A., and Wilhide, J. D. 1994. A larval mosquito survey in northeast Arkansas including a new record for *Aedes albopictus*. *Journal of the American Mosquito Control Association* **10:** 236-239.

Joy, J. E., Hanna, A. A., and Kennedy, B. A. 2003. Spatial and temporal variation in the mosquitoes (Diptera: Culicidae) inhabiting waste tires in Nicholas County, West Virginia. *Journal of Medical Entomology* **40:** 73-77.

Jupp, P. G. and Kemp, A. 1992. *Aedes albopictus* and other mosquitoes imported in tires into Durban, South Africa. *Journal of the American Mosquito Control Association* **8:** 321-322.

Kalpage, K. S. P. and Brust, R. A. 1973. Oviposition attractant produced by immature *Aedes atropalpus*. *Environmental Entomology* **2:** 729-730.

Kaufman, P. E., Harrington, L. C., Waldron, J. K., and Rutz, D. A. 2005. The importance of agricultural tire habitats for mosquitoes of public health importance in New York State. *Journal of the American Mosquito Control Association* **21:** 171-176.

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

Laird, M. 1988. The natural history of larval mosquito habitats. London, UK: Academic Press, Limited.

Laird, M., Calder, L., Thornton, R. C., Syme, R., Holder, P. W., and Mogi M. 1994. Japanese *Aedes albopictus* among four mosquito species reaching New Zealand in used tires. *Journal of the American Mosquito Control Association* **10:** 14-23.

Lampman, R. L., Hanson, S., and Novak, R. 1997. Seasonal abundance and distribution of mosquitoes at a rural waste tire site in Illinois. *Journal of the American Mosquito Control Association* **13:** 193-200.

Lardeux, F., Sechan, Y., Loncke, S., Deparis, X., Cheffort, J., and Faaruia, M. 2000. Integrated control of peridomestic larval habitats of *Aedes* and *Culex* mosquitoes (Diptera: Culicidae) in atoll villages of French Polynesia. *Journal of Medical Entomology* **39:** 493-498.

Lee, J. and Rowley, W. A. 2000. The abundance and seasonal distribution of *Culex* mosquitoes in Iowa during 1995-97. *Journal of the American Mosquito Control Association* **16:** 275-278.

Lounibos, L. P. 2002. Invasions by insect vectors of human disease. *Annual Review of Entomology* **47:** 233-266.

MacDonald, R. S., Madder, D. J., and Surgeoner, G. A. 1981. Diel periodicity of oviposition by *Culex pipiens* and *Culex restuans* in southern Ontario. *Proceedings of the Entomological Society of Ontario* **112:** 39-40.

Manitoba Tire Stewardship Board. 2005. April 1, 2004 – March 31, 2005 Annual Report. 27pp.

McLintock, J. and Iversen, J. 1975. Mosquito and human disease in Canada. *The Canadian Entomologist* **107:** 695-704.

Mitchell, C. J., Haramis, L. D., Karabatsos, N., Smith, G. C., and Starwalt, V. J. 1998. Isolation of La Crosse, Cache Valley, and Potosi viruses from *Aedes* mosquitoes (Diptera: Culicidae) collected at used tire-sites in Illinois during 1994-1995. *Journal of Medical Entomology* **35:** 573-577.

Moore, C. G., Francy, D. B., Eliason, D. A., Bailey, R. E., and Campos, E. G. 1990. *Aedes albopictus* and other container-inhabiting mosquitoes in the U. S.: results of an eight-city survey. *Journal of the American Mosquito Control Association* **6:** 173-178.

Morris, C. D. and Robinson, J. W. 1994. Distribution of mosquito larvae in a waste tire pile in Florida -- an initial study. *Journal of the American Mosquito Control Association* **10:** 174-180.

Nawrocki, S. J. and Craig, G. B., Jr. 1989. Further extension of the range of the rock pool mosquito, *Aedes atropalpus*, via tire breeding. *Journal of the American Mosquito Control Association* **5:** 110-114.

Niebylski, M. L. and Craig, G. B., Jr. 1994. Dispersal and survival of *Aedes albopictus* at a scrap tire yard in Missouri. *Journal of the American Mosquito Control Association* **10:** 339-343.

Novak, R. 1995. A North American model to contain the spread of *Aedes albopictus* through tire legislation. *Parazitologiya* 129-139.

Novak, R., Steinly, B. A., Webb, D. W., Haramis, L., Clarke, J., Farmer, B., and Geslik, R. 1990. Penetration rate of two pesticide carriers at a large used-tire storage facility in Chicago, Illinois. *Journal of the American Mosquito Control Association* **6:** 188-196.

Pafume, B. A., Campos, E. G., Francy, D. B., Peyton, E. L., Davis, A. N., and Nelms, M. 1988. Discovery of *Aedes bahamensis* in the United States. *Journal of the American Mosquito Control Association* **4:** 380.

Paulson, S. L. and Grimstad, P. R. 1989. Replication and dissemination of La Crosse virus in the competent vector *Aedes triseriatus* and the incompetent vector *Aedes hendersoni* and evidence for transovarial transmission by *Aedes hendersoni* (Diptera: Culicidae). *Journal of Medical Entomology* **26:** 602-609.

Peyton, E. L., Campbell, S. R., Candeletti, T. M., Romanowski, M., and Crans, W. 1999 . *Aedes japonicus japonicus* (Theobald), a new introduction into the United States. *Journal of the American Mosquito Control Association* **15:** 238-241.

Pratt, Jr., J. J., Heterick, R. H., Harrison, J. B., and Haber, L. 1946. Tires as a factor in the transportation of mosquitoes by ships. *Military Surgeon* **99:** 787-788.

Rai, K. S. 1986. Genetics of *Aedes albopictus*. *Journal of the American Mosquito Control Association* **2:** 429-436.

Reisen, W. K and Siddiqui, T. F. 1978. The influence of conspecific immatures on the oviposition preferences of the mosquitoes *Anopheles stephensi* and *Culex tritaeniorhynchus*. *Pakistan Journal of Zoology* **10:** 31-41.

Reiter, P. and Darsie, D. F. 1984. *Aedes albopictus* in Memphis, Tennessee (USA): an achievement of modern transportation? *Mosquito News* **44:** 396-399.

Reiter, P. and Sprenger, D. 1987. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *Journal of the American Mosquito Control Association* **3:** 494-501.

Romi, R., Sabatinelli, G., and Pontuale, G. 1997a. *Aedes atropalpus*, a new mosquito introduced by discarded tires in Italy. *Parasite* **4:** 343-349.

Romi, R., Sabatinelli, G., Savelli, L. G., Raris, M., Zago, M., and Malatesta, R. 1997b. Identification of a North American mosquito species, *Aedes atropalpus* (Diptera: Culicidae) in Italy. *Journal of the American Mosquito Control Association* **13:** 245-246.

Rueda, L. M., Patel, K. J., Axtell, R. C., and Stinner, R. E. 1990. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* **27:** 892-898.

Rueger, M. E., Olson, T. A., and Price, R. D. 1964. Larval habitats of *Culex tarsalis* (Coq.) (Diptera: Culicidae) in Minnesota. *Mosquito News* **24:** 39-42.

Rupp, H. R. 1977. *Culiseta melanura* larvae in tires -- a recurring phenomenon? *Mosquito News* **37:** 772-773.

Sardelis, M. R., Dohm, D. J., Pagac, B., Andre, R. G., and Turell, M. J. 2002a. Experimental transmission of eastern equine encephalitis virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *Journal of Medical Entomology* **39:** 480-484.

Sardelis, M. R. and Turell, M. J. 2001. *Ochlerotatus j. japonicus* in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. *Journal of the American Mosquito Control Association* **17:** 137-141.

Sardelis, M. R., Turell, M. J., and Andre, R. G. 2002b. Laboratory transmission of La Crosse virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *Journal of Medical Entomology* **39:** 635-639.

Savage, H. M., Ezike, V. I., Nwankwo, A. C. N., Spiegel, R., and Miller, B. R. 1992. First record of breeding populations of *Aedes albopictus* in continental Africa: implications for arboviral transmission. *Journal of the American Mosquito Control Association* **8:** 101-103.

Savignac, R., Back, C., and Bourassa, J. 2002. Biological notes on *Ochlerotatus japonicus* & other mosquito species new to Quebec. *Abstracts: 68th Annual Meeting of the American Mosquito Control Association and the West Central Mosquito & Vector Control Association* 21-22.

Simmons, J. S., St. John, J. H., and Reynolds, F. H. K. 1930. Transmission of dengue fever by *Aedes albopictus* Skuse. *Philippine Journal of Science* **41:** 215-229.

Sprenger, D. and Wuithiranyagool, T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *Journal of the American Mosquito Control Association* **2:** 217-218.

Suleman, M., Arshad, M., and Khan, K. 1996. Yellow fever mosquito (Diptera: Culicidae) introduced into Landi Kotal, Pakistan, by tire importation. *Journal of Medical Entomology* **33:** 689-693.

Sutherst, R. W. 2004. Global change and human vulnerability of vector-borne diseases. *Clinical Microbiology Reviews* **17:** 136-173.

Thomson, A. L. 2004. Are sewer systems nurseries for blood-thirsty insects: a survey of Winnipeg catch basins for immature mosquitoes? Honours Thesis, University of Winnipeg.

Trexler, J. D., Apperson, C. S., Zurek, L., Gemeno, C., Schal, C., Kaufman, M., Walker, E., Watson, D. W., and Wallace, L. 2003. Role of bacteria in mediating the oviposition responses of *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* **40:** 841-848.

Turell, M. J., Dohm, D. J., Sardelis, M. R., O'Guinn, M. L., Andreadis, T. G., and Blow, J. A. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *Journal of Medical Entomology* **42:** 57-62.

Turell, M. J., O'Guinn, M. L., Dohm, D. J., and Jones, J. W. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology* **38:** 130-134.

Turell, M. J., Sardelis, M. L., O'Guinn, M. L., and Dohm, D. J. 2002. Potential vectors of West Nile virus in North America. *Current Topics in Microbiology and Immunology* **267:** 241-252.

Wallis, R. C. 1954. A study of oviposition activity of mosquitoes. *American Journal of Hygiene* **60:** 135-168.

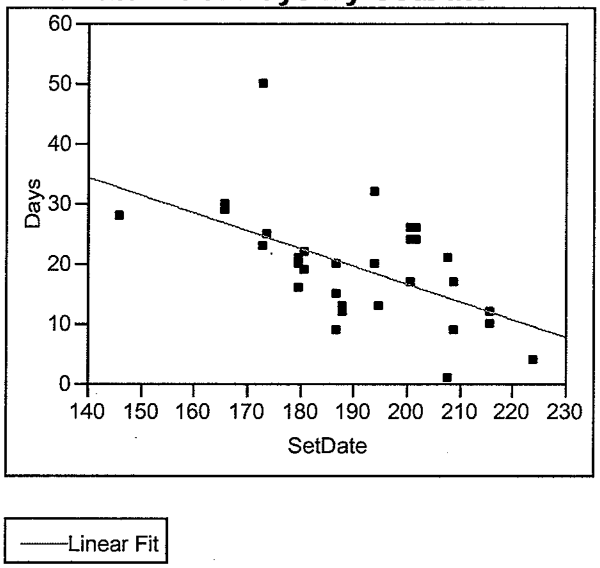
Weber, R. G. and Tipping, C. 1993. Preoviposition drinking by *Culex restuans* (Diptera: Culicidae). *Journal of Insect Behavior* **6:** 343-349.

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

Wood, D. M., Dang, P. T., and Ellis, R. A. 1979. The insects and arachnids of Canada. Part 6. The mosquitoes of Canada (Diptera: Culicidae). Publication 1686, Agriculture Canada, Biosystematics Research Institute.

Appendix 1

Bivariate Fit of Days By SetDate



Linear Fit

Days = 75.92 - 0.30(SetDate)

Summary of Fit

RSquare	0.296947
RSquare Adj	0.273512
Root Mean Square Error	7.933894
Mean of Response	19.5
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	797.5997	797.600	12.6710
Error	30	1888.4003	62.947	Prob > F
C. Total	31	2686.0000		0.0013

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	75.92015	15.91189	4.77	<.0001
SetDate	-0.29612	0.083188	-3.56	0.0013