INTRODUCTION

Interest in waste tires as oviposition sites for mosquitoes in North America increased when Sprenger and Wuihiranyagool (1986) discovered an established population of the exotic Asian Tiger Mosquito, Aedes albopictus (Skuse), at a roadside tire dump in Houston, Texas, in 1985. Tires have frequently been implicated in the spread and introduction of mosquitoes in many parts of the world (Hedberg et al. 1985, McLintock and Iversen 1975, Mitchell et al. 1998, Paulson and Grimstad 1989, Romi et al. 1997, Reiter and Darsie 1984, Savage et al. 1992, Suleman et al. 1996, Lounibos 2002). There are recycled and stockpiled tires in modest to large accumulations in Manitoba, Canada, and so have the potential to contribute to mosquito populations and to affect the epidemiology of mosquito-borne pathogens such as West Nile Virus. Despite an active waste tire recycling program in Manitoba, not all used tires can be processed and recycled rapidly enough to eliminate the need for stockpiles. As a result, used tires, including marketable stock and those tires destined for recycling, frequently are stored outdoors in semi-permanent or permanent piles that can serve as larval habitats for mosquitoes. Rapid infestation of new stock can also occur as it arrives when adult mosquitoes are present in the area (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 both nuisance and vector mosquitoes.

In Manitoba, there were 306 waste transfer stations in 2003 where tire piles may have occurred throughout the year (pers. comm. Wendy Ralley at Manitoba Water Stewardship Water Quality Management Section). In addition, there were 868 tire dealerships that may have kept used scrap or marketable tire stock outdoors (Manitoba Tire Stewardship Board, 202-1100 Concordia Av. Winnipeg, Manitoba R2K 4B8). Under the Waste Reduction and Prevention Act (WRAP 1995), the Manitoba Tire Stewardship Board (TSB) was created in 1995 to manage recycling of tires. From 1995 to 2005, tire-recycling corporations in Manitoba collectively processed more than 9.5 million tires (Manitoba Tire Stewardship Board, 202-1100 Concordia Av. Winnipeg, Manitoba R2K 4B8). Even with extensive recycling, used tires accumulate in the environment for various periods of time. Generally, it has not been possible for recyclers to remove all tires from the environment quickly enough to preclude the emergence of mosquitoes. In 2002, the TSB provided research funds to assess the risk that tires and their associated mosquitoes could pose to public health in Manitoba and to investigate ways in which such risk could be mitigated. A survey of used tires was carried out to investigate the potential relationship between mosquitoes and tires in Manitoba. The objectives of the survey were: 1) to determine which species of mosquitoes are present in tires in Manitoba, 2) to determine if there is measurable, geographical, and seasonal variation within Manitoba in mosquito prevalence or abundance in tires, and 3) to correlate mosquito numbers with tire attributes such as size, orientation, and water...
volume to identify potential management solutions related to tire arrangement.

MATERIALS AND METHODS

Terminology
To examine the role of tires in providing suitable habitat for immature mosquitoes, a few terms need to be defined. Intensity is the average number of mosquitoes per tire that contained mosquitoes. Because the distribution of mosquito larvae across tires approximated a negative binomial, intensity provides more information than a simple average. Prevalence is the percentage of tires in which immature mosquitoes were collected. Tires at most of the collection sites occurred in piles from which tires were selected and assigned to either horizontal (tire on its side, parallel to the ground) or vertical (tire on its tread, perpendicular to the ground) categories.

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 TSB provided the names and addresses of the 868 registered tire dealers in Manitoba. Manitoba was stratified into five regions: Northern, Eastern, Western, Central, and Winnipeg (Figure 1). The Northern region was not included in the survey because the distribution and abundance of Culex species is lower in the north than the south (Wood et al. 1979, Darsie and Ward 2005) and the primary objective of the study was to assess potential impact of tires on the distribution and abundance of mosquito species relevant to arbovirus transmission. Potential sites were assigned numbers, and five waste management grounds and five tire dealerships were randomly selected from three of the remaining four regions using a random number generator. There were no waste management grounds in Winnipeg. Ten tire dealers were selected within the city limits.

Proprietors at each of the selected sites were contacted to determine the number of tires and the length of time the tires might spend outside at each site. Sites with fewer than the desired sample size of thirty tires, or where tires were exposed less than two weeks, were discarded, and new sites were randomly selected. Two weeks was chosen as the minimum exposure period to approximate estimated development time for immature Culex spp. in Manitoba (Buth et al. 1990).

Reliable Tire Corporation and the Tire Recycling Corporation (tire recycling companies) were considered waste disposal grounds in the central region; tires from a wide region around the central site accumulated from a large area in the central region, in much the same way as for a waste transfer station. Each site was visited at least once; Sturgeon Tire and the Letellier Waste Management ground were sampled twice, while Denray Tire was sampled three times.

Sampling
Upon arriving at a site, a straight stick was thrown randomly to determine the starting point of a transect line, 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 wet 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, where there were hazardous conditions (e.g., broken glass, sharp metal). No tire was sampled more than once. Sampling involved recording the orientation of the tire in one of two categories: horizontal or vertical. Tires were also assigned to one of four sizes. The four categories in increasing size were car, truck, semi-trailer (hereafter referred to as semi), and tractor tires with outside tire diameters of approximately 64, 76, 102, and 178 cm, respectively. The entire contents of each tire were removed using a small dipper and passed through a 212 μm sieve into a graduated pail, where volume was recorded (to the nearest 0.5 liter). This was repeated until thirty wet tires were sampled. The percentage of wet tires was not recorded. In June and July, all collected egg rafts and immature mosquitoes were brought back to the lab and stored at room temperature (21°C) until they could be counted, approximately one week or less. Egg rafts were allowed to hatch in small plastic containers. Larvae in the 1st instar were provided a bovine liver powder solution and kept at room temperature until they developed to the 4th instar and then identified using the keys in Wood et al. (1979). Successfully hatched larvae were counted and added to the total from each tire. If a larva died in the 1st instar, the key in Dodge (1966) was used to make the identification. In August, due to observed larval mortality and decomposition of accumulated organic matter in samples prior to processing, collections were stored at 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 (16.5 cm x 16.5 cm x 16.5 cm), and identified using the key in Wood et al. (1979).

Individuals were counted and recorded, keeping note of the number of each species. Statistical analyses included logistic regression for categorical dependent variables and standard regression/ANOVA for continuous dependent variables. Voucher specimens were deposited in the J. B. Wallis Museum, Department of Entomology, University of Manitoba.

RESULTS
A total of 1,142 tires was sampled at different sites during June, July, and August of 2003 (Figure 1). For the following results, variation is reported as standard error. 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 the Winnipeg region (364), western region (230), and eastern region (165). Four hundred and ten car
Figure 1. A map of southern Manitoba to indicate sites within the three survey regions (E, Eastern region, C, Central Region, W, Western Region), plus the greater Winnipeg area, visited during the survey. Square, tire dealership; circle, waste transfer station.
tires were sampled, followed by semi tires (300), tractor tires (245), and truck tires (187). Tires that had immature mosquitoes contained a mean of 100.3 ± 7.4 (mean intensity), and over ¼ of the tires contained larvae (26.8%). *Culex restuans* (Theobald) accounted for 95.3% of the total larvae found. The remaining 4.7% was comprised of *Culex tarsalis* Coquillett (2.5%), *Culiseta inornata* (Williston) (1.8%), and *Ochlerotatus triseriatus* (Say) (0.4%). *Culex restuans* represented at least 95% of the total larvae and pupae collected over the entire summer, while *Cx. tarsalis* nearly doubled its proportion from July (1.8%) to August (3.4%). *Ochlerotatus triseriatus* was not found in June and was never abundant, but peaked in August. The prevalence of *Cs. inornata* peaked in July at 3.1% (Table 1).

Factors affecting prevalence

Mosquito prevalence increased significantly as summer progressed ($\chi^2 = 3.98$, df = 1, p = 0.046). Prevalence increased from June (11.7%) to July (28.7%) to August (36.1%). Sampling region significantly affected prevalence, ($\chi^2 = 28.8$, df = 3, p < 0.0001) and the interaction between sampling region and sampling date was significant ($\chi^2 = 9.64$, df = 3, p = 0.022). Prevalence was much higher in the east than in the other regions, but prevalence in all three regions increased as the summer progressed.

Mosquito prevalence was significantly affected by tire size (Likelihood Ratio 68.21, df = 3, p < 0.0001). Prevalence was similar for three of the tire classes increasing in size, 18.8% of car tires contained immature mosquitoes, 19.8% of truck, 26.7% of semi tires, whereas 47.8% of tractor tires contained immatures (Table 2). Using Tukey-Kramer’s HSD, only tractor tires were significantly different from the other tire types (Table 2) (p < 0.05). The interaction of tire type and region was also significant ($\chi^2 = 20.62$, df = 9, p = 0.014). The interaction of tire size and sampling date was also significant (F = 2.64, df = 3, p = 0.048). The prevalence of immature mosquitoes collected from semi tires decreased across the summer, while the prevalence in the other three tire types increased.

Tire orientation also significantly affected mosquito prevalence (Likelihood Ratio 20.934, df = 1, 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 collected 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 = 1.633, df = 3, p = 0.18). The interaction between sampling date and orientation was not significant (F = 1.06, df = 1, p = 0.303).

Factors affecting intensity

Intensity (the average number of mosquitoes per tire containing mosquitoes) was not significantly affected by date (t ratio = 1.85 df = 1, p = 0.065). Sampling region significantly affected intensity (Table 3), (F = 6.4, df = 3, p = 0.0003) and the interaction between sampling region and sampling date was not significant (F = 0.733, df = 3, p = 0.532). That is, the numbers of mosquitoes increased similarly in all three regions as the summer progressed.

Mosquito intensity was significantly affected by tire size (F = 47.14, df = 3, p < 0.0001). Intensity increased significantly in tractor tires, but not between the other three size classes. On average, car tires contained 12.1 immature mosquitoes, with 16.7, 21.5, and 71.1 mosquitoes per tire

<table>
<thead>
<tr>
<th>Month</th>
<th>Abundance (% of monthly total)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>3,332 (97)</td>
<td>12.2</td>
</tr>
<tr>
<td>July</td>
<td>14,551 (95)</td>
<td>28.2</td>
</tr>
<tr>
<td>August</td>
<td>13,032 (95)</td>
<td>35.0</td>
</tr>
<tr>
<td>June</td>
<td>68 (2)</td>
<td>1.1</td>
</tr>
<tr>
<td>July</td>
<td>278 (2)</td>
<td>1.5</td>
</tr>
<tr>
<td>August</td>
<td>487 (4)</td>
<td>5.9</td>
</tr>
<tr>
<td>June</td>
<td>21 (1)</td>
<td>0.5</td>
</tr>
<tr>
<td>July</td>
<td>475 (3)</td>
<td>1.6</td>
</tr>
<tr>
<td>August</td>
<td>82 (1)</td>
<td>3.5</td>
</tr>
<tr>
<td>June</td>
<td>0 (0)</td>
<td>0.0</td>
</tr>
<tr>
<td>July</td>
<td>27 (0)</td>
<td>0.3</td>
</tr>
<tr>
<td>August</td>
<td>121 (1)</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 1. Total abundance (numbers of larvae and pupae) and prevalence (percentage of tires containing each species in that month) of the four species of mosquitoes found in tires in southern Manitoba in the summer of 2003.
Table 2. Mean intensity (± 95% CI) and prevalence found in four different sizes of tires in Manitoba in the summer of 2003 (values followed by different letters within columns differ significantly at the p = 0.05 level using Tukey-Kramer’s HSD).

<table>
<thead>
<tr>
<th>Tire size</th>
<th>Mean</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Car</td>
<td>12.1 ± 1.8a</td>
<td>18.78a</td>
</tr>
<tr>
<td>Truck</td>
<td>16.7 ± 4.0a</td>
<td>19.79a</td>
</tr>
<tr>
<td>Semi</td>
<td>21.45 ± 3.2a</td>
<td>26.67a</td>
</tr>
<tr>
<td>Tractor</td>
<td>71.1 ± 9.6b</td>
<td>47.76b</td>
</tr>
</tbody>
</table>

in truck, semi, and tractor tires, respectively. Using Tukey-Kramer’s HSD, only tractor tires had a significantly different mean intensity than the other tire types (p < 0.05). The interaction of tire type and region was also significant (F = 6.94, df = 9, p < 0.0001). Tractor tires in the Eastern region had a large increase in intensity. The intensity of mosquitoes was similar in the other tire sizes in the east. The interaction of tire size and sampling date was also significant (F = 5.84, df = 3, p = 0.0006). The increase in intensity of immature mosquitoes in tractor tires later in the season was greater than the increase in the other three sizes.

Tire orientation did not significantly affect mosquito intensity (F = 2.24, df = 1, p = 0.135), nor was the interaction between tire size and orientation significant (F = 0.22, df = 3, p = 0.88). A mean of 18.2 ± 3.3 immature mosquitoes was found in horizontally stacked tires, whereas 33.0 ± 3.4 were found in vertically stacked tires. The interaction between region and orientation was not statistically significant (F = 1.36, df = 3, p = 0.25). The interaction between sampling date and orientation was significant (F = 4.28, df = 1, p = 0.0387).

Table 3. Mean intensity (± 95% CI) and prevalence found in tires in four different regions in Manitoba in the summer of 2003 (values followed by different letters within columns differ significantly at the p = 0.05 level using Tukey-Kramer’s HSD).

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>13.8 ± 3.1a</td>
<td>16.09a</td>
</tr>
<tr>
<td>Central</td>
<td>27.03 ± 3.8a</td>
<td>27.67a</td>
</tr>
<tr>
<td>Winnipeg</td>
<td>25.2 ± 4.3a</td>
<td>25.27a</td>
</tr>
<tr>
<td>East</td>
<td>56.4 ± 10.4b</td>
<td>46.06b</td>
</tr>
</tbody>
</table>

The interactions of tire size, orientation, and date were significant (F = 4.46, df = 3, p = 0.0040), as well as the interactions of region, orientation, and date (F = 2.86, df = 3, p = 0.0361). Whereas overall, the prevalence increased in the three regions, if the orientation is considered, horizontal tires contained more mosquitoes early in the season, while vertical tires contained more mosquitoes later in the season.

DISCUSSION

Based on the literature, we did not expect the mosquito fauna of tires in Manitoba to include species other than those previously found in tires elsewhere in North America. Culex restuans was, by far, the dominant species. Culex tarsalis, Cs. inornata, and Oc. triseriatus were present, but in low numbers. Culex restuans appears to be found in more habitats than other mosquito species, 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). 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.

Nearly 27% of the tires surveyed contained mosquito larvae, similar to Baumgartner (1988) who found approximately 30% of tires surveyed in Illinois contained mosquito larvae. He also found 82% of the larvae to be Cx. restuans. Our result of 95% Cx. restuans larvae is higher, but the general dominance by collections of Cx. restuans is similar. Berry and Craig (1984) surveyed tire stations in Indiana and found that Cx. restuans accounted for 90% of the total larvae collected, also in close agreement with our findings.

Beier et al. (1983a, b) found a much higher prevalence of mosquitoes in tires in Indiana, reporting 60 to 100% of the tires they examined contained immature mosquitoes. In addition, they found Oc. atropalpus (Coquillett) to be the most common species and Oc. triseriatus was more common than Cx. restuans in tires. This is very different from what we found in Manitoba, which is at the northern limit of the range of Oc. triseriatus, where it has never been found in large numbers. Ochlerotatus atropalpus is not known to occur in Manitoba. In addition to biogeographic considerations, their selection of tires with at least 800 ml of water (Beier et al. 1983a, b) may have affected the prevalence they observed as some larvae may have escaped detection in tires with low levels of water.

The interaction of region and date in our survey was not significant. Mosquito intensity and prevalence in tires in all four regions increased similarly as the summer progressed. The number of tires in a site would potentially influence the number of mosquitoes present, depending on the amount of other habitat. However, no estimate of total numbers of tires at a site was made. The increase in intensity and prevalence with date is expected due to the increase over time of mosquito populations during most Manitoba
Tire size was a significant factor in determining intensity and prevalence. Tractor tires (the largest) more often contained mosquitoes and had more immature mosquitoes than the three other smaller tire sizes. This may have been a function of the surface area and volume of water held in larger tires. Further research is required to determine whether gravid mosquitoes preferentially lay eggs in larger tires, or whether the numbers of mosquitoes are similar when these factors are standardized for surface area or volume of water. The interaction between region and tire size was also significant for both prevalence and intensity. The number of mosquitoes in tractor tires was much higher in the Eastern region. In fact, tractor tires in the eastern region of Manitoba, in general, had significantly greater numbers and prevalence of mosquitoes than the other three tire sizes. 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 intensity 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.

The prevalence of immature mosquitoes was significantly affected by the proportion of tires in each orientation and the time of the season. 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. None of the other interactions between factors affected either prevalence or intensity except date and orientation. Horizontal tires contained more mosquitoes on average earlier in the season than vertical. Vertical tires had a higher average prevalence 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. We examined only tires which contained water, so the prevalence of water in tires cannot be calculated. Vertically stacked tires have deeper pools of water than horizontally stacked tires. Therefore, these pools should persist longer, which increases the likelihood of discovery by gravid mosquitoes and the survival of the larvae until they are sampled.

If the intensity, or the average number of immature mosquitoes per positive tire, is 100, but the average across all tires is 28, clearly 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 mainly on tires that are heavily infested. If it could be determined which tires are more likely to become heavily infested based on position, orientation, or environmental variables, control efforts would become more time, cost, and labor efficient.

There is a high degree of variation in the microhabitat characteristics within and even among tire yards. Work needs to be done to determine what makes tires attractive, neutral, or repellent to female mosquitoes and how large an impact tires have on naturally occurring mosquito populations under different conditions of natural larval habitat ecology. Also, we need to know what may influence the importance 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 oviposition. Further research into these subjects should shed more light on the complex biology of this system.

Acknowledgments

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