A Spatial Analysis Evaluation of DeltaGard®20EW Operational Efficacy in Winnipeg, Manitoba for Adult Mosquito Control

Prepared By:
Jennifer Emma Pawluk

Supervisor:
Dr. R. Anderson

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University of Winnipeg
Graduate Department of Bioscience, Technology, and Public Policy
Department of Biology
Winnipeg, Manitoba, Canada

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List of Terms

AFA – Adulticiding Factor Analysis
AMCA – American Mosquito Control Association
BMP – Best Methods Practice
Bti – *Bacillus thuringiensis israelensis*
CDC – Center for Disease Control
CEC – Clean Environment Commission
CNS – Central Nervous System
CoW – City of Winnipeg
EPA – Environmental Protection Agency
ICB – Insect Control Branch
IMA – Insect Management Area
IPM – Integrated Pest Management
NJLT – New Jersey Light Trap
OC – Organochlorines
OP – Organophosphates
PBO – Piperonyl Butoxide
PCA – Principal Component Analysis
PMRA – Pesticide Management Regulatory Agency
PNS – Peripheral Nervous System
SP – Synthetic Pyrethroids
ULV – Ultra-low Volume
WEE – Western Equine Encephalitis
WEEv – Western Equine Encephalitis virus
WNd – West Nile disease
WNv – West Nile virus

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1.0 Abstract

Background: Mosquito abatement includes the management and surveillance of nuisance and potential vector mosquitoes. The major nuisance mosquito in Winnipeg, Manitoba is *Ae. vexans*, a floodwater mosquito. The City of Winnipeg Insect Control Branch uses surveillance, source elimination, larvicide, and adulticide in their mosquito abatement program. Adulticide application is the last resort when the other methods are not sufficient. This study tested DeltaGard®20EW adulticide efficacy operationally on wild mosquitoes in Winnipeg, Manitoba as a replacement for the previously used Malathion 95ULV® while considering the effect of landscape features.

Methods: New Jersey Light and Centre for Disease Control mosquito trap data from July 2010 and July 2020 were used to statistically detect changes in adult mosquito activity before and after treatment with Malathion 95®ULV (2010) and DeltaGard®20EW (2020). Landscape features surrounding traps that were frequently mosquito hot spots and traps that were never hot spots were compared by applying spatial analysis tools. Kriging analysis was performed to estimate changes in mosquito activity citywide. Wing lengths were used as a proxy for adult mosquito body size to determine if body size is positively correlated with mosquito longevity.

Results: No significant difference was detected when comparing post-spray mosquito trap counts in treated and untreated (experimental control) locations in July 2010 or July 2020. When daily changes in mosquito activity were analyzed in the treatment group, a significant decrease in mosquito activity was detected in the group treated with Malathion 95ULV® the day after treatment with the effect lasting for two days. No significant daily changes in mosquito activity were detected after DeltaGard®20EW application. There were no significant differences between mosquito activity hot spot locations and non-hot spot locations when tree density, proximity to rivers, proximity to parks/open spaces, or parks/opem spaces density were analyzed spatially. However, hot spots were found to have more trees in a 50m radius and to be closer to rivers. A significant positive correlation between longevity and adult mosquito body size (*Ae. vexans*) was detected.

Conclusions: The lack of significance when comparing traps in areas treated with DeltaGard®20EW and untreated areas indicates that changes may be due to natural background fluctuations in mosquito activity and population. Significant daily decreases in mosquito activity in the treatment groups were detected the day following Malathion 95ULV® treatment. Thelack of significance in the DeltaGard®20EW trials may be due to issues with modelling like a lack of untreated trap locations for comparison, a starting population that was too low to detect significant changes, a lack of specific knowledge about the cumulative egg bank and *Ae. vexans* biology, too few trap locations, traps being placed strategically instead of randomly, the challenge of measuring background mosquito activity and population dynamics, and a lack of meteorological and landscape data specific to trap locations. Measuring adulticide efficacy in wild mosquitoes and creating models to analyze changes in their activity is challenging. A significant positive correlation between longevity and adult mosquito body size was detected for *Ae. vexans* mosquitoes, although further research should track specific cohorts of mosquitoes over time.

2.0 Introduction

Mosquito abatement is an important approach to the management of nuisance and potentially vector mosquitoes which can spread pathogens. During non-freezing seasons in Manitoba (and elsewhere), nuisance mosquitoes such as *Aedes vexans* (Meigen) hinder recreational activities and enjoyment of humans by biting to take blood meals (Wood, Dang and Ellis 1979). They also harass pets and livestock with deleterious effects. *Ae. vexans* is the most abundant nuisance species in Manitoba. *Culex tarsalis* Coquillett and *Cx. restuans* Theobald have been important vectors in outbreaks of West Nile disease (WNd) in Manitoba since 2003 (123 human cases that year) and Western Equine Encephalitis (WEE) since 1975 (14 human (no deaths) and 261 equine cases that year) (Tulchinsky 1976; Drebot *et al*. 2003). Reduction of nuisance mosquitoes and the control and/or prevention of West Nile virus (WNv) and Western Equine Encephalitis virus (WEEv) transmission to people in Manitoba has been accomplished by the adoption and use of an Integrated Pest Management (IPM) strategy (City of Winnipeg 2022; Manitoba Health, Seniors and Active Living 2022).

The City of Winnipeg (CoW) is the largest metropolitan area in Manitoba and has a well-staffed and organized Insect Control Branch (ICB) that uses several mosquito abatement methods as part of their IPM strategy. The first line of defense against adult mosquito emergence is source reduction by way of habitat management. This may involve drainage, removal, or alteration (where possible) of aquatic habitats to make them unsuitable for mosquitoes. Larviciding is another approach that involves the application of appropriate insecticides to larval habitats which can't be eliminated to target mosquitoes in their aquatic, larval form. Source reduction and larviciding may fail to adequately reduce mosquito populations. For example, this may occur when short-term weather events conducive to increased mosquito abundance and activity occur. High temperatures can speed up larval development such that the window of opportunity for larvicide application is too short in comparison to operational capability. Significant rainfall immediately after an application of *Bacillus thuringiensis israelensis* (*Bti*) may dilute this larvicide sufficiently to require re-application. In these instances, adulticide application may become necessary. For adulticide application to occur, the threshold index set by the CoW-ICB and Manitoba's Clean Environment Commission (CEC) must be reached and a decision-making algorithm called the Adulticiding Factor Analysis (AFA) is used to make decisions about when and where to apply adulticide. The threshold and the AFA will be discussed in more detail in section 3.4 of the subsequent literature review.

In cases of dangerous infection levels with WNv and numbers of vector mosquitoes, a health order from the Manitoba government is required to adulticide to reduce pathogen transmission (Manitoba Health, Seniors and Active Living 2022). Manitoba Health uses several epidemiological criteria, which include information on mosquito activity levels, to help decide when to issue this health order. Once these criteria are met and the health order has been received, the CoW-ICB conducts an adult mosquito control program for WNv control. Buffer zones are not in effect during WNv-prevention adulticide treatments (CoW 2022). Buffer zones are areas of the CoW that are not treated with adulticide applied for nuisance mosquito control because residents have registered to exempt a 90m radius around their primary residence during an adulticiding treatment (CoW 2022).

Malathion (an organophosphate adulticide applied with ultra-low volume (ULV) truck-mounted technology) was used by CoW-ICB as the adulticide of choice from 1983 to 2015 (CoW 2022). Since this time, deltamethrin (a synthetic pyrethroid applied by ULV truck-mounted technology) has been adopted for use by the CoW-ICB (CoW 2022). ULV applications use the minimum volume of active ingredient (malathion or deltamethrin) that is effective neat (without dilution) and disperse adulticide by producing droplets of active ingredient that kill adult mosquitoes on contact (Rey *et al*. 2012). It is important to note that the change to deltamethrin (formulated as DeltaGard®20EW) in 2015 was due to the unavailability of malathion (CoW 2022). Deltamethrin has been assessed as an active ingredient of "low risk" to public health by the Pest Management Regulatory Agency (PMRA) of Health Canada (Health Canada 2018). Toxicological aspects of both malathion and deltamethrin will be discussed in section 3.5 of the subsequent literature review.

The CoW-ICB tested DeltaGard®20EW in preliminary trials with caged live mosquitoes in 2015 (Nawolsky and Wade 2016). These studies yielded promising results: applications of 1g of deltamethrin per hectare led to 96% mortality in caged mosquitoes located up to 90 meters away from the point of the insecticide release (Nawolsky and Wade 2016). This result was corrected for the natural mortality of the untreated caged mosquitoes. Mortality of caged mosquitoes that were not treated with deltamethrin and acted as experimental controls in these trials was less than 3.5% (Nawolsky and Wade 2016). However, Winnipeg's cityscape is not homogenous and deltamethrin application must be tested operationally in a wider range of conditions, including on free-flying mosquitoes, than that represented by the initial trials.

The CoW accounts for meteorological conditions, street length, and optimal speed when applying adulticide from truck-mounted equipment by following guidelines written on the pesticide use label for DeltaGard®20EW and the recommendations of the American Mosquito Control Association (AMCA) Best Methods Practice (BMP) for Mosquito Abatement (CoW 2022). The CoW-ICB also follows the recommendations of the PMRA when applying pesticides (CoW 2022). Other aspects of the cityscape, such as differences in tree density and proximity to parks/open spaces as well as distance to rivers (which are no-spray zones due to toxicity of synthetic pyrethroids to aquatic organisms) must be considered when determining efficacy. Another important spatial consideration is the placement and number of mosquito traps and their ability to effectively monitor adult mosquito activity. The number and location of mosquito trap sites operated by the CoW-ICB is restricted due to limited resources to operate the traps and because permission is required to set traps on private property. Traps are located based on convenience. These spatial features may influence the ability to evaluate deltamethrin application and measures of efficacy and will be discussed further in section 3.6 of the following literature review.

Much of the research performed to determine DeltaGard®20EW efficacy is based on caged mosquito trials and does not take real-life operational control and background mosquito population and activity fluctuations into consideration (Brill and Morrison 2013; Nawolsky and Wade 2016; Dennet *et al.* 2017). Data are not available for the efficacy of DeltaGard®20EW under operational mosquito control conditions in Winnipeg. Comparisons between trap count data before and after treatment with adulticide will determine the efficacy of DeltaGard®20EW as a replacement for Malathion 95ULV® in Winnipeg. Efficacy means a statistically significant decrease in mosquito activity after adulticide treatment.

Finally, adult mosquito body size as indexed by wing length should be considered. Adult mosquito body size is highly correlated with wing length (Katz *et al*. 2020). Body size is predictive of mosquito longevity (larger mosquitoes are thought to live longer than smaller mosquitoes) (Hawley 1985). If areas within the CoW mosquito abatement zone produce smaller mosquitoes, those locations may need to be prioritized less for management efforts than areas that produce larger mosquitoes because smaller mosquitoes should have shorter lives. Expected longevity of a cohort of mosquitoes is one criterion that should be considered in decision-making for adulticide treatments.

I hypothesize that in comparison to malathion, DeltaGard®20EW will be an effective adult mosquito control agent in Winnipeg when tested operationally. I predict the following:

a) That DeltaGard®20EW will cause a statistically significant decrease in adult mosquito activity in Winnipeg within the spatially complex operational context of the urban landscape.

b) That trap quantity and locations are sufficient to adequately measure mosquito activity in Winnipeg.

c) That wing length distributional data will provide insight into the temporal decline in adult mosquito activity (irrespective of adulticide treatment) and that it will be useful for the CoW-ICB's decision-making process.

3.0 Literature Review

3.1 The Importance of Mosquito Control

During Manitoba summers when adequate temperatures for development (8ºC or higher depending on mosquito species) coincide with rainfall events and/or snowmelt, the City of Winnipeg is faced with the emergence of adult biting mosquitoes. Adult female mosquitoes bite because they require blood meals for reproduction (Wood, Dang and Ellis 1979). The feeding activity of these mosquitoes is both bothersome and a potential route by which parasitic pathogens are introduced to and acquired from host blood tissue (Wood, Dang and Ellis 1979).

In Winnipeg, mosquitoes are primarily a nuisance issue. The biting activity of females hinders outdoor summer recreational activities, tourism, and quality of life. Once mosquito trap counts reach an average of 25 female mosquitoes citywide for 2 nights in a row, this equates to approximately 2 bites per minute (CoW 2022). This biting pressure is enough to trigger a citywide mosquito insecticide-application program to target adult mosquitoes (adulticide) and reduce the nuisance. Without the implementation of an adulticiding program, mosquito biting nuisance can become high enough to reduce enjoyment of the outdoors in Manitoba in the summer.

Although serious mosquito-borne pathogens (such as malaria parasites, Dengue viruses, and Yellow Fever viruses) do not exist in Manitoba, potential exposure to WEEv and WNv exists. Clinical WNv human cases have been low (fewer than 10 per year) for most of the years since its introduction to western Canada in 2002. However, outbreaks of WNd have led to as many as 123 (in 2003) and 582 (in 2007) cases in Manitoba (Government of Manitoba 2022). A WEE epidemic occurred in Manitoba in 1975 where there were 14 human cases with no deaths and 261 equine cases (Tulchinsky 1976). The risk to livestock and public health makes mosquito management necessary.

Due to nuisance factors and disease risk, it is crucial to monitor and control the adult female biting mosquito population in some parts of Manitoba. A comprehension of mosquito biology is necessary for understanding the CoW-ICB's approach to mosquito management and control.

3.2 General Mosquito Biology

The following section was primarily derived from *The Mosquitoes of Canada: Diptera, Culicidae* by Wood, Dang, and Ellis (1979). Any deviations from this source material have been cited.

Mosquito development includes four distinct stages: egg, larval, pupal and adult. Female adult mosquitoes oviposit (lay their eggs) in different locations depending on the species. *Anopheles*, *Culex*, and *Culiseta* genera lay their eggs directly in still bodies of water. These aquatic habitats can be permanent or temporary, natural or artificial and some examples include containers in back yards, puddles, ponds and even hoof marks left by livestock that fill with water (Day 2016). Different mosquito species thrive in different levels of water cleanliness/organic content, with some doing well in significantly nutrient-rich conditions (Kaur *et al*. 2003; Omolade and Adetutu 2018). Mosquito species belonging to these three genera have eggs that generally hatch within 48 hours of being laid directly on the water surface.

Mosquitoes within the genera *Aedes* and *Ochlerotatus* are called floodwater species because they lay their eggs on damp soil containing olfactory (i.e. moisture content and odours indicative of the presence of micro-organisms on which mosquito larvae feed) and visual cues used by female mosquitoes to select useful sites that are prone to inundation (Day 2016). Floodwater mosquito eggs then usually experience delayed hatching as soil dries out before the next inundation. Freshly laid eggs of this type generally do not hatch right away even if wet because embryonation takes some time to occur. Upon immersion in water, eggs display instalment hatching (i.e. not all the eggs in a single brood hatch with each subsequent rainfall event and some of the eggs enter diapause when development stops due to unfavorable conditions for a season). Floodwater mosquito eggs require at least a few centimeters of water at temperatures of 8ºC or higher to hatch and/or with an oxygen content of approximately 10% depending on the species (as discussed further in section 3.3). *Aedes* and *Ochlerotatus* mosquitoes usually overwinter in the egg stage.

The larvae of all the mentioned genera (floodwater species or otherwise) grow through 4 instars and feed actively. In general, mosquito larvae filter-feed or graze detritus, algae and other microorganisms from their aquatic habitat. Air exchange generally occurs through spiracles enclosed within a siphon. Larvae suspend themselves by way of surface tension at the surface of the water to breathe from the air above. *Coquillettidia perturbans* larvae and pupae are adapted to pierce the inner tissues of aquatic plants which allows them to live near the bottom of aquatic habitats and breathe through the aerenchyma (air-conducting tissue) of emergent plants. This means that they avoid predation and larvicide efforts at the surface of the water. Development speed through the 4 instars of the larval stage is dependent on temperature, available nutrition, and predation risks (Yeap *et al.* 2011; Barreaux *et al*. 2018; Guitiérrez *et al.* 2020). The larval stage may take anywhere from 4 days to 1 month. After the fourth larval instar, pupation occurs. The pupal stage lasts from 1.5-4 days and no feeding occurs in this stage. Instead, metamorphosis from larvae to adult occurs within the pupa.

Adult mosquitoes emerge from the pupal stage (eclosion), and, once hardening of the cuticle occurs, there is no further growth. *Anopheles*, *Culex*, and *Culiseta* genera usually overwinter as adults. Adult males swarm within a few days of eclosion and pass sperm to the females entering the swarm during flight. Females only require one copulation early in their lives because the sperm obtained from this event is stored and used to fertilize each of the egg batches she produces in her lifetime. For most species, a blood meal is then required for a female to obtain enough protein to fully develop the eggs for each batch. The dependence on blood as a reproductive resource is called anautogeny, whereas blood-free egg production is called autogeny.

After blood-meal digestion and maturation of an egg batch, the female mosquito finds a suitable oviposition site to lay her eggs. The suitability of oviposition sites varies depending on the species of mosquito as discussed above. Floodwater mosquitoes, such as the notorious biter, *Ae. vexans*, lay their eggs on damp soil that is prone to inundation (Shäfer and Lundström 2006). Floodwater mosquito biology and oviposition site selection will be discussed next in section 3.3. Non-floodwater mosquitoes, such as the *Culex* genera, lay their eggs directly into more permanent standing water.

3.3 Manitoba Floodwater Mosquito Biology

Manitoba's most abundant nuisance species (*Ae. vexans*) is called a floodwater mosquito because it lays its eggs in or on moist soil that is prone to inundation (being covered in water) (Wood, Dang and Ellis 1979). *Ae. vexans* account for over 90% of the species composition in most mosquito trap collections in Winnipeg over the mosquito season (Balcaen 2020). Although *Ae. vexans* is not the only floodwater mosquito species that exists in Manitoba, it appears in the highest numbers in traps and is the main target for adulticiding efforts in Winnipeg. The other major floodwater mosquito species, *Ochlerotatus sticticus* (Meigen), lays its eggs on moist soil on riverine banks which are flooded in the spring and lead to adult emergences at that time of year. These emergences are generally not large enough to meet the trap count threshold for adulticide application and are therefore not a major target for adult mosquito control. Consequently, *Oc. sticticus* will not be discussed further. The focus of this section will be *Ae. vexans* and the factors that contribute to their high relative abundance in mosquito trap collections in Winnipeg.

Ae. vexans is a floodwater mosquito species, characterized by oviposition in areas that are prone to inundation and subsequent drying events (Horsfall 1955). *Ae. vexans* lay their eggs in or on moist soil throughout the spring and summer seasons and into the fall in warm years (Horsfall 1955). Female gravid floodwater mosquitoes begin by using long-range visual and olfactory cues to search for a suitable oviposition location in their preferred habitat (Day 2016). Permissive conditions for mosquito search flights are warm, moist, humid, and calm conditions (Day 2016). Gravid mosquitoes then use site-specific olfactory cues (possibly organic enrichmentor previously oviposited eggs) and chemotactile receptors to assess the oviposition site quality (Day 2016).

Due to their preference for ephemeral egg-laying habitats, floodwater mosquitoes have several adaptations which make their eggs resistant to desiccation and capable of surviving until the return of suitable conditions. *Ae. vexans* eggs enter a necessary delay in hatching in the mosquito season to allow embryonation up to the stage where the immature mosquito can hatch when inundated (O'Malley 1990). The exact time for this process has not been determined, but the embryo requires 8-10 days to fully develop in the egg stage (Horsfall 1955). Eggs that do not hatch during the regular *Ae. vexans* emergence season in the summer and eggs that are laid too late in the season to undergo inundation enter diapause. Diapause is a state of dormancy that involves a significant decrease in metabolism and an incapability to hatch until after a suitable amount of time passes, even if the right conditions occur (dependent on species and not clearly determined) (Diniz *et al*. 2017). Diapause ensures the survival of the egg during and after dry periods (including overwintering) when larval emergence under inappropriate conditions would lead to death (Diniz *et al*. 2017). Eggs are believed to remain viable during diapause for approximately two years if the oviposition substrate stays moist, although exact survival times have not been determined (Gjullin *et al.* 1950). Another important attribute of floodwater mosquito eggs is installment hatching. This means that only a percentage of a given brood will hatch under appropriate conditions (Logan *et al*. 1991). Some hatch with the first flood, while others require more than one flooding event to hatch. The reason why only some eggs hatch with each inundation has not been clearly determined, but installment hatching is interpreted to be a kind of ecological bet-hedging which permits some embryos to be reserved for subsequent hatching opportunities, thus not depleting the stock if a flood event is insufficient to take the hatched larvae through to pupation (Schäfter and Lundström 2006). Unhatched, viable eggs contribute to an accumulation in the soil surrounding regular inundation sites. This accumulation of eggs will be referred to as the egg bank.

Hatching is stimulated by as little as a few centimeters of rain on dry soil. Water temperatures must be at least 8ºC with an oxygen content of approximately 10% (Trpiš and Horsfall 1969; Brust and Costello 1969; Wood, Dang and Ellis 1979). This phenomenon means that *Ae. vexans* larvae are usually the last to appear in late spring (Breeland *et al*. 1961). Because larval development is temperature-dependent and requires temperatures of 8ºC or greater, when water temperatures are colder than this, the development from larvae to adult takes too long and the water source may dry up or freeze first (Schäfter and Lundström 2006). A rise in temperature and bacterial growth after inundation depletes oxygen in the water which is the cue for hatching and signals the availability of larval food. In waters that are too oxygen-rich (over 10% oxygen content), larvae may not emerge due to the risk of predation from fish and other organisms which lurk in these waters (Rydzanicz *et al*. 2011).

Floodwater mosquito (*Ae. vexans*) population dynamics can be hard to predict due to instalment hatching, diapause, and reliance on rainfall events. Large emergences can occur with rainfall events if the egg bank has built up over several dry years. Generations of floodwater mosquitoes are usually discrete and do not overlap (Figure 1), but if rainfall events happen closer together, there is a possibility of overlap increasing the number of mosquitoes present from different generations at a given time. The primary goal of adulticide treatment is to reduce population peaks.

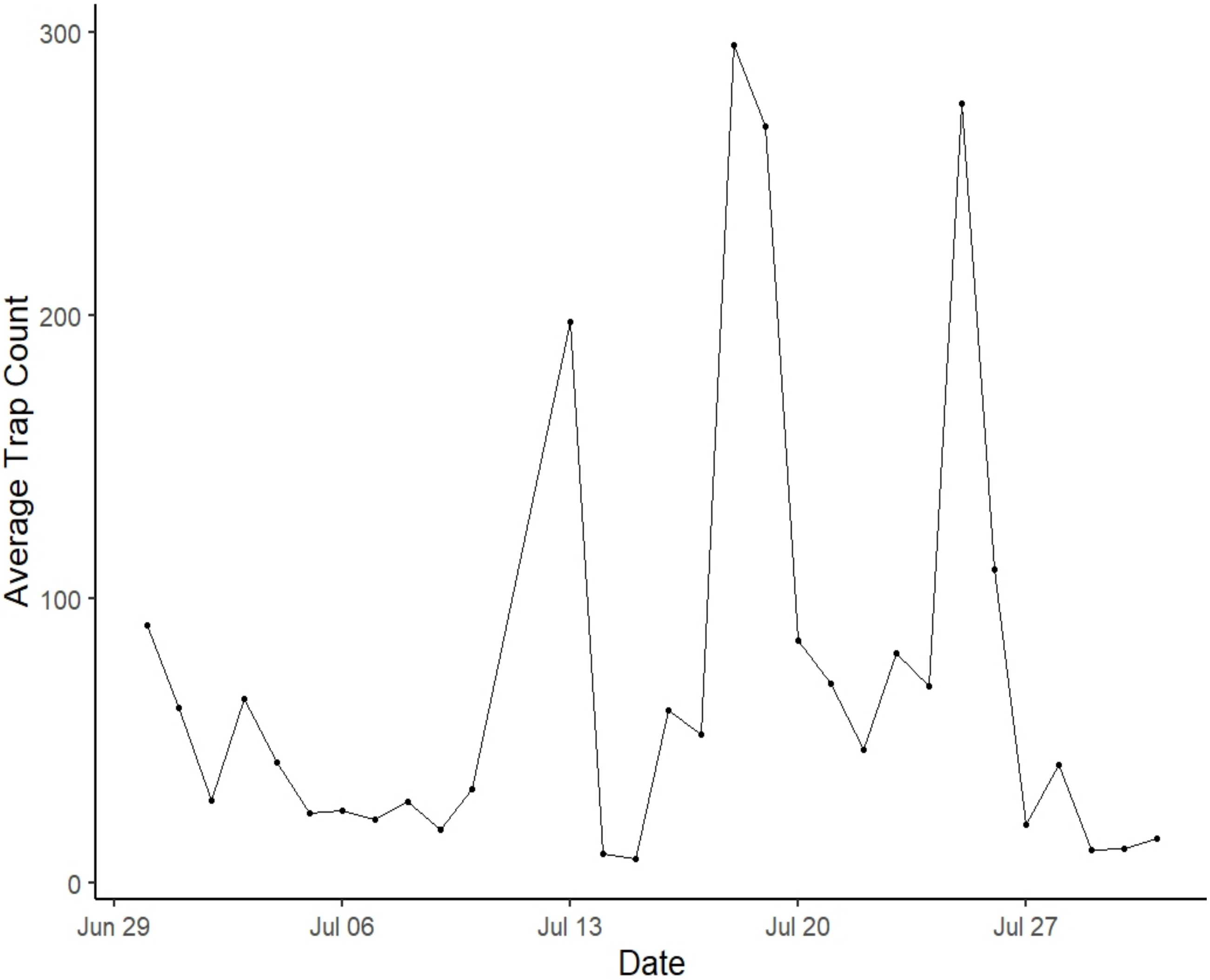


Figure 1. *Ae. vexans* population dynamics from 2020 data collected at trap locations that were not treated with adulticide that year. Population peaks are mostly discrete and not overlapping, although the period from mid to late July shows some cumulative population presence.

Adult female *Ae. vexans* are persistent biters that have been recorded taking 1-8 blood meals before death with 1-3 happening in the pre-oviposition period when females are fertilized but have not yet laid their eggs (Breeland and Pickard 1964). *Ae. vexans* can lay as many as 546 eggs in their lifetime, although this is a rare occurrence (Breeland and Pickard 1964). Hearle (1926) found that the Canadian form of *Ae. vexans* deposited an average of 108-182 eggs per brood. The number of broods oviposited by a single female is not known and depends on nutritional availability and lifespan of the mosquito. However, the species is multivoltine, meaning that they are capable of laying two or more broods per year (Horsfall 1955; Wood, Dang and Ellis 1979; Clements 1992). Blood feeding activity of females for egg development begins on the second day after adult emergence if insemination has occurred (Horsfall 1973). Due to their multivoltine nature, adult *Ae. vexans* appear in abundance several times in a mosquito season, usually during the summer months, and are a nuisance to humans and livestock (Crans 2004). *Ae.vexans* are the major nuisance species controlled for by the CoW-ICB.

3.4 Winnipeg's Integrated Pest Management Strategy

The information included in this section was obtained from the CoW-ICB website and their Adult Mosquito Control Policy when not otherwise cited (CoW 2022). The CoW-ICB IPM program covers mosquito abatement within Winnipeg (Figure 2) and a surrounding 12km buffer that includes parts of 13 surrounding rural municipalities that do not appear in the figure. Several approaches are used together in IPM because each control tool has some proportional contribution to the overall goal of adult mosquito reduction.

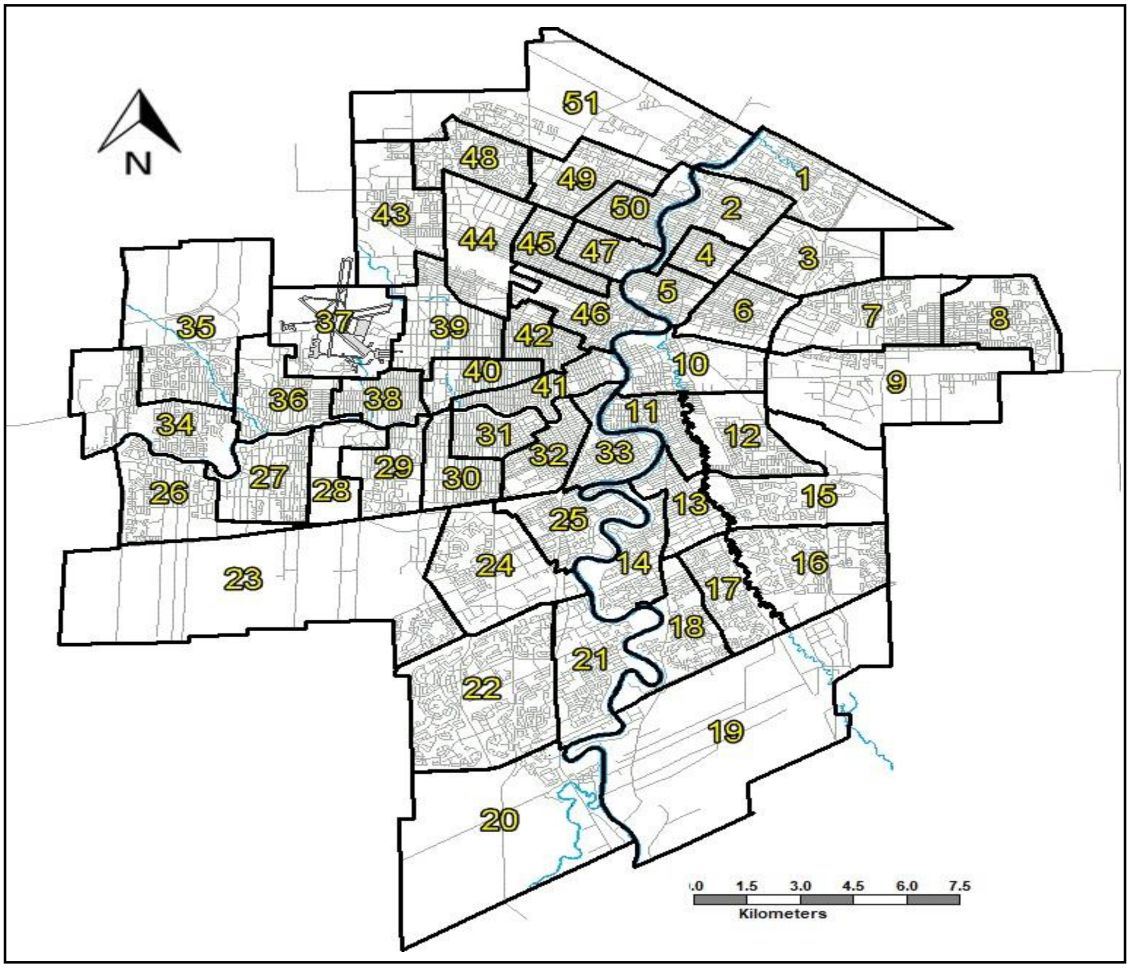


Figure 2. CoW-ICB mosquito abatement areas covering Winnipeg (shown in figure) and 13 surrounding rural municipalities (not shown in figure). An IPM program including surveillance, source reduction, larvicide, and adulticide efforts is carried out in these areas. Numbers indicate insect management area polygons.

Source Reduction & Public Education

Source reduction involves draining, dumping, or covering standing water from May until August to eliminate mosquito habitats wherever possible. The ICB engages in source reduction and encourages private land owners to also participate by eliminating standing water sources within private holdings. In cases where standing water cannot be eliminated or drained without causing other problems, standing water must be altered to make it unappealing to female mosquitoes for oviposition. The ICB addresses standing water on public and CoW-owned property wherever possible with approaches such as installing proper drainage systems in ditches and aerating ponds in golf courses. Public education encourages homeowners to dump containers and contribute to back-yard source reduction. In cases where standing water cannot be modified in these ways, it may be treated with larvicide to reduce mosquito populations.

Surveillance

The CoW-ICB uses surveillance to provide a rational basis for decisions about which mosquito control tools to employ. Surveillance for adult and larval mosquitoes is carried out by the CoW-ICB from May until September every year. For larval surveillance, samples of water are taken from known larval sites in the CoW and surrounding areas by use of a larval dipper. Larval dippers are white cups attached to long wooden handles and are used to scoop water samples that may contain larvae (Figure 3). Mosquito larvae are dark and contrast against the white cup such that the number of larvae can be estimated (based on multiple samples), and by extension, the relative density of mosquito larvae in such habitats is predicted. Areas that produce more larvae can be assigned priority for larviciding efforts.

Figure 3. A mosquito larval dipper used to take samples of water from known larval sites for surveillance (John W. Hock Company, Gainesville, FL 2022).

For adult mosquito surveillance, New Jersey Light Traps (NJLT) are used to trap adult mosquitoes in approximately 35-40 permanent locations inside the ICB's mosquito abatement area. Some locations are added or removed yearly. NJLTs attract mosquitoes to light emitted by incandescent lightbulbs in the traps. When the mosquitoes fly toward the light, a fan pulls the mosquitoes into a jar, trapping them. Another type of adult mosquito trap used is the Center for Disease Control (CDC) miniature light trap which uses dry ice or carbon dioxide canisters to release CO_2 as an olfactory attractant in addition to light. Lights can be removed from CDC traps to reduce capture of male mosquitoes and to collect essentially only females because CO_2 only acts as an olfactory attractant to females searching for a blood meal. Light attracts both males and females. These traps also contain a fan that pulls the mosquitoes into the trap jar. Mosquitoes collected in these traps are then counted and the major nuisance mosquito species are identified. This information contributes to the decision-making algorithm that determines whether adulticide will be applied (as described in the adulticiding part of this section). A study by Slaff *et al.* (1983) found that CDC traps baited with dry ice closely agreed with mosquito collections made with human bite counts where humans counted the number and species of mosquitoes biting them. Collections from the NJLTs were not found to accurately reflect nuisance mosquito populations (Slaff *et al.* 1983). Most mosquito abatement programs use NJLTs because they don't require CO_2 canisters or dry ice to be carried out to traps, saving money and resources. However, it is possible that they are not sufficient indicators of species diversity and nuisance mosquito activity.

Larvicide

When bodies of standing water cannot be drained because they are necessary or when flooding events and heavy rainfall occur and the ICB cannot keep up with eliminating the sources of mosquitoes, methoprene (trade name Altosid®) or *Bti* larvicide (trade names Vectobac® 200G and Vectobac® 1200L) is applied to these bodies of water. This is the largest component of the ICBs IPM program because mosquito populations are at their most concentrated when located in standing water. The ICB uses truck-mounted equipment, backpacks, ATVs, and helicopters to apply larvicide.

Methropene is a juvenile hormone mimic that impairs or halts arthropod growth and molting (Krieger 2010). Methoprene is not specific to mosquitoes, and for this reason, it should only be used in locations where mosquitoes are the major species and where other invertebrates are not abundant (Krieger 2010). The PMRA classifies all pesticides that are applied directly to water as “restricted” and this includes methoprene. Application equipment must be certified to use restricted class products (Health Canada 2010).

Bti is a bacterium that exists naturally in soils worldwide and produces a Delta-endotoxin which can be crystalized for storage and later used for formulation/application. The crystal-shaped proteins are then applied in bodies of water and ingested by feeding larvae. The Delta-endotoxin is cleaved into its lethal components by enzymes in the mosquito midgut (Lacey 2007). The larvae quit feeding within hours and die quickly. Larvicide is used to control both floodwater and non-floodwater mosquitoes in their larval form and is not effective against eggs, adults, or pupae (because the pupal stage does not feed). *Bti* only has a brief residual effect (field half-lives ranging from 0.5-4 days) because its toxins are rapidly denatured and/or bound to organic matter in the organically enriched environments to which they are generally applied (Beegle *et al.* 1981; de Lara Haddad *et al.* 2005; Lacey 2007; Saiful *et al.* 2012).

Due to the narrow operational window of the larvicide and when heavy rainfall or flooding occurs and larviciding efforts cannot keep up, adult mosquito populations can still occur in high numbers and adulticide application may become necessary.

Adulticide

Adulticide application is the ICB's last-resort mosquito control tool. When larval populations cannot be controlled sufficiently, large numbers of nuisance and pathogen-transmitting mosquitoes may emerge. This occurs when precipitation (as little as 2 cm depending on the species) and warm spring and summer temperatures (19°C - 25°C) coincide and a large and rapid adult mosquito population build-up occurs (Wood, Dang and Ellis 1979; Dodson *et al*. 2012).

The ICB uses a decision-making algorithm called the AFA to decide when and where to apply adulticide. The AFA level is determined by soil moisture conditions, forecasted rainfall, adult mosquito traps, temperature, and larval development site status (number and instar stage of larvae in known development sites). The AFA level must be "high" to trigger a residential adulticiding program. The guidelines for a "high" AFA level include medium soil moisture levels conducive to pooling water on the ground, forecasted significant rainfall (> 2.2 cm) over the next week, NJLT collections meeting the set threshold (described next), temperatures conducive to mosquito activity, and larval surveys that indicate continued re-emergence of adult mosquitoes for more than a week.

The NJLT threshold is considered "high" when the city-wide average trap count reaches 25 female mosquitoes for 2 nights in a row with one quadrant of the city being in the range of 100 female mosquitoes. Consequently, adulticiding commences to target nuisance mosquitoes. This threshold was set by Manitoba's CEC in 1975 to reduce the use of insecticide for adult mosquito control (CoW 2022). The average count of 25 female mosquitoes per trap, citywide, equates to approximately 2 bites per minute which was considered a high enough biting pressure by the CEC to outweigh the consequences (i.e. environmental impact on non-target organisms and waterways and public health impacts) of adulticide use (CEC 1982). These concerns will be described further in section 3.5 of this literature review.

In cases of pathogen transmission risk and significant populations of vector species, a health order from the Manitoba government may be issued for adulticide application to reduce such risk (Manitoba Health, Seniors and Active Living 2022). Manitoba Health follows their own epidemiological criteria when issuing a health order. The CoW-ICB acts as a contractor to conduct a WNv adult mosquito control program where publicly-requested buffer zones are not in effect.

Adulticide Components and Application

Pesticides are usually purchased as formulations (which can vary depending on operational use). These formulations may contain stabilizers/carriers with several functions such as acting as a solvent to help the active ingredient penetrate the substrate on which it lands, better application by preventing caking or foaming, extending the product’s shelf life, and protecting the pesticide from degradation due to sunlight (EPA 2022). These additives can be found on insecticide labels and differ depending on the insecticide. Larvicides and adulticides typically come in varying formulations within each of their respective categories (such as organophosphates or synthetic pyrethroids). In the case of DeltaGard®20EW (being tested as an adulticide by CoW-ICB), EW stands for emulsion-in-water which creates a smaller particle size than those created using emulsifiable concentrate (EC) formulations that create an oil-in-water emulsion.

Synergists are added to some adulticides to make them more effective (Krieger 2010). Synthetic pyrethroids are insecticides which have a rapid knock-down effect with a high recovery rate because they are metabolized quickly by arthropods and mammals. To slow this metabolic breakdown, the synergist piperonyl butoxide (PBO) is often added to synthetic pyrethroid formulations to inhibit the mixed function oxidase system of insects (Casida 1970

Different application methods of adulticides require the active ingredient to be formulated in different ways. Low-volume (LV) and high-volume (HV) applications require diluents such as water or oil to be mixed with the active ingredient for thermal fogging dispersal (Mount 1985). Thermal fogging requires diluents because it vaporizes the liquid solution for application. The introduction of ULV applications (mechanical or pressure-created aerosols) have mostly phased out thermal fogging. ULV applies the minimum volume of liquid adulticide formulation (usually less than 500ml/ha) per unit area that provides the maximum efficiency in killing adult mosquitoes (Mount 1985). ULV requires no diluent (applied “neat”) or little diluent (Mount 1985). Adulticide application in Winnipeg is done with ULV ground-based equipment in specific areas or throughout the city as needed.

Adulticide is applied from public streets and lanes, in major parks and golf courses, and in cemeteries owned and operated by the CoW. Adulticide application occurs during the dusk-to-dawn period (between 9:30pm and 6:30am) because most mosquitoes of concern are most active at that time. Certain meteorological conditions are also required for application. The standard for ULV and thermal fogging applications is a temperature between 12.8ºC – 29.4ºC and a wind speed between 1.5kph - 8kph (Krieger 2010). A slight breeze is ideal for dispersal, but during very windy days, the insecticide may spread to unwanted areas (designated buffer zones or close to waterways). Additionally, high wind speeds make uniform dispersal difficult to achieve, and may mean the concentration of the applied product is below that useful for killing mosquitoes in some locations. In Winnipeg, adulticides are applied in the evening to target *Ae. vexans* when they are most active. Depending on the insecticide and application method used, the manufacturer of the equipment and/or adulticide provides best use practice standards for different meteorological conditions. The CoW-ICB follows guidelines written on the pesticide use labels and the recommendations of the AMCA BMP for mosquito abatement that includes frameworks imposed by the PMRA via the Pest Control Products Act (CoW 2022).

An important aspect of ULV efficacy is droplet size and dispersal. For ULV adulticides to work, droplets must directly contact mosquitoes while they are in flight or resting (Krieger 2010). Specific residual effects and drift of deltamethrin and malathion will be discussed in Section 3.5.

Droplets that are too small may evaporate too quickly in the air and never reach their target or may not contain enough adulticide to kill. Droplets that are too large will not disperse far enough and will reach the ground too quickly. Droplets require a specific size range to achieve maximum efficacy of the adulticide and this information is also included in best use practice standards (Krieger 2010).

The CoW-ICB used malathion (an organophosphate) applied with truck-mounted technology from 1983 to 2015. Malathion is no longer available for purchase in Winnipeg and the city has adopted DeltaGard®20EW (deltamethrin) synthetic pyrethroid for adult mosquito control. Malathion 95ULV® was used in Winnipeg in 2010 (the year from which data was obtained for analysis in this thesis). Specific information regarding operational use and toxicology of malathion and deltamethrin will be discussed next.

3.5 Adulticide Toxicology

Adulticide applications used for mosquito control are based on very small amounts and are not likely to add significantly to active ingredients already applied for agricultural and other urban insecticide and garden use. However, the broad-spectrum action of some of these agents means that they may be toxic to non-target arthropods and other organisms, and that they may pose acute and/or chronic risks to humans and other mammals (Thier 2001; Farajollahi and Williams 2013). All insecticides are potentially hazardous, but the degree of hazard depends on the chemistry, the application rate, exposure, and biological identity of the non-target organism in question (Krieger 2010). The major classes of adulticides used in mosquito control are organochlorines (OC), organophosphates (OP), carbamates, and synthetic pyrethroids (SP). Of these, malathion (OP) and deltamethrin (SP) will be discussed in detail as they have been used most recently in Winnipeg for adult mosquito control and are the focus of this study.

Before a detailed description of the toxicology of malathion and deltamethrin, it is important to establish what this term means. Toxicological risk is primarily determined by dose and exposure to the adulticide (Krieger 2010). Dose is the amount of active ingredient per unit of body mass required to kill or make organisms ill (Krieger 2010). Dose is usually fine-tuned to be just enough to kill the target organism without killing other susceptible organisms (unless they happen to be of a similar size). Dose is generally determined by the LD_{50}: the lethal dose of adulticide that kills 50% of the target organism. LD_{50} is measured in amount per kilogram of body weight (mg/kg_{bw}) (Krieger 2010). It can also be listed as the application rate in grams/hectare (g/ha). Exposure may be acute (short-term) and/or chronic (long-term), and may vary with route (pathway to internal tissues such as oral, dermal, and inhalation). Each application can have different duration of exposure and procedures (such as timing and method) that could contribute to variation in the amount of toxin to which organisms are exposed (Krieger 2010).

3.2.1 Malathion Toxicology

Efficacy and Mode of Action

Malathion is an OP insecticide that inhibits acetylcholinesterase from breaking down the neurotransmitter, acetylcholine, increasing its level and duration in the central nervous system (CNS) (Reigart and Roberts 1999). This results in overstimulation of the nervous system and death of the mosquito and other organisms if the dose is high enough.

There are no published studies with specific evidence of malathion efficacy in Winnipeg. However, ULV applications of malathion tested on caged mosquitoes in Chicago resulted in statistically significant reductions ($p < 0.001$) in *Ae. vexans* populations averaging 21% over the 3 days following application (Geery *et al.* 1983).

Dose and Application Rate

Malathion is applied at a rate of 60.8 g/ha for ULV ground application (Health Canada 2003). ULV equipment is adjusted to make sure droplet diameter is, on average, 17 microns and that no droplets can exceed 32 microns (EPA 2022). This ensures that the droplets are the right size to disperse through the environment and kill mosquitoes upon contact. The specific dose in each droplet targets mosquitoes, but it may affect non-target flying arthropods of similar size that are active or exposed during the crepuscular/nocturnal periods when the adulticide is applied in Winnipeg.

The LD_{50} has been studied in birds (2140 mg/kg) and rats (14850 mg/kg) in laboratory settings (WHO 2016). The LD_{50} for humans and mosquitoes is not readily available. Human clinical signs of toxicity are mild and generally occur at dose levels much higher than those used for mosquito control. Adverse effects at low dose levels necessary to kill mosquitoes are minimal or not observed (Krieger 2010).

Exposure

Malathion breaks down rapidly in the environment (1-17 days in soil and water) and therefore has little residual effect (Krieger 2010). Malathion is more effective at killing mosquitoes during direct contact rather than from residual exposure. Also, acute intoxication of non-targets, usually because of accidental spills, is more common than chronic intoxication due to the minimal lasting effect of malathion (Krieger 2010). Malathion does not accumulate in tissues (Krieger 2010).

Routes of malathion exposure can be oral, dermal, or by inhalation (Krieger 2010). In mammals, malathion is oxidized to form malaoxon, a cholinesterase inhibitor that causes acetylcholine to accumulate in the nervous system and causes overstimulation with lethal effects at appropriate doses (Krieger 2010). However, the amount of malathion used for adult mosquito control forms very little malaoxon in mammals (Krieger 2010). This, combined with detoxifying enzymes explains its low toxicity in mammals. Malathion has been denoted as a Class III pesticide (slightly hazardous) by the EPA (2022).

During its operational use, malathion was applied by the CoW-ICB using ULV ground-based technology. This application method minimizes drift due to small droplet sizes that evaporate quickly in the air if they do not contact a mosquito (Mount 1985). The use label for Malathion 95ULV® states that precautions should be taken and it should not be applied in dead calm or near sensitive plants and arthropods when wind velocity and direction posea risk of spray drift (Loveland Products 2018). Application in wind speeds exceeding 10-15 kph are prohibited (Loveland Products 2018). Malathion is applied in the evening and early morning hours only to target nuisance mosquitoes during their most active time and to prevent non-target deaths of insects like butterflies and bees which are more active during the day (CoW 2022).

3.2.2 Deltamethrin Toxicology

Efficacy and Mode of Action

Deltamethrin is a SP that targets the axonal voltage-gated sodium channel (Krieger 2010). The insecticidal action of SPs and other pyrethrins (pyrethroids derived from *Chrysanthemum*) can be separated into two types: Type I pyrethroids with rapid paralyzing knockdown and Type II pyrethroids (like deltamethrin) with slow-developing kill (Sawicki and Thain 1962).

Type I pyrethroids produce T-syndrome in which an acute poisoning occurs that mostly affects the peripheral nervous system (PNS), producing hyperexcitation, prostration, whole body tremors, and tonic seizures before death at lethal doses in both mammals and insects (Gammon *et al*. 1981; Lawrence and Casida 1982). Lethal doses for mammals are very high relative to those that kill mosquitoes. The symptoms associated with T-syndrome indicate repetitive firing of the axons due to a transient modification of an open channel and leads to a fast knockdown effect with a high recovery rate because insects and mammals both metabolize SPs quickly by oxidation (Gerolt 1975). PBO synergist is used to increase the toxicity of these pyrethrins by slowing their metabolic breakdown.

Type II pyrethroids act primarily on the CNS and can lead to so-called CS-syndrome in which mammals profusely salivate, paw and burrow with writhing of the body (Lund and Narahashi 1983). This class of pyrethroids works by keeping sodium channels in an open state and blocking the action potential in both mammals and insects. Due to the long slow-developing kill that is characteristic of Type II pyrethroids, deltamethrin does not require a synergist.

Insects and mammals both metabolize SPs quickly by oxidation, however SPs penetrate the CNS and other target tissues of insects relatively quickly (Casida *et al.* 1971; Elliot *et al.* 1972; Gerolt 1975; Class *et al.* 1990). When deltamethrin contacts a mosquito directly, it penetrates through the cuticle and epidermis and is taken up by hemolymph carrier proteins to be distributed throughout the mosquito's body (Schleier and Peterson 2011).

The CoW-ICB tested DeltaGard®20EW in preliminary trials with caged live mosquitoes in 2015 (Nawolsky and Wade 2016). These studies yielded promising results: applications of 1g deltamethrin/ha resulted in 96% mortality in caged mosquitoes located 90 meters away from the point of the insecticide release (Nawolsky and Wade 2016). This result was corrected for the natural mortality of the untreated caged mosquitoes. Mortality of caged mosquitoes that were not treated with deltamethrin and acted as experimental controls in these trials was less than 3.5% (Nawolsky and Wade 2016).

Dose and Application Rate

DeltaGard®20EW is applied at a rate of 0.5-1.5g/ha for ULV ground application (Brill and Morrison 2013). Spray equipment is adjusted to ensure that the median droplet diameter is between 8 – 30 microns so that direct contact will kill adult mosquitoes (Brill and Morrison 2013). The size of the droplets and rate of application ensures that the dose is sufficient to cause significant adult mosquito mortality. However, other similarly sized arthropods are at risk if they are exposed during crepuscular hours when adulticide is applied in Winnipeg. Pyrethroids are toxic to honeybees with a contact LD_{50} of 1.95×10^{-4} mg/kg (Lynn and Hoxter 1991). An average droplet of DeltaGard®20EW ULV contains approximately 8.38×10^{-8} mg of deltamethrin. Therefore, approximately 262 droplets of DeltaGard®20EW ULV would be required to kill a honeybee.

In mammals, acute oral LD_{50} values are between 50-500 mg/kg for deltamethrin products that are registered for use in North America and are considered moderately toxic to mammals by the EPA (category II) (Soderlund *et al.* 2002). Intoxication with single pyrethroid dose of these

LD_{50} values result in transient symptoms that begin within minutes to a few hours after exposure (Soderlund *et al.* 2002). At high, near-lethal doses (100X and often much higher than levels to which humans are normally exposed to deltamethrin applications for adult mosquito control when used according to labels), mammals that survive acute intoxication recover and appear normal within 1-14 days after treatment (Soderlund *et al.* 2002).

There is approximately 8.38×10^{-11} g of deltamethrin in each ULV droplet when truck-mounted equipment is dispensing 0.5 g/ha of insecticide at 10 km/hr and the aerosol spreads with a width of 8 meters and a height of 3 meters within a few seconds (the approximate average amount of drift). With these parameters, truck-mounted ULV equipment produces a swath of 167 meters per minute and that swath consists of approximately 4000 cubic meters of air. A human weighing 63 kg would require 3.70×10^{7} droplets received orally to cause acute LD_{50} symptoms (at the lowest previously listed value of 50 mg/kg). In this scenario, 3 grams of deltamethrin would be dispensed in the swath produced by ULV application (4000 cubic meters of air) per minute. It takes approximately 167 minutes for a human to breathe through a cubic meter of air which means that it would take over 11,000 hours or 458 days of breathing in freshly released DeltaGard®20EW to get 3000 mg of deltamethrin, the dose for serious effects. This means that it takes approximately 13 million times as much active ingredient to kill a human as to kill a mosquito, based on weight.

Exposure

Deltamethrin has a longer half-life than malathion (~90 days versus 1-17 days for malathion) (Krieger 2010). Due to its longer environmental persistence, it needs to be applied less often, but it is more likely to result in chronic exposure. When tested as a residual barrier applied on vegetation, laboratory results indicated that deltamethrin allowed reasonable control of 80% of the mosquitoes resting in vegetation for almost two weeks after initial application (Bengoa *et al.* 2013). Deltamethrin has also been approved by the WHO as a safe residual treatment for bed nets (Ehiri *et al.* 2004; Bengoa *et al.* 2013). Environmental contamination of bodies of water is avoided by application protocols followed by the CoW-ICB because pyrethroids are highly toxic to fish and some aquatic insects and crustaceans on which fish feed (Bridges and Cope 1965; Mauck *et al.* 1976).

Mammalian pyrethroid toxicity varies depending on the route of administration. Dermally, pyrethroids have very low acute toxicity because their absorption through the skin is limited (Woollen *et al.* 1992; Clark *et al.* 1995). Direct intravenous or intracerebral injection greatly enhances acute pyrethroid toxicity, but these routes of administration do not happen during ULV pesticide application (Gray and Soderlund 1985). Acute inhalation toxicity of deltamethrin is considered low to moderate and oral acute toxicity ranges from low to high depending on dose (Krieger 2010). Mammals rapidly metabolize pyrethroids *in vivo* (Krieger 2010). Deltamethrin is not a skin or eye irritant (Krieger 2010). Synthetic pyrethroids are also more insecticidal at low temperatures and are generally applied during nighttime hours for adult mosquito control when they are more effective (Krieger 2010; Gammon 1978). Insect body temperatures are approximately 10°C lower than mammalian body temperatures, contributing to the more selective toxicity of pyrethroids (Krieger 2010).

Due to the general insecticidal properties of SPs, other flying insects are at risk. Application of the insecticide in the evening decreases this risk because few non-target beneficial organisms such as bees and butterflies are active at this time. When this theory was tested in Greece, no measureable negative effects were found on non-target species when deltamethrin was applied at night, and beehives exposed to the insecticide remained healthy and productive (Chaskopoulou *et al*. 2014).

DeltaGard®20EW is applied by ULV ground-based equipment in small droplets (median diameter between 8 – 30 microns) that contact mosquitoes while they are in flight (Mount 1985). Droplets that do not contact mosquitoes evaporate in the air before falling into water and onto land and don't drift much (Mount 1985). Of all the SPs, only deltamethrin does not require PBO synergist which has been classified as a possible human carcinogen by the EPA (Thier 2001). When diluted, DeltaGard®20EW is applied with FFAST (film-forming aqueous spray technology) which is water-based instead of oil-based (Brill and Morrison 2013). Oil-based diluents can wreck application equipment and require organic solvents for spill clean-up and can also damage foliage.

Summary

Table 1 summarizes the toxicological comparison of deltamethrin and malathion for ULV ground-based adult mosquito control.

Table 1. A comparison of deltamethrin and malathion toxicology when applied with ULV ground-based equipment for adult mosquito control according to use labels followed by the CoW-ICB.

	Deltamethrin	**Malathion**
Mode of Action	Axonal voltage-gated sodium channel	Acetylcholinesterase inhibitor
Application Rate	0.5-1.5 g/ha	60.8 g/ha
ULV Droplet Diameter Median	8-30 microns	17-32 microns
Recorded LD$_{50}$	50-500mg/kg in mammals 1.95x10^{-4}mg/kg in honeybees	1485mg/kg in rats 214mg/kg in birds
Half-Life	~90 days	1-17 days

3.6 Spatial Considerations in Mosquito Control

Spatial analysis is important for understanding ecological systems (such as those where operational mosquito control is done) because population dynamics and adulticide efficacy can vary from location to location depending on several landscape features that will be discussed later in this section. Spatial heterogeneity has always been important in ecological studies, but a lack of available spatial tools meant that it could not be explored in detail (O'Sullivan and Unwin 2010). Spatial tools and methods of analysis had to be developed.

When spatial tools for analyzing heterogeneity in a study area started being developed in the 1960s, they were initially used by researchers with strong mathematical and programming backgrounds (O'Sullivan and Unwin 2010). However, as tools developed over time, geographers, archeologists, and ecologists began to quantify spatial heterogeneity and to understand relationships between spatial variables (O'Sullivan and Unwin 2010). Spatial analysis determines the relationship between two or more variables and accounts for spatial characteristics of data such as coordinates, clustering, patterns, and trends (O'Sullivan and Unwin 2010). The process of spatial analysis helps to extract new information about sample data, variables, ecology, and landscape through visual and quantitative assessments, analyses, etc. The results of spatial analysis allow us to better understand any variable that changes in space, determine spatial suitability (i.e. habitats) and predict future patterns. Spatial analysis of landscapes can determine if populations are significantly clustered or dispersed or if organisms exists randomly in their environment (O'Sullivan and Unwin 2010). Species patchiness implies that nearby observations of species abundance tend to be similar and are more closely clustered than by random chance (Wagner and Fortin 2005). Patchiness may be due to species dispersal, competition for space and resources, spatial dependence on certain habitats, or underlying environmental conditions (Wagner and Fortin 2005).

In the field of mosquito biology, a focus on spatial analysis has led to significant improvements in control methods. Although the use of spatial statistics to improve mosquito control has focused on tracking and controlling vector mosquitoes that can transmit pathogens (Gimnig *et al*. 2005; Koenraadt *et al*. 2007; Trawinski and Mackay 2009; Azil *et al*. 2014; LaCon *et al*. 2014; Sudsom *et al*. 2015), they have also been used to target nuisance populations. For example, spatial statistics can be employed to determine the optimal location and/or number of mosquito traps and to identify mosquito activity hot spots that should be prioritized for control and the landscape features that surround these hot spots (Ryan *et al*. 2004; Jacob *et al*. 2010; Cianci *et al*. 2015).

The dispersal of mosquito populations in Winnipeg depend on several landscape and habitat features. *Ae. vexans* populations are clustered within riparian areas and they convene in wetlands and forested areas, although they are strong fliers that travel out of these habitats to feed (Wood, Dang and Ellis 1979; Jensen and Washino 1994; Balcaen 2020). Riparian locations are more prone to flooding and inundation which is conducive to floodwater mosquito oviposition (Wood, Dang and Ellis 1979). Forests and areas with more dense vegetation provide shade for mosquitoes that are otherwise prone to desiccation (Wood, Dang and Ellis 1979; Strickman 1982).

Parks in Winnipeg often have large mosquito trap collections relative to the rest of the city. In 2019, only parks and cemeteries reached the trap count threshold for adulticide application (CoW 2022). In 2020, parks and cemeteries reached threshold levels weeks before the rest of Winnipeg (CoW 2022). High relative mosquito activity in parks may be explained by the frequent presence of rivers, forests, and dense vegetation.

Besides providing a riparian habitat for floodwater mosquito oviposition, waterways in Winnipeg act as buffer zones where DeltaGard®20EW cannot be applied to avoid contamination (CoW 2022). Mosquitoes in habitats closer to rivers may avoid contact with the adulticide for this reason. Despite the approximately two-week residual barrier effect exhibited by DeltaGard®20EW, it is still possible that dense vegetation will hinder adulticide efficacy by blocking drift and by giving adult mosquitoes a place to hide from aerosol droplets containing active ingredient. The DeltaGard®20EW use label states that it should be applied at its maximum rate in densely vegetated areas because penetration is hindered and the likelihood of directly contacting a mosquito is lowered (CoW 2022). Deltamethrin is an effective repellent for *Ae. aegypti* and *Ae. albopictus* (Chattopadhyay *et al*. 2013; Bibbs and Kaufman 2017; Bowman *et al.* 2018). *Ae. vexans* may also be repelled by residual deltamethrin, avoiding chronic residual toxicological effects by avoiding treated vegetation, although the effect on this species must be determined and is outside of the scope of this study.

Different species are likely to respond to their environment in various ways depending on the scale considered and their movement ranges. The density and distribution of forested and riparian environments may influence mosquito activity, population, and the efficacy of DeltaGard®20EW. Local landscape structure across space should be considered as it may be experienced by the organism of interest (i.e. mosquitoes and their breeding sites and frequently visited habitats) (Wagner and Fortin 2005). Organisms are generally not spread randomly across the landscape and the pattern of individuals in space is the focus of spatial statistics in landscape ecology (Krebs 1998). There are a number of spatial analysis tools available and the ones relevant to this study are discussed next.

Spatial Autocorrelation

Spatial autocorrelation measures and analyzes the degree of dependency of two coordinates in a geographical location (O'Sullivan and Unwin 2010). Moran's I tool is used to detect spatial autocorrelation and to determine if spatial data is significantly clustered or dispersed or if it exists randomly in space. Further spatial analysis can only be performed on data that is significantly clustered or dispersed because the point of spatial analysis is to analyze spatial patterns in data (O'Sullivan and Unwin 2010).

Spatial Interpolation

Spatial interpolation estimates variables at unobserved locations compared to those at observed locations (O'Sullivan and Unwin 2010). In the context of this study, spatial interpolation can be used to estimate trap count data at unmeasured locations where mosquito traps are not operated. Predictive maps can then be used to visualize mosquito activity levels in Winnipeg despite the limitations involved in placing traps. This can help to control for background mosquito population dynamics when determining the effect of DeltaGard®20EW and to visualize changes in mosquito activity before and after treatment.

The number of mosquito traps and their locations are limited in the CoW-ICBs IMA. The CoW-ICB must place mosquito traps on CoW owned and operated land or on private property only with the permission of the owner. Therefore, traps are placed based on convenience and cannot be randomly dispersed, which interferes with the study designs aimed at evaluating efficacy of different insecticide applications. The number of traps is also limited by CoW-ICB resources and adequate personnel to set, collect, and monitor the traps. Traps are often placed in areas with dense vegetation and shade that foster high levels of mosquito activity (Wood, Dang and Ellis 1979; Balcaen 2022). This may skew results because it is selective to locations that are more likely to catch mosquitoes. DeltaGard®20EW may affect mosquitoes at these trap locations unequally depending on surrounding tree density and distance from waterways and parks/open spaces which will be discussed in the Grouping Spatial Analysis part of this section.

There are two major types of interpolation techniques: deterministic and geostatistical approaches (O'Sullivan and Unwin 2010). Deterministic methods use predefined mathematical equations to predict values at unsampled locations without capturing the spatial structure of the data (O'Sullivan and Unwin 2010). Geostatistical approaches assume that there is knowledge that the data has spatially correlated distances or directions associated with it and fit a spatial model while giving an estimate of accuracy for the predictive surface (O'Sullivan and Unwin 2010). There are several spatial interpolation methods available including triangular interpolation network (TIN) and inverse distance weighing (IDW) (deterministic) and kriging (geostatistical).

TIN creates a triangular network where each corner of the triangle is a point feature (O'Sullivan and Unwin 2010). For instance, in the context of this study, triangles would be created to cover the entire surface of CoW with each corner of the triangle being an individual mosquito trap location. The attribute values (predicted mosquito trap counts) are then calculated by weighing the values of the three apexes of the triangle (O'Sullivan and Unwin 2010). Due to the limited trap locations in this study and the uneven dispersal of those traps, the accuracy of this method would be minimized. When trap locations are clustered, neighboring observations will be given a relatively good prediction. However, isolated trap locations would create much larger triangles with imprecise or flawed predictions. This method of interpolation requires many observations in the study site.

IDW predicts attribute values at unsampled locations based on the distance of known observations (O'Sullivan and Unwin 2010). For instance, observations closest to the unsampled location are given larger weights than those further from the unsampled location (O'Sullivan and Unwin 2010). Again, due to the uneven dispersal and limited number of trap locations, this smoothing parameter may blur interesting variations or make mosquito activity across the city appear more homogenous than it actually is.

Kriging, being a geostatistical method, considers both the distance between observations and the spatial structure in data (O'Sullivan and Unwin 2010). This interpolation method is most appropriate to mosquito trap count data in this study because known patterns in mosquito activity and selective placement of mosquito trap locations means that there is already a known spatial trend in the data. Kriging creates an interpolation surface from trap locations with attributes (i.e. trap count). A leave-one-out cross validation of the kriging surface (a form of jackknifing to test the validity of a model) may give insight into whether the number and location of mosquito traps in Winnipeg accurately measure city-wide mosquito activity. Researchers have then used linear models comparing cross-validation outputs to evaluate the relationship between observed (measured) and predicted (interpolated) mosquito count numbers to see how accurately the experimental design measures what it intends to measure (Ryan *et al.* 2004).

Grouping Spatial Analysis

Multi-ring buffer analysis and the "Generate Near Table" and "joins" functions in ArcGIS (ArcGIS, Version 10.4.1, Environmental Systems Research Institute (ESRI) Inc., Redlands, CA) can be used to count the number of landscape features and to measure the distance (in meters) between mosquito traps and different landscape features respectively (ESRI 2022). This can aid in determining which landscape features may be most important in mosquito population dynamics and adulticide efficacy in Winnipeg. However, the size of buffers surrounding trap locations must be decided and changes in their size can alter the analysis. Also, barriers to mosquito migration, such as tall buildings, may influence distance measurements to nearest rivers.

Hot spot analysis can be used to reveal which mosquito trap locations have been hot spots of mosquito activity (higher than usual numbers surrounded by high neighboring numbers) using ArcGIS (ArcGIS, Version 10.4.1, ESRI Inc., Redlands, CA). Trap locations that were hot spots at some point can be analyzed to determine if certain landscape features surrounding them are characteristic. Kruskal-Wallis analysis can then be used to compare landscape features between hot spot and non-hot spot locations to check for significant differences relevant to operational mosquito control.

Landscape ecology expands our understanding of dynamic ecological patterns based on the principal that organisms move within their environment. Mosquitoes travel at various distances depending on species to locate blood meals and oviposition sites. Landscape ecologists call landscapes “mosaics” because they are complex patterns composed of interconnected and repeating habitats, ecosystems, and land use/land cover over a specific area. The landscape patterns that we see today are the product of disturbances, abiotic environmental/physical constraints such as climate, landforms, and geology that create a geomorphic template on which the final contributing factor, the biological landscape, is constructed (Bannerman 1997). The inclusion of spatial analysis tools is crucial when testing adulticide efficacy and for mosquito surveillance because the location of mosquito traps differ with respect to surrounding landscape features and mosquito population dynamics are dependent on spatial characteristics.

3.7 Mosquito Wing Length and Longevity

Another aspect that must be considered in adult mosquito control programs is the longevity of mosquitoes because those with longer lives should be targeted as a sub population likely to produce nuisance for a longer time. The average lifespan of adult *Ae. vexans* (the major nuisance species in Winnipeg) is 3-6 weeks (Horsfall 1955). Some experiments have collected *Ae. vexans* females 55, 104, and 113 days after release (Herms, James and Harwood 1969). Various studies have found that temperature and availability of nutrients during development and body size produced the best explanation of variation in adult mosquito longevity (Yeap *et al.* 2011; Barreaux *et al.* 2018; Guitiérrez *et al.* 2020).

Longevity of mosquitoes is generally believed to be positively correlated with body size with larger mosquitoes living longer (Packer and Corbet 1989; Yeap *et al.* 2011). Wing length is used to measure adult mosquito body size and has been found to correlate well with dry body weight (Packer and Corbet 1989). Therefore, wing length is used as a measure of body size in this experiment. Adult mosquitoes emerge from pupae the size they will remain for their whole lives (Wood, Dang and Ellis 1979).

Various studies provide evidence of the positive correlation between longevity and adult mosquito body size. Experimental manipulation of factors known to be important to mosquito growth showed that wing length was found to decrease as temperatures increased from 21-29°C because development speeds up in warmer temperatures (Barreaux *et al.* 2018). Cutting food availability in half was also found to result in smaller mosquitoes (Barreaux *et al.* 2018). Healthier (larger) mosquitoes are more likely to bite more than once, survive longer, may have better flight range, are more accomplished at locating hosts, are better at attaining blood meals and at locating oviposition sites, and have increased fecundity and reproductive success (Packer and Corbet 1989; Yeap *et al.* 2011; Guitiérrez *et al.* 2020). Larger body mass also means having higher energy reserves and higher mass to surface area ratio making those individuals less prone to desiccation than smaller mosquitoes (Yeap *et al.* 2011). Teneral (between adult emergence and flight) lipid content and body size are positively correlated making larger females better equipped to invest in reproduction without compromising longevity (Johnson 1974; Guittiérrez *et al.* 2020). For floodwater mosquitoes such as *Ae. vexans*, transient larval habitats mean that sometimes the larvae must develop quickly and smaller mosquitoes emerge (Guitiérrez *et al.* 2020). Smaller mosquitoes must invest in immediate reproduction at the expense of longevity because they have less teneral lipid content and need to feed quickly after insemination to increase this lipid content for egg development (Guittiérrez *et al.* 2020).

Despite most evidence supporting the claim that longevity is positively correlated with body size, there are some studies that dispute this. Barreaux *et al.* (2018) found that overall, the relationship between body size and longevity was weak when studied in *Anopheles gambiae*. They found that larger mosquitoes lived longer in some environments, but not in others. At 25°C there was no clear relationship between body size and longevity. At 29°C, the relationship was negative when fed normally and positive when food supply was cut in half. At 21°C the relationship was positive when fed normally, and negative when food supply was halved (Barreaux *et al.* 2018). The authors state that longevity often increases with body size, but that this positive correlation does not always exist and can even be negative (Barreaux *et al.* 2018). Variation may be due to the fact that survival and adult body size respond differently to diverse environmental factors such as temperature and nutritional availability (Barreaux *et al.* 2018). Yan *et al*. (2021) found that the effect of body size in *Ae. aegypti* was only noticeable on increased fecundity, but not on longevity.

The correlation between longevity and body size is usually positive, but developmental temperature and nutritional supply may need to be considered in future studies. In the context of this project, if longevity is correlated with larger body size, the CoW-ICB could potentially target certain locations in Winnipeg that produce larger (healthier) mosquitoes. The average size of *Ae. vexans* specific to Winnipeg and the relationship between their longevity and body size has not been determined.

4.0 Materials and Methods

4.1 Study Site

The study area for this thesis project included the City of Winnipeg and a 12km-wide zone outside of the city including areas within surrounding municipalities (Figure 4). Winnipeg is in Manitoba, Canada and covers 464.08km^2 (Statistics Canada 2016). In Winnipeg, several major and minor rivers dissect the city. The northbound Red River and the eastbound Assiniboine River are the largest two by flow volume and meet at a point called “The Forks” (CoW 2022). The Seine River and other smaller watercourses also pass through Winnipeg. The region is prone to flooding due to its riverine location, minimal relief, average annual precipitation of 450-700 mm, and soils with low permeability (Government of Manitoba 2022). This regularly leads to transient sitting water, especially after overland flooding or large rainfalls. In the summer months, Winnipeg’s average relative humidity is 63% and average daytime temperature is 26°C.

Figure 4. Winnipeg, Manitoba and a surrounding 12km radius was the study site for testing DeltaGard®20EW adulticide efficacy operationally (World Atlas 2022).

Winnipeg's extensive tree canopy is dominated by American elm, bur oak, ash, and aspen with a ground cover of mixed prairie grass species (Looman and Best 1987). In 2018, the citywide canopy was determined to cover 17% of Winnipeg's surface area with an estimated 3,075,000 trees in the city (Diamond Head Consulting Ltd. 2021). The distribution of these trees is shown in Figure 5. Winnipeg's dense vegetation, riparian environments, and meteorological conditions are conducive to mosquito production.

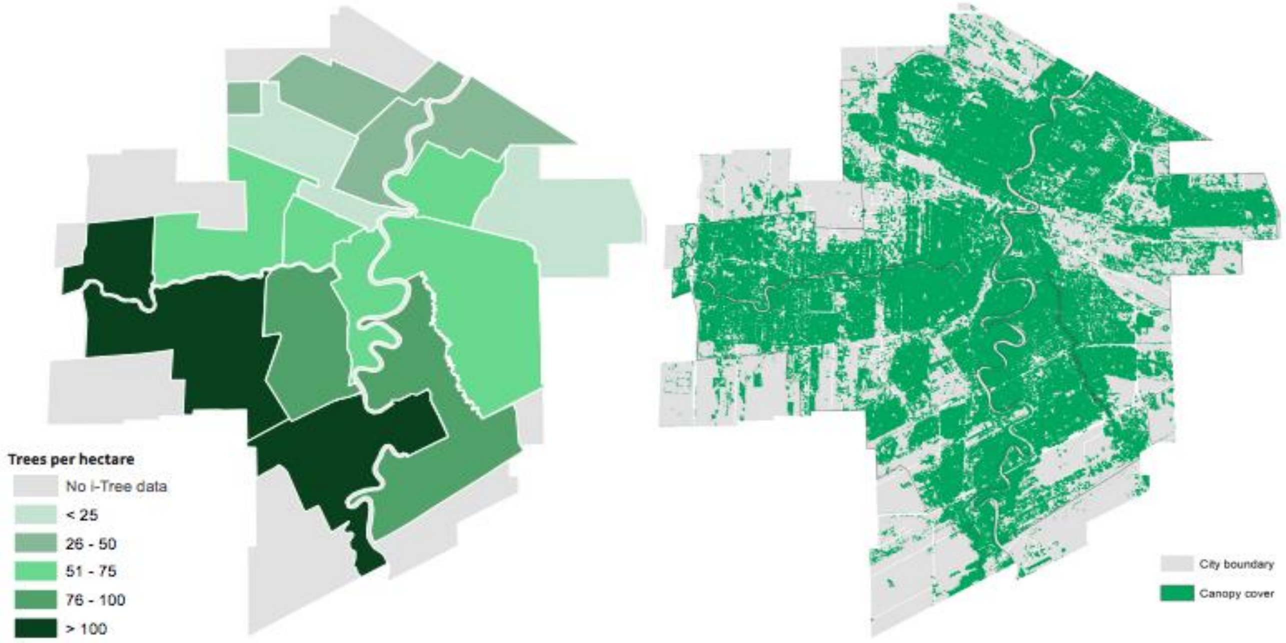


Figure 5. Right: The approximate distribution of trees and canopy across Winnipeg using the City's inventory and satellite data of tree canopy from the University of Maryland (Diamond Head Consulting Ltd. 2021). Left: Tree density per hectare for the whole urban forest by ward (Diamond Head Consulting Ltd. 2021).

4.2 General Methods Overview

Historical records supplied by the CoW-ICB and data I collected in 2020 were used to compare treatment with Malathion 95ULV® in July 2010 to treatment with DeltaGard®20EW in July 2020. Both adulticides were applied with ULV ground-based equipment. NJLTs and CDC traps were used to monitor adult mosquito activity before and after adulticide treatment. Trap collections were obtained daily at most trap location sites for at least three days before and three days after a treatment event. Trap collections were then counted and sorted by sex and species with emphasis on *Ae. vexans*, *Cx. tarsalis*, *Cx. restuans*, and *Cq. perturbans*. Collections from 2020 with 30+ *Ae. vexans* were separated and 30 random *Ae. vexans*' wings were dissected for length measurement as an index of body size.

The analysis was divided into three main components. First, spatial analysis was applied to the data to determine clustering of trap counts and differences in the landscape features surrounding traps. Second, trap counts were analyzed to compare traps in treatment and non-treatment locations post-adulticide treatment using a non-parametric version of ANCOVA called the T.aov function which is used to compare treatment groups while controlling for baseline mosquito activity (pre-treatment trap counts). Wilcoxon signed rank test was used to detect significant daily increases and decreases in mosquito trap counts. Finally, wing length measurements were used to determine the average size of *Ae. vexans* in different parts of Winnipeg and to determine if longevity (measured by the cohort duration in days to 90% depletion) was positively correlated with body size. More details about the programs used for these analyses are described in the following sections.

4.3 Mosquito Trapping

In 2010, the CoW-ICB regularly monitored 28 NJLTs inside of the CoW limits and 9 NJLTs in the surrounding municipalities (Figure 7). Traps were placed based on convenience on private property with permission of the owner or on CoW-owned land. The number of traps that were set and collected was also limited by resources and the number of CoW-ICB personnel. NJLTs (John W. Hock Company, Gainesville, FL 2022) work by attracting mosquitoes to a light source (25-watt light bulb) (Figure 6). Once mosquitoes fly toward the trap, an interior fan pulls them in to the trap funneling them into a collection jar. The traps were run continuously from May – September of 2010 except in cases of disruptions such as vandalism or light bulbs burning out. In July 2010, mosquitoes were collected from the traps daily at all locations, except when the traps had been disturbed or when they had become dysfunctional for any reason.

Figure 6. Left: a CDC-light trap baited with dry ice in a blue canister sublimating into CO_2 and operated by a rechargeable 12-volt, 6-amp DC battery. Right: a NJLT with light as a mosquito attractant and plugged directly into an AC power source (John W. Hock Company, Gainesville, FL 2022).

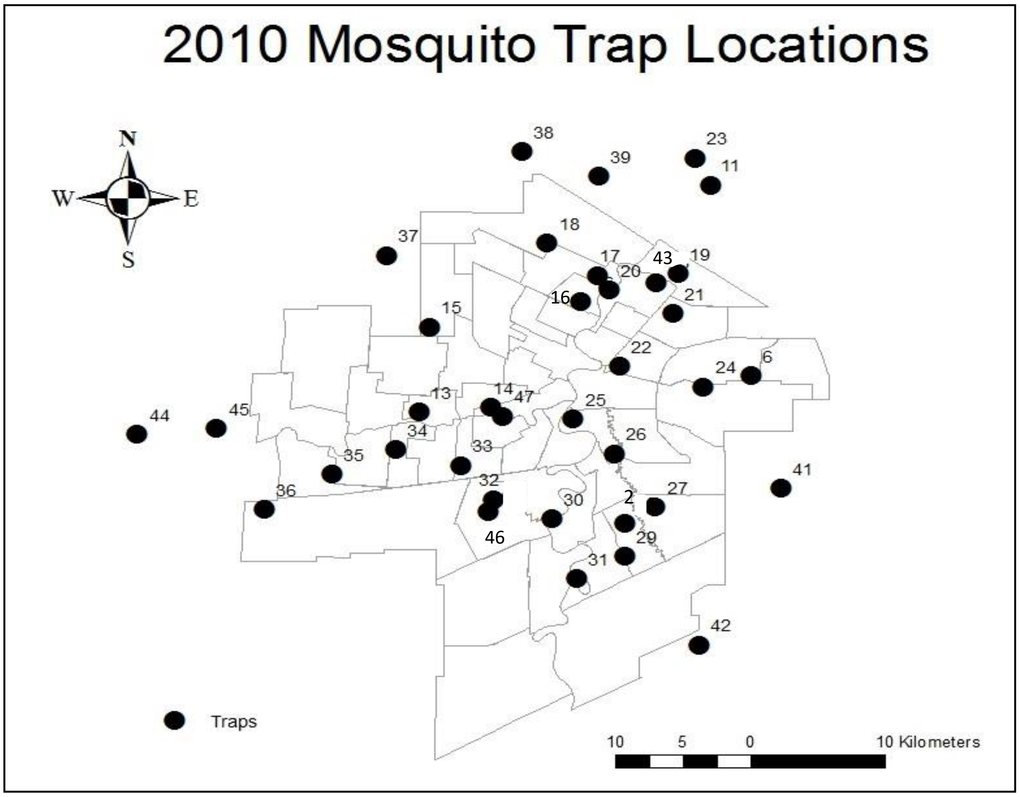


Figure 7. Approximate locations of NJLTs executed by the CoW-ICB in 2010 within and outside Winnipeg, Manitoba. Trap numbers were randomly assigned.

In 2020, the CoW-ICB regularly monitored 33 NJLTs inside of the CoW limits and 9 NJLTs in the surrounding municipalities (Figure 8). The traps ran continuously from May – September of 2020 except when disrupted. In July 2020, mosquitoes were collected daily except on July 11-13 and July 18-20 when collections were made once at the end of the three days. For these two collections, the total trap count data was divided by three and that value was used for each of the three dates described to account for missing daily data.

Along with the city-operated NJLTs, in 2020, I set up 11 CDC-light traps baited with dry ice and with the lights removed (Figure 8). These traps were connected to a 12-volt, 6-amp DC battery to operate an internal fan that sucked mosquitoes into the trap. By removing the lights, males who are primarily attracted to light and not CO_2 were avoided during collection (Figure 6). These traps were again positioned on private property or CoW-owned locations but were not set consistently for two reasons. Sometimes, traps could not be set up due to inadequate warning of when treatment events would occur. In other cases, because dry ice cannot be stored for long periods of time due to sublimation, these traps could not be set up when treatment events occurred on weekends, as the supplier was not open.

Both CDC and NJLTs are placed in shaded areas surrounded with vegetation and they are suspended a few feet from the ground. These habitats are known to be sites of mosquito activity. Descriptions of the land cover and land use surrounding the sites where these traps are placed are described by Balcaen (2020); the spatial arrangement of the trees, rivers, and parks/open spaces surrounding the traps will be further investigated in this thesis as described in the spatial analysis section.

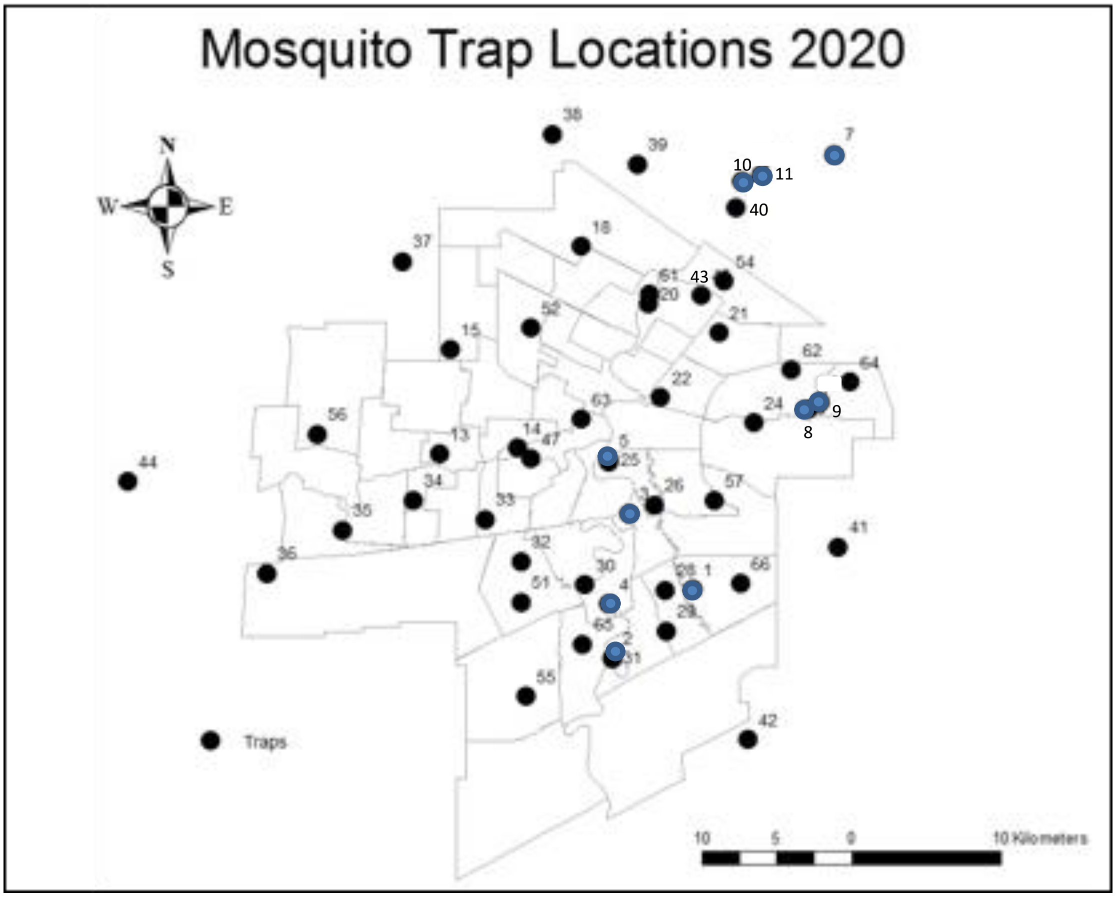


Figure 8. Approximate locations of NJLTs operated by the CoW-ICB and CDC traps I operated in 2020 within and outside Winnipeg, Manitoba. Trap numbers were randomly assigned. NJLTs are shown in black and CDC light traps are shown in blue.

It is important to monitor adult mosquito activity before adulticiding to account for changes in mosquito activity that are due to natural population dynamics and to weather variables that affect activity (i.e. temperature, humidity, and wind). These pre-treatment trap counts are then compared to post-treatment trap counts in treated and untreated areas to assess the effectiveness of the adulticide in reducing mosquito nuisance. Increases and decreases in mosquito activity were also analyzed for significance by comparing trap counts on a day-to-day basis. Mosquito trap collections were obtained at least two days before and two days after treatment with adulticide (Malathion 95ULV® in 2010 and DeltaGard®20EW in 2020).

Figure 11 (Section 4.4) shows that the entire CoW was sprayed in rotation within a four-day span. Any traps located in untreated areas that would remain untreated for the two days before and two days after adulticide application (depending on the day of the spraying event) were used as experimental controls. For instance, the polygons shaded in orange were treated on the evening of July 24, 2020. Only traps in the polygons shaded in yellow and blue could then be used as proper experimental controls because they would remain untreated for the two days following the treatment event in the orange polygons.

More traps were operated in 2020 than in 2010. Four NJLTs had been added between 2010 and 2020 (Table 2). The 11 CDC light traps were operated by me in 2020 and added to the amount of data collected in that year (Table 2).

Table 2. A summary of the adulticide applied in July 2010 and July 2020 and the number of mosquito traps (NJLT and CDC) that were operated in both years.

	July 2010	**July 2020**
Adulticide Applied	Malathion 95ULV®	DeltaGard®20EW
Number of Traps Used	37	53
Number of NJLTs Used	37	42
Number of CDC Traps Used	0	11

4.4 ULV Application and Treatment Zones

Malathion 95ULV® (in 2010) and DeltaGard®20EW (in 2020) were applied with truck-mounted ULV technology from streets and lanes, and in cemeteries, golf courses, and parks owned and operated by the CoW. Adulticiding only occurred when pre-treatment trap counts met AFA action thresholds.

Conditions must be meteorologically conducive to ULV adulticide application. Winds cannot be greater than 15-20 kph but must be greater than 0 kph for adulticide dispersal (CoW 2022). Temperatures must be above 13°C and it cannot be raining during application (CoW 2022). The CoW-ICB follows the adulticide label for specific application procedures.

In 2010, Malathion 95ULV® was applied according to label directions. Ground-based equipment delivered 60.8 g/ha for ULV application (Health Canada 2003). Trucks operated at a speed of 15 kph and ULV equipment was adjusted to make sure droplet diameter was an average size of 17-32 microns (EPA 2022). GPS-enabled computers tracked vehicle position and adjusted flow rate depending on truck speed to ensure correct droplet size and that the right amount of active ingredient was dispersed through the environment to kill adult mosquitoes upon contact. Malathion 95ULV® was applied between 9:30 p.m. and 6:30 a.m.

In July 2010, citywide treatment with Malathion 95ULV® was performed on the nights of 2, 3, 4, 5, 10, 11, 27, 28, and 29. The municipalities of East St. Paul, Manitoba and West St. Paul, Manitoba were treated on the evenings of 4 and 28. Only treatment events on July 10 and 11 were used in this analysis because they were far enough from other treatment events to relevantly separate dates into before and after treatment and to have untreated experimental control locations (Figure 9). Treatment events also occurred in June and August of 2010 but are not included in this analysis.

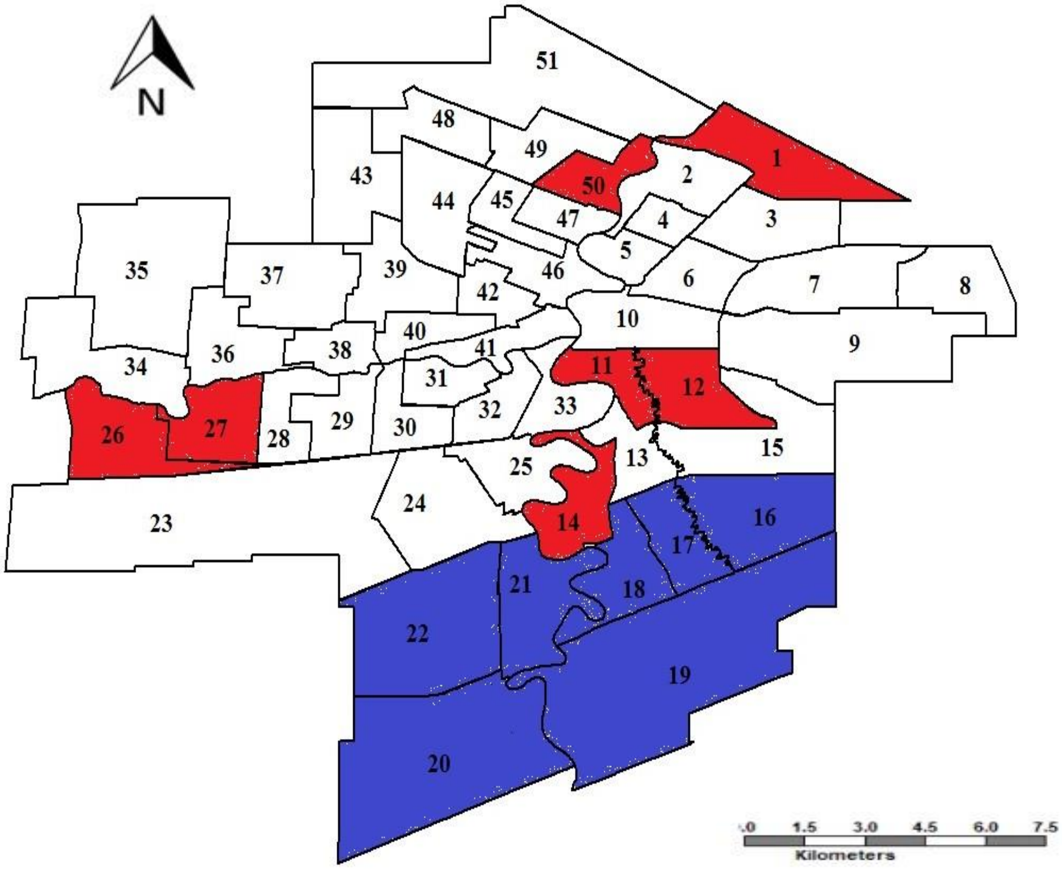


Figure 9. Areas treated with Malathion 95ULV® in Winnipeg, Manitoba on the evenings of July 10 (red) and 11 (purple), 2010. ULV-ground based equipment was used. Polygon treatment area numbers are indicated.

In July 2020, DeltaGard®20EW was applied according to its label (CoW 2022). Ground-based ULV equipment delivered 0.5-1.5 g/ha of active ingredient at a truck speed of 15-20 kph. Spray equipment was adjusted to ensure that droplet diameter was between 8-30 microns to kill mosquitoes upon contact (Brill and Morrison 2013). Re-application was limited to once every 3 days and could not exceed 10 applications per year (Brill and Morrison 2013). The ULV equipment automatically adjusted flow rate to compensate for changes in speed (CoW 2022). DeltaGard®20EW was applied at night between 9:30 p.m. and 6:30 a.m. by the CoW-ICB.

In July 2020, parks and cemeteries were treated with DeltaGard®20EW on the nights of 3, 6, and 14. Only the 14th was included in this analysis because it was distinct enough from other park and cemetery treatment events to relevantly separate dates into before and after treatment (Figure 10). Citywide treatment occurred in rotation from July 24-27 (Figure 11).

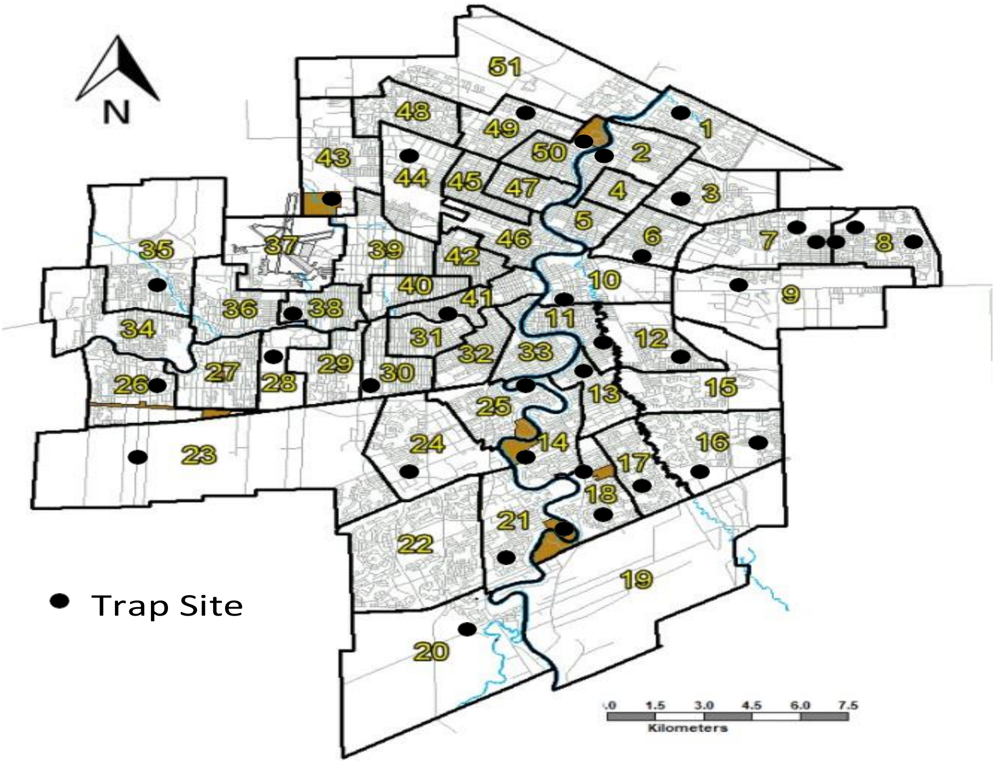


Figure 10. Parks and cemeteries treated (shaded in orange) with DeltaGard®20EW in Winnipeg, Manitoba on July 14, 2020. ULV-ground based equipment was used. Polygon treatment area numbers and trap locations are indicated (CoW 2022).

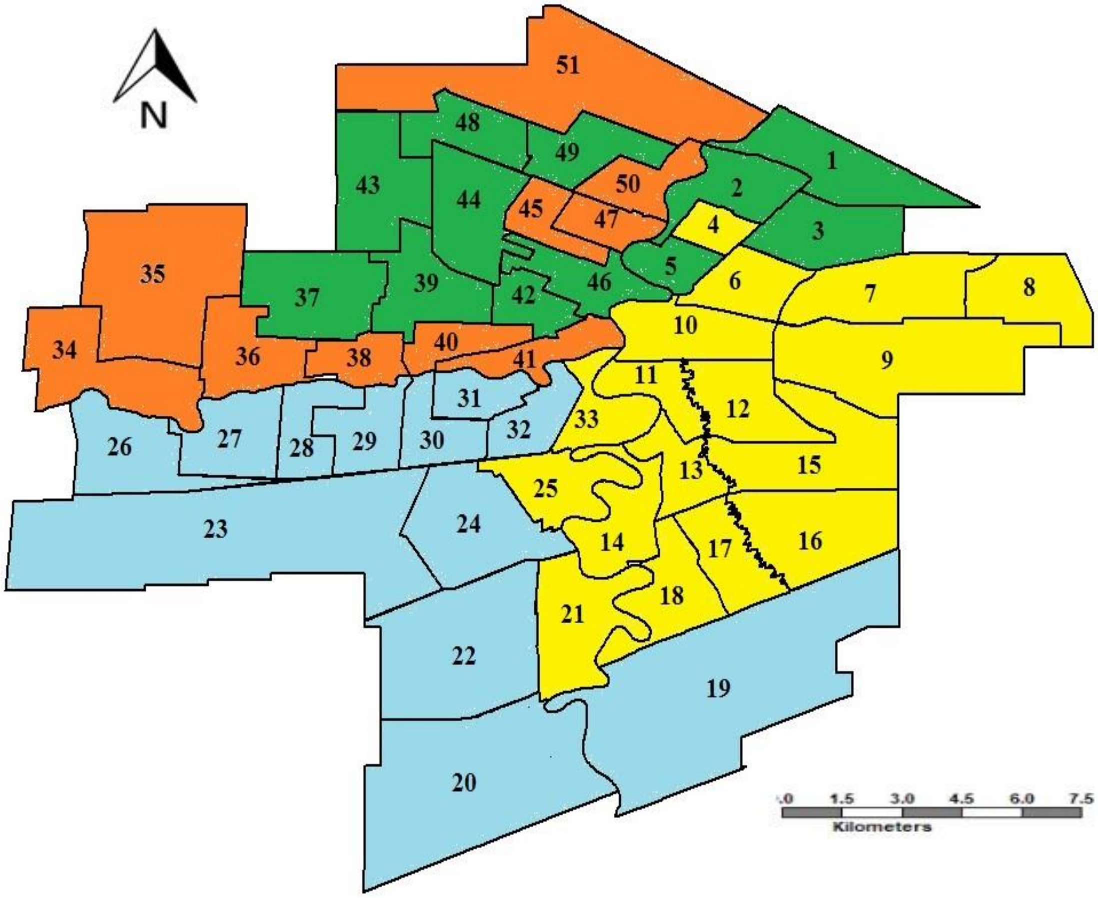


Figure 11. Areas treated with DeltaGard®20EW in Winnipeg, Manitoba on the evenings of July 24 (orange), 25 (green), 26 (yellow), and 27 (blue), 2020. ULV-ground based equipment was used. Polygon treatment area numbers are indicated.

4.5 Trap Collection and Processing

Mosquitoes from NJLTs operated in 2010 and 2020 by the CoW-ICB were counted and sorted by sex and major species. The major species were *Ae. vexans*, *Cx. tarsalis*, *Cx. restuans*, and *Ae. dorsalis* in 2010. *Ae. dorsalis* was replaced by *Cq. perturbans* as a species of interest in 2020. Some mosquitoes were deemed unidentifiable due to their condition, but were included in the total trap catches for analysis. Most of these mosquitoes were assumed to be *Ae. vexans* due to their relative abundance. In 2010 and 2020 respectively, 23% and 42% of the sampled mosquitoes were unidentified.

In 2020, I collected the CDC traps and the mosquitoes were taken back to the lab to be frozen, then counted and sorted into sex and major species. Species were identified using the dichotomous key of Wood, Dang and Ellis (1979). All mosquitoes were identified in my own trap collections except for ~30 samples with well over 400 mosquitoes that were separated into four approximately equal sub-samples. The sub-sample was sorted and counted and multiplied by 4. This method is common in mosquito trap catch processing and is considered reliable (Reinert 1989; Jaworski et al. 2019; Balcaen 2020).

Wing lengths were measured in 2020 samples supplied by the CoW and my own samples that contained 30+ *Ae. vexans*. The samples were from different days and different trap locations. Wings were dissected from 30 randomly selected adult *Ae. vexans* females from each sample and were measured under a microscope. Wing lengths were used as an index of body size to assess a potential correlation between size and longevity.

4.6 Data Entry and Organization

Archival mosquito surveillance data were obtained from the CoW-ICB which had digitized records of NJLT collections from 1991-2022. Treatment events with DeltaGard®20EW in 2020 only occurred in July. I selected 2010 as my historical Malathion 95ULV® comparison year because it had many treatment events in July as well. For the spatial analysis, all the data from July 2010 and 2020 were included (with the exception of the kriging analysis which only included July 2020 data). For the non-parametric ANCOVA analysis (described in Section 4.8), only data two days before and after my selected treatment event dates (2010: July 10 and 11; 2020: July 14 and 24-27) were retained. Treatment event dates were selected if they were far enough from other treatment events to avoid a confound with lingering adulticide effects.

The reduced data set was then cleaned by removing any lines where species identification totals or female and male totals did not add up to total trap count. Any rows with missing or N/A data were also removed. X, Y coordinates were entered for each trap location. The data were divided into two treatment groups (2 days before spray; 2 days after spray). Any traps that fell into both categories for different treatment dates were removed to avoid the confounding effects of lingering adulticide. Traps used as experimental controls that were in untreated locations were designated in the same way (2 days before the traps in the treated locations were sprayed; and 2 days after they were sprayed). *Ae. vexans* made up 63% and females made up 82% of the trap collections so total trap collections were used instead of sub-dividing into sex and species. Unidentified species made up 33% and a large proportion of these were most were likely *Ae. vexans*. The data were entered in Microsoft Excel. Preliminary analysis indicated that the data were not normally distributed but were instead left-skewed with generally low trap count numbers and high outliers.

Spatial data were obtained from the CoW open source maps website in the form of shapefiles for rivers (polyline), trees (point), trap location (point), and parks/open spaces (polygon) for spatial analysis in ArcGIS 10.4.1 (ESRI 2022; CoW 2022). The maps were most recently updated in 2022 and are considered the same for 2010 and 2020 for the purposes of this thesis (CoW 2022).

Adulticide application only occurs under certain meteorological conditions as described previously. A principal component analysis (PCA) was performed in RStudio and was used to reduce the dimensionality of humidity, temperature, wind speed, and humidity in July 2010 and July 2020 by creating uncorrelated principal components that successively explain variance in the dataset (R Core Team 2022). The PCA biplot produced is included in the Appendix (Figure XXVIII). However, only the Richardson International Airport weather station continuously tracks daily weather, so the available data are not specific to trap locations.

The average meteorological conditions from July 2010 are summarized in Table 3. A total of 45,606 mosquitoes were collected and the average daily trap count was 44 (Table 3). The average daily trap counts in July 2010 are shown in Figure 12. Malathion 95ULV® was applied and 37 NJLTs were used. Of the total mosquito trap collections, 70% were *Ae. vexans*, 3% were *Cx. tarsalis*, 23% were unidentified (but likely primarily *Ae. vexans* due to their relative abundance) and <1% were other species. Females composed 87% of the trap collections.

The average meteorological conditions from July 2020 are summarized in Table 3. A total of 160,453 mosquitoes were collected and the average trap count was 105 (Table 3). The average daily trap counts in July 2020 are shown in Figure 12. DeltaGard®20EW was applied and the CoW-ICB operated 41 NJLTs and I operated 11 CDC traps. Of the total mosquito trap collections, 56% were *Ae. vexans*, 42% were unidentified (but likely primarily *Ae. vexans* due to their relative abundance), and <1% were other species. Females composed 76% of the trap collections.

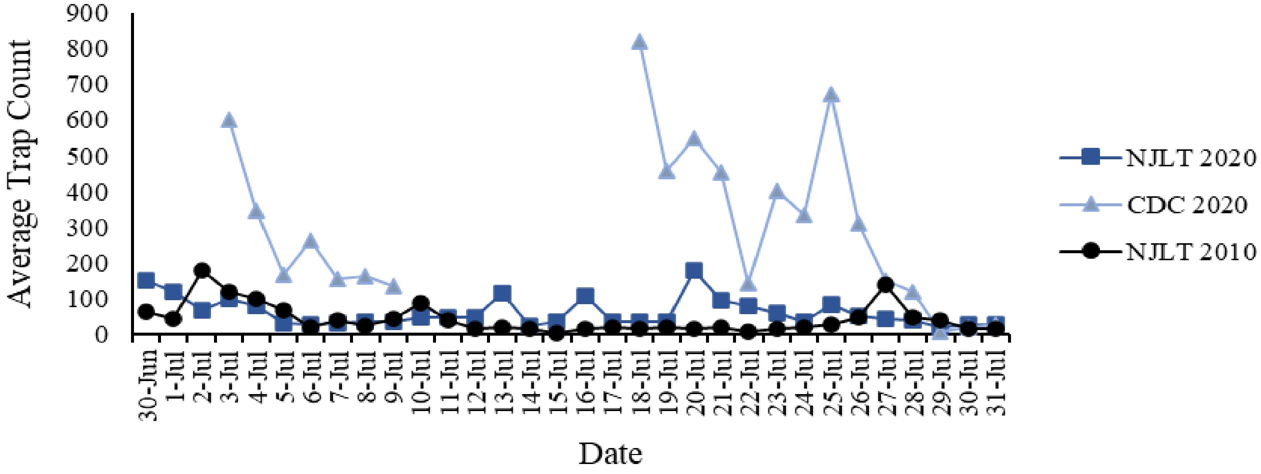


Figure 12. Total average citywide mosquito trap count per day in July 2010 and 2020. Averages are rounded to the nearest whole number and include both traps in treated and untreated locations. CDC and NJLTs are separated into different lines for the 2020 trap count data.

Table 3. Summary of mosquito trap collections in July 2010 and 2020. Most traps ran continuously throughout July of both years.

	July 2010	**July 2020**
Adulticide Applied	Malathion 95ULV®	DeltaGard®20EW
Number of Traps Used	37	53
Total Mosquitoes Collected	45,606	160,453
Average Daily Trap Count	44	105 (CDC=323; NJLT=76)
% *Ae. vexans* in Collection	70%	56%
% Female Mosquitoes in Collection	87%	76%
% Unidentified Mosquitoes in Collection	23%	42%
% Other Species in Collection	3% *Cx. tarsalis* <1% all others	<1% all others
Meteorological Conditions		
Average Precipitation	2.2mm	1.3mm
Average Temperature	20.1°C	21.1°C
Average Humidity	78.4%	73.4%
Average Wind Speed	30.0kph	12.3kph

4.7 Spatial Analysis

Each spatial layer was projected as UTM 14 NAD 1983 Transverse Mercator, and all of the spatial analyses described below were performed in ArcGIS (ArcGIS, Version 10.4.1, ESRI Inc., Redlands, CA). Shapefiles containing the location of rivers, trees, and parks/open spaces in Winnipeg were obtained from the CoW open-source map page (CoW 2022). The following description of the spatial analysis methods used in this thesis are derived from the ESRI website (2022) unless otherwise cited.

Spatial Autocorrelation

Trap count data were tested for daily spatial autocorrelation from July 2010 and July 2020 because patterns in spatial data may change daily depending on mosquito activity and the clustering of high trap counts around Winnipeg. Trap count data that meets the threshold set by CoW (described previously) may lead to adulticide treatment. Spatial autocorrelation was determined using Moran's I analysis which produces three possible spatial patterns in the landscape: 1) Positive correlation 2) Negative Correlation 3) Random.

Positive spatial autocorrelation means that the spatial data are significantly clustered and is determined by a p-value ≤ 0.10 and a z-value ≥ 1.65 and a positive Moran's index. Positive spatial autocorrelation means that geographically nearby values of a variable tend to be similar on a map. High values tend to be located close to other high values and low values near low values. Negative spatial autocorrelation means that the data are significantly dispersed. Significance is determined by a p-value ≤ 0.10 and a z-value ≤ -1.65 and a negative Moran's index. Negative spatial autocorrelation refers to a geographic distribution of values, or a map pattern, in which the neighbors of locations with large values have small values. Any other p-value and z- score combination means that the spatial data are random and are not spatially autocorrelated. In this case, the data is random and there is no pattern to the distribution.

Moran’s I analysis measures spatial autocorrelation based on the location of the features being measured (traps) and their attribute values (trap count) using the Global Moran’s I statistic. This tool determines the mean and variance for a trap count and then subtracts the mean trap count from all the adjacent trap locations to create a value for the deviation from that mean. The deviation values are multiplied together for all neighboring features to create a cross-product that is included in the numerator for the Global Moran’s I statistic. When trap counts in neighboring traps are either all larger than the mean or all smaller than the mean, the cross-product is positive. When some neighboring trap counts are smaller and some are larger than the mean, the cross-product will be negative. The larger the deviation from the mean, the larger the cross-product value. The Moran’s index, z-score, and p-value are then computed to indicate if the spatial pattern is significantly clustered or dispersed.

Hot Spot Analysis

Hot spot analysis was applied to all daily trap count data from July 2010 and July 2020. Hot spot analysis identifies statistically significant hot and cold spots (i.e. higher than normal trap counts and lower than normal trap counts respectively) using Getis-Ord Gi* statistic. The output of the analysis includes confidence level bins for each of the determined hot spots (90%, 95%, and 99% confident hot spots). The hot spot analysis tool works by looking at each trap within the context of neighboring traps. A trap with a high trap count may be interesting but may not be a statistically significant hot spot because it is not surrounded by other traps that also have high trap counts.

The sum of an individual trap count and its neighbors is called the “local sum”. This local sum is compared proportionally to the sum of all the other trap location counts on the landscape (expected local sum). A statistically significant z-score indicates that the local sum is very different from the expected local sum and that the difference is too large to be the result of random chance. The higher a statistically significant positive z-score, the more intense the clustering of high values indicating a hot spot. The lower the statistically significant negative z-score, the more intense the clustering of low values indicating a cold spot. The intensity of these hot and cold spots is binned into different confidence levels as summarized in Table 4.

Table 4. Critical z-scores for different confidence levels when Getis-Ord Gi* statistic is used to determine hot spots and cold spots in a landscape based on attribute values (i.e. mosquito trap counts) of locations (i.e. mosquito traps).

Z-Score	**Confidence Level**
< -1.65 or > +1.65	90%
< -1.96 or >+1.96	95%
<-2.58 or >+2.58	99%

I used an inverse distance band so that all features (traps) were considered neighbors of all other features. Hot spot trap locations from 2010 and 2020 were then compared by summing the number of hot spots in July for each trap location regardless of confidence level and year. The features surrounding hot spot traps were compared to non-hot spot traps to check for similarities and differences in their locational attributes as described next. Hot spots with confidence levels of 90%, 95%, and 99% were all included. For instance, trap number 11 was a hot spot on 4 different days in July 2010 and was a hot spot on 4 different days in July 2020 meaning that it had been a hot spot a total of 8 times during the experimental trials. Any traps that had been a hot spot at any time during these two experimental months were designated to the frequent hot spot group versus locations that were never hot spots (non-hot spot locations).

Distance and Proximity Analysis

Buffer analysis creates polygons around input features (trap locations) to a specified distance. Multi-ring buffer analysis was used to create arbitrary 50m, 100m, and 150m circular buffers around each trap location from July 2010 and July 2020. Many of these traps remained in the same locations for both years. The number of trees and the number of parks/open spaces within each concentric radial band around these traps were estimated using the "joins" feature in ArcGIS 10.4.1(ESRI 2022). This count is an estimation because the CoW open source inventory does not include most of the trees within parks or surrounding traps located outside of the city. For this reason, those traps were not included in the tree density analysis.

The "Generate Near Table" analysis tool was then used to calculate proximity information between features in one or more feature class or layer. This tool was used to check proximity of each trap to the nearest river and nearest park/open space in meters. The names of the rivers and parks/open spaces were also generated. The "joins" feature was used to connect this information to the specific trap locations. For this analysis, it was important to set the same equidistance projected coordinate system for all layers in the analysis to ensure scale accuracy. All layers were projected in UTM 14 NAD 1983 Transverse Mercator. Direct distances were measured without respect to barriers such as roads and buildings.

The information from the multi-ring buffer analysis and near table analysis were entered in Microsoft Excel to compare similarities and differences amongst trap locations. Kruskal-Wallis analysis (a non-parametric version of ANOVA) was performed in RStudio on each of the above described landscape features to compare trap locations that were and were not hot spots on any day in July 2010 or 2020 (RStudio, Version 4.1.0, R Core Team, Vienna, Austria). This may explain locational differences in adulticide efficacy and mosquitopopulation dynamics and/or mosquito activity patterns generated by day-to-day weather variation.

Kriging Analysis

Kriging analysis was applied to daily trap count data from July 2020 at each of the trap locations. Kriging generates an interpolation surface from a scattered set of points with attributes (z-values). Kriging is based on the assumption of randomness and the selective placement of mosquito traps in Winnipeg hinders its use in our study. Kriging is distinct from other interpolative tools because itinvolves an investigation of spatial interactions between trap location and the associated trap countdata. Kriging is most appropriate when spatial autocorrelation occurs and assumes that significantclustering or dispersal of data can be used to explain variation in the landscape to predict values (i.e. trap count data) in locations that are not explicitly measured. These predicted values are the expected values at each of the unmeasured locations.

I standardized the color scale in the kriging outputs so that the same color represented the same mosquito trap count range in each day in July 2020. To test the accuracy of the kriging surface, I performed a leave-one-out cross validation where random traps were removed and expected and observed results were compared. Ideally, expected and observed results would create a 1:1 linear relationship when graphed against one another. Cross-validation is a jackknifing method that tests the validity of a model by removing each known value (i.e. mosquito trap) subsequently and re-producing the kriging interpolative surface without each data point. The predicted value at the location where the mosquito trap has been removed is then compared to its actual known value in leave-one-out cross validation analysis to measure the accuracy of the interpolative surface.

4.8 Non-Parametric Model Development: T.aov

A non-parametric version of the ANCOVA was used to test the equality of non-parametric curves based on an ANOVA-type statistic using RStudio (RStudio, Version 4.1.0, R Core Team, Vienna, Austria). The “T.aov” function in the “fANCOVA” package in RStudio was used to determine the effect of DeltaGard®20EW and Malathion 95ULV® on adult mosquito activity. “T.aov” compares two non-parametric curves with an automatic smoothing parameter selected using AICc. A bootstrap of 20,000 was applied. The same trap locations were sampled over a 4-day period surrounding an adulticide event in both treated and untreated locations. The post-spray trap count was compared to check for significant differences ($p < 0.05$) between traps in treated and untreated locations while controlling for baseline mosquito activity (pre-treatment trap collections) by including this as a covariate.

Total trap count data was used regardless of sex or species of the collected mosquitoes. Data were classified as “treated” or “control” (untreated) and into pre-treatment (2 days before treatment date) and post-treatment (2 days after treatment date) subgroups. Any data that did not fall in to one of these categories were removed. The covariate was the 2-day pre-treatment collection to control for baseline mosquito activity or presence in trap catches. The effect assessed for significance ($p < 0.05$) was the post-treatment trap count (mosquito activity) and how it was affected by adulticide use after controlling for baseline population activity.

For the 2010 data, T.aov tests were run for the treatment events on July 10 and 11. Malathion 95ULV® was applied on both evenings. For the treatment event on July 10, any IMA polygons that were sprayed were designated as within treatment. The July 9 and 10 trap collections were designated as pre-treatment (baseline) because the trap collection for the evening of the 10th when the spray event occurred would be collected on the morning of the 11th. The July 11 and 12 trap collections were designated as post-treatment. Any IMA polygons that were treated on the night of the 11th were removed from the analysis because they could not be included as true untreated, experimental control trap locations. This procedure was repeated for July 11. The procedure described above was also applied to the trap collection data from July 14 and 24-27 of 2020 with DeltaGard®20EW application. DeltaGard®20EW and Malathion 95ULV® events were compared and the 4-day events were graphed to show the change in daily mosquito activity.

The Wilcoxon signed rank test was then applied to data obtained from traps in the treatment group to determine if daily changes in mosquito activity were significant ($p < 0.05$). RStudio was used for this analysis (RStudio, Version 4.1.0, R Core Team, Vienna, Austria). The following daily changes were tested for significance:

1) 2 days prior to 1 day post adulticide
2) 2 days prior to 2 days post adulticide
3) 1 day prior to 1 day post adulticide
4) 1 day prior to 2 days post adulticide

4.9 Wing Length Analysis

Mosquito trap collections from July 3 to August 21 of 2019 were scanned to find population peaks. Any samples within these population peaks (from different days and locations) with 30+ *Ae. vexans* were separated. Thirty *Ae. vexans* were randomly selected from each of these samples and their wing lengths were measured in micrometers under a microscope. The average wing length and the longevity of each selected cohort was determined (average cohort wing length). The overall average wing length of all the *Ae. vexans* measured was also determined to be 3.98 micrometers (overall average wing length). Longevity was recorded as the number of days to 90% depletion of the trap count. The data were entered in Microsoft Excel.

For each sample of wing lengths, an average, median, and measure of skew was determined. Given that the wing length distributions were normal, longevity and wing length data were analyzed with ANOVA to determine whether any of the distributional characteristics of wing length explain variations in longevity or cohort persistence. ANOVA was performed in RStudio (RStudio, Version 4.1.0, R Core Team, Vienna, Austria). The groups tested for significant differences with ANOVA were cohorts with an average wing length less than the overall average wing length of *Ae. vexans* in Winnipeg, cohorts with an average wing length greater than the overall average wing length, and cohorts that were of the same value as the overall average wing length. First, average wing length of all the *Ae. vexans* from 2020 was determined to be 3.98 micrometers (overall average wing length). Next, the average wing length of each individual sample of 30+ *Ae. vexans* from different trap locations and dates (a cohort) was determined (average cohort wing length). The difference in average wing length of the three groups were also visualized with violin plots. In the violin plots, each dot indicates the average wing length of a cohort of 30+ *Ae. vexans*.

5.0 Results

5.1 Spatial Analysis

Spatial Autocorrelation

When historical trap count data from July 2010 were analyzed, Moran's I tests revealed spatial autocorrelation and more specifically, spatial clustering ($p \leq 0.10$) of trap count data on July 25 ($p = 0.05$), 27 ($p = 0.00$), 28 ($p = 0.07$) and 29 ($p = 0.01$). When trap count data from July 2020 were analyzed, Moran's I tests revealed spatial autocorrelation and more specifically, spatial clustering ($p \leq 0.10$) of trap count data from July 2020 on July 3 ($p = 0.00$), 14-26 ($p = 0.00$), and 28-31 ($p = 0.00$). Moran's I output (Moran's I, z-score, p-value, variance, and expected I) for daily spatial autocorrelation tests for July 2010 and July 2020 are summarized in Tables I and II in the appendix. These tables show that significant clustering of trap count data was detected during the treatment events from July 27-29, 2010 and surrounding all the treatment events in July 2020 except for the 6th.

Hot Spot Analysis

In 2010, 64 hot spots were revealed in July and were limited to 16 trap locations (of the 37 traps operated that year) as summarized in Table 5. This means that 43% of sites were hot spots on 4 of the 31 days in July 2010 or 13% of the days in that month. In July of 2020, 97 hot spots were revealed and were limited to 25 trap locations (of the 53 traps operated that year) (Table 5). This means that 48% of sites were hot spots on 8 of the 31 days in July 2020 or 58% of the days in that month. Several of the trap locations were hot spots on more than one date. The confidence level of each of the hot spots per location and year is included in the appendix (Table III). Daily maps showing the approximate location of all the experimental mosquito traps and whether they were detected as hot spots are included in the appendix (Figure I for 2010; Figure II for

2020). The confidence levels of the hot spots decrease after adulticide is applied and disappear in approximately 2-3 days post-treatment. There were no cold spots in either year.

Table 5. The number of traps that were hot spots based on their mosquito trap count in July 2010 and July 2020. This table combines hot spots of all confidence levels (90%, 95%, 99%). Blanks indicate that no hot spots occurred or that the trap was not used that year. Only traps that were hot spots on any day in either year are included. For example, trap number 11 was detected as a hot spot on 4 different days in July 2010 and on 4 different days in July 2020.

Trap Number	2010	2020	Total
1		2	2
2		8	8
3		2	2
4		4	4
5		3	3
7		6	6
8		1	1
10		8	8
11	4	4	8
15	2	1	3
17	6		6
18	1		1
19	2		2
20	1	7	8
25		3	3
26	1		1
27	2		2
29	8		8
30	1	3	4
34		3	3
35	13		13
37		5	5
39	1	1	2
40		3	3
41	2		2
42	3		3
43	3	1	4
44	14	8	22
52		1	1
54		5	5
56		1	1
61		3	3
63		13	13
64		1	1

Distance and Proximity Analysis

Table IV in the appendix summarizes the output of the multi-ring buffer and “near” analyses. The number of trees and open/spaces within 50m, 100m, and 150m radii surrounding trap locations, the distance in meters to the closest river and park/open space, and the names of those rivers and parks/open spaces for each trap location are included (Table IV).

The results of the Kruskal-Wallis analysis tests for differences in landscape features between hot spot locations and non-hot spot locations were not significant (Table 6). Figures 13-15 depict these indices and are included despite their lack of a significant difference because some of the violin plots indicate trends in higher mosquito trap counts (hot spots) associated with some of the spatial features.

Table 6. Summary of Kruskal-Wallis test outputs for landscape features comparing mosquito hot spot locations and non-hot spot locations from July 2010 and 2020 combined.

Spatial Feature	p-value
Number of Trees within 50m	0.155
Number of Trees within 100m	0.833
Number of Trees within 150m	0.342
Number of Parks/Open Spaces within 50m	0.529
Number of Parks/Open Spaces within 100m	0.895
Number of Parks/Open Spaces within 150m	0.854
Distance to Nearest River (m)	0.085
Distance to Nearest Park/Open Space (m)	0.591

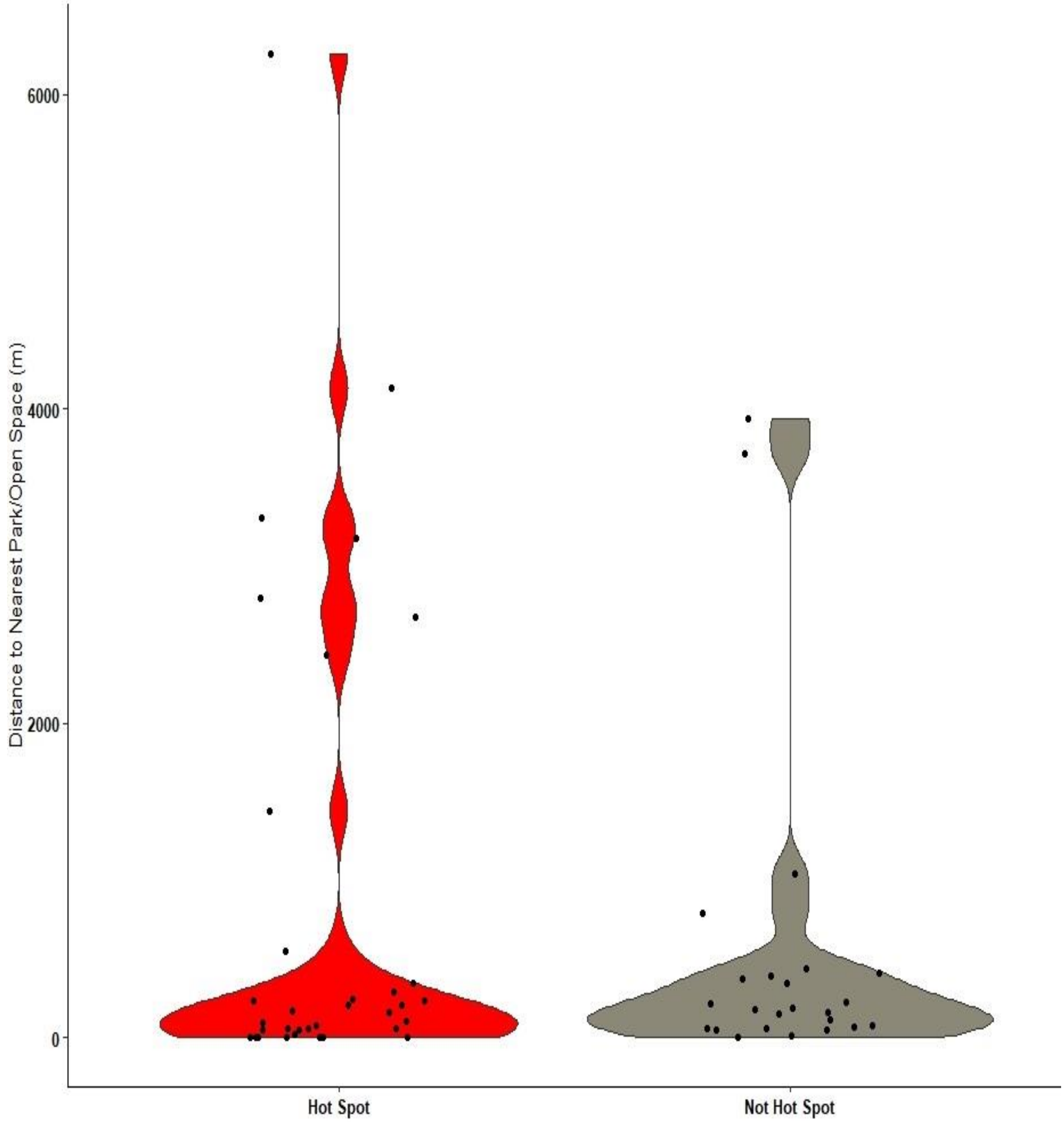


Figure 13. Distance (m) from mosquito trap locations from July 2010 and 2020 to the nearest park/open space. Trap locations that were hot spots at some point in either month are in red (left) and traps that were never hot spots during those two months are in grey (right).

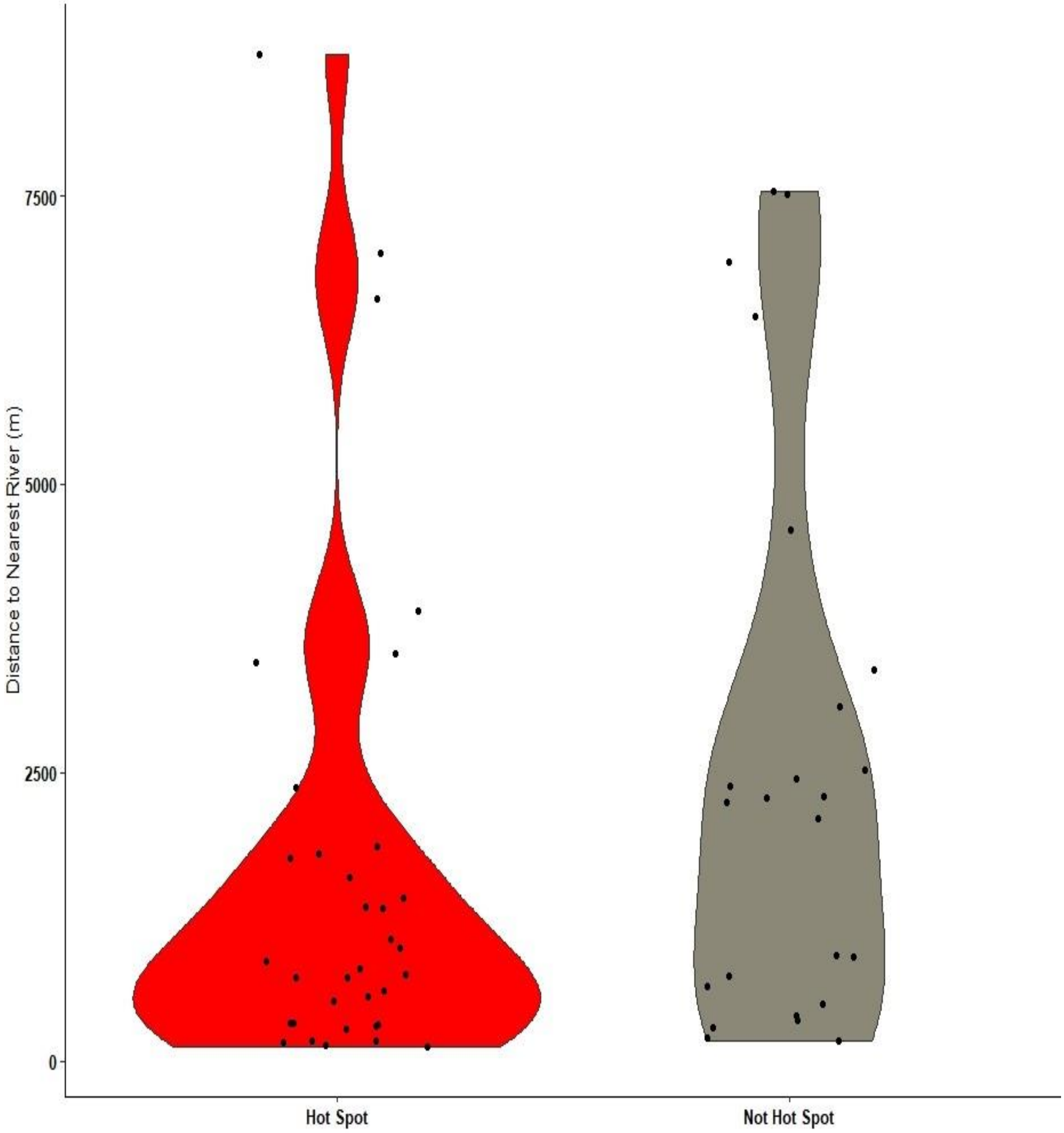


Figure 14. Distance (m) from mosquito trap locations from July 2010 and 2020 to the nearest river. Trap locations that were hot spots at some point during either month are in red (left) and traps that were never hot spots during those two months are in grey (right).

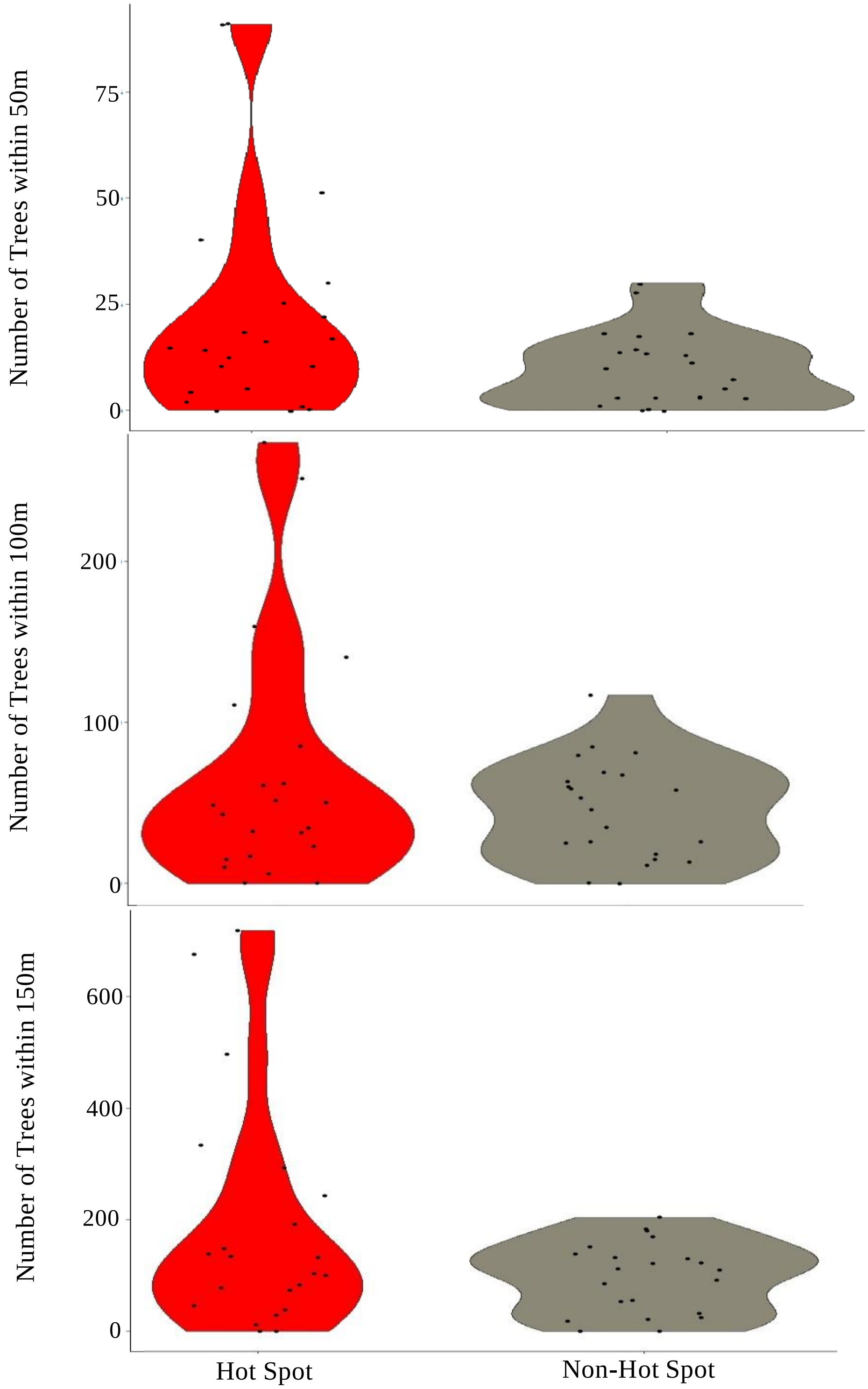
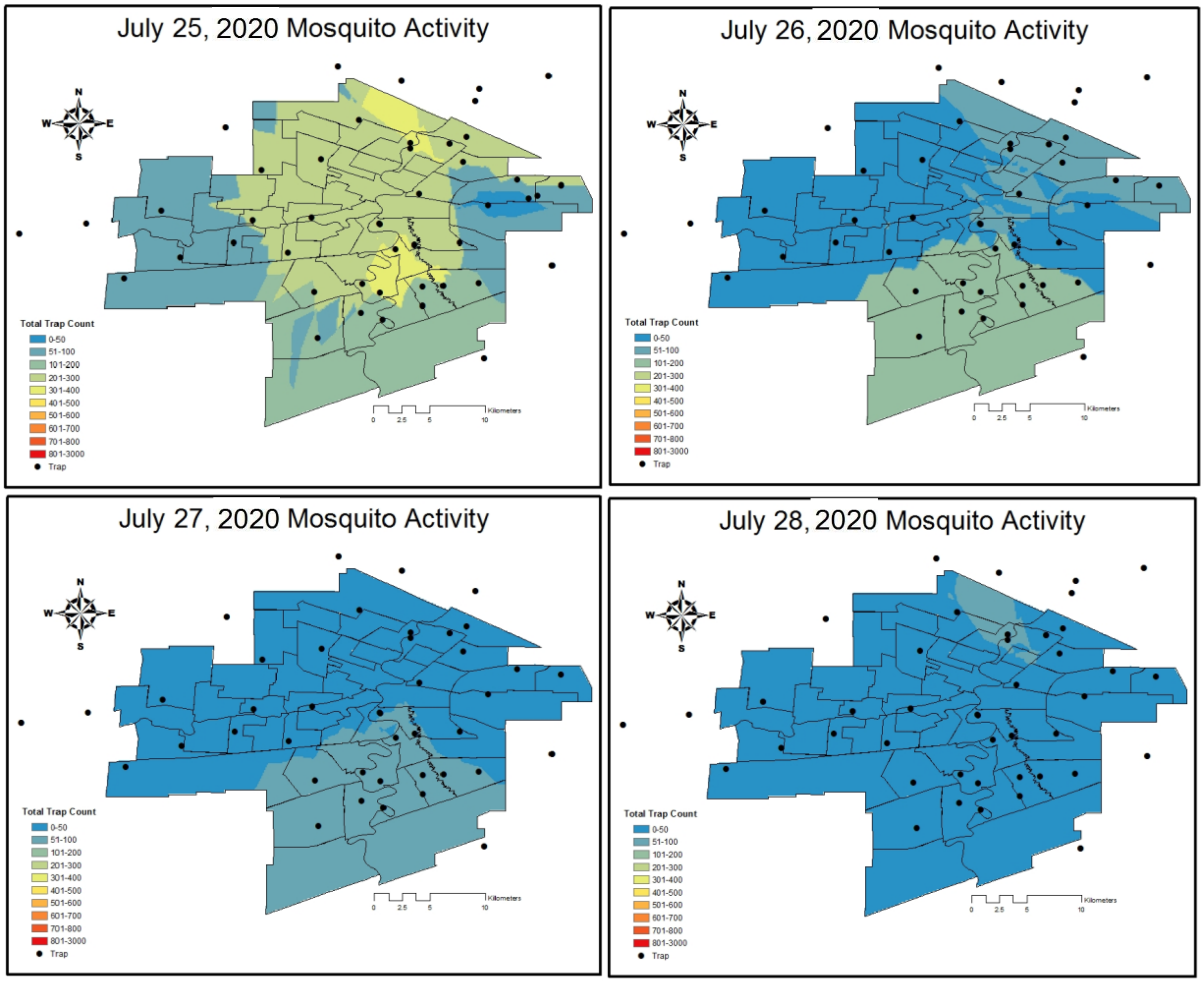


Figure 15. The number of trees in a 50m, 100m, and 150m radius surrounding mosquito trap locations from July 2010 and July 2020. Trap locations that were hot spots at some point during either month are in red (left) and traps that were never hot spots during those two months are in grey (right). Note that the y-axis scale differs among radii distances.

Kriging Analysis

Daily kriging maps are available in the appendix (Figures III – XXVII). The interpolative surfaces show an increase in mosquito activity near parks and cemeteries before theyare treated on July 3 and 6. However, this pattern is not detected surrounding the July 14 treatment event. The interpolative surface shows a decrease in mosquito activity occurring in areas of the city that are treated with DeltaGard®20EW on the nights of the 24th and 26th (northern part of Winnipeg), the night of the 26th (eastern), and the night of the 27th (south-western) (Figure 16). See Figure 11 in methods for the treatment pattern.


July 25, 2020 Mosquito Activity

July 26, 2020 Mosquito Activity

July 27, 2020 Mosquito Activity

July 28, 2020 Mosquito Activity

Figure 16. Kriging interpolative surfaces showing the change in mosquito activity (low = blue, high = yellow) estimated from trap count data. The northern part of the city was treated on the evenings of the 24th and 25th, the east on the 26th, and the south-west on the 27th.

Leave-one-out cross-validation analysis output is shown in Figure 17 for July 25, 26, 27, and 28, 2020. The general trend shows that kriging analysis overestimated small trap count values and underestimated large trap count values with its predictions. The blue regression lines shown in the figures below deviate significantly from an ideal 1:1 relationship between measured and predicted values.

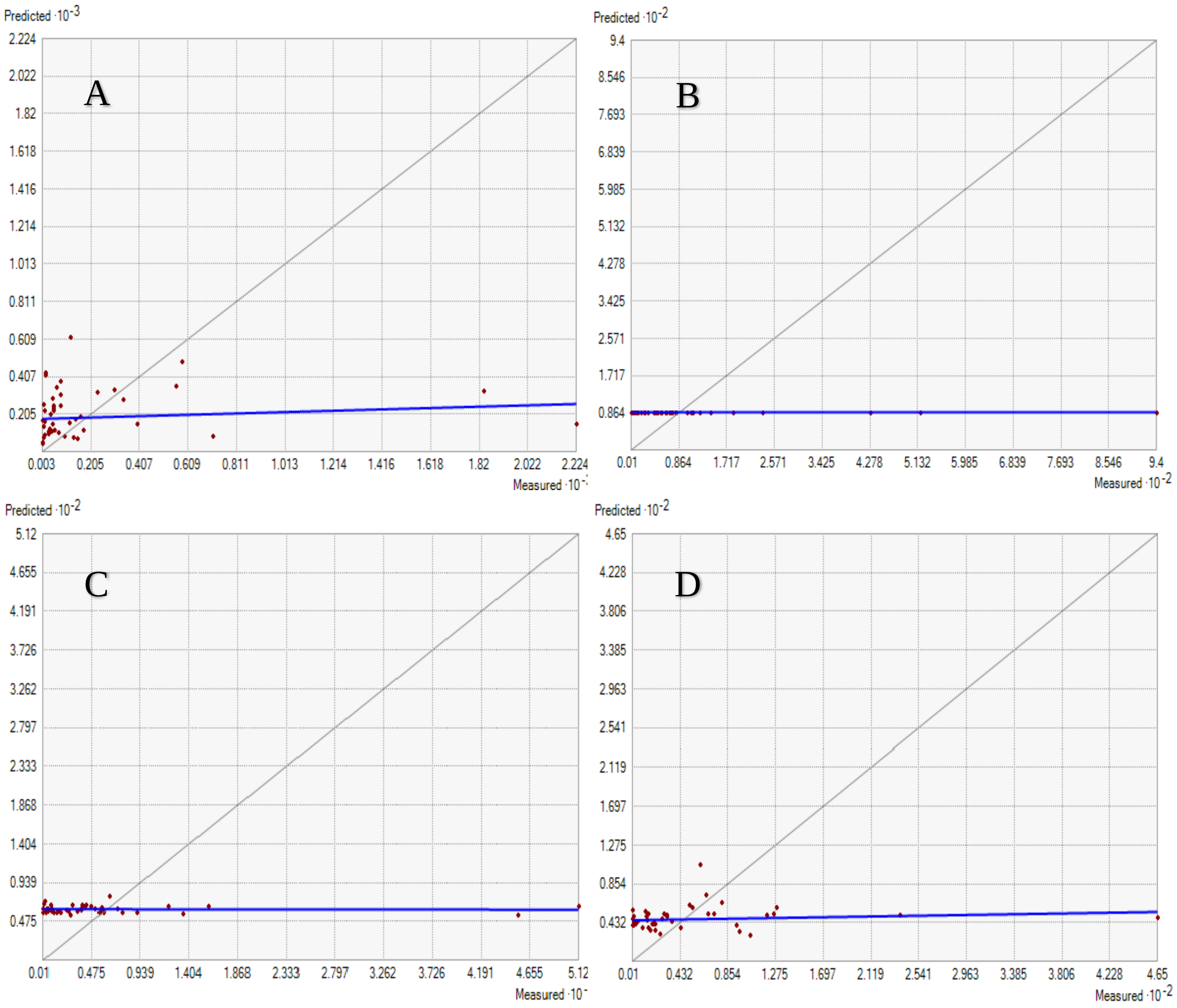


Figure 17. Predicted versus measured plots of kriging surfaces on July 25 (A), 26 (B), 27 (C), and 28 (D), 2020. The grey lines represent the ideal 1:1 relationship between measured and predicted values, the blue lines represent the actual regression lines calculated in each leave-one-out cross-validation.

5.2 T.aov Analyses

2010: Malathion 95ULV® Treatments

Treatment Evening: July 10, 2010

Figures 18 and 19 show the average daily and total daily mosquito activity in treatment and non-treatment locations for the treatment evening of July 10, 2010. T.aov shows that the post-spray count did not differ significantly between traps in untreated and treated locations after controlling for baseline variation in trap catches (pre-treatment count) (p = 0.4157) (Table 7). Wilcoxon signed rank test of temporal changes in the treatment group show that mosquito activity decreased significantly from July 10 to July 12 (p = 0.01563), from July 10 to July 11 (p = 0.01563), and from July 9 to July 12 (p = 0.02539). The change in mosquito activity from July 9 to July 11 was not significant (p = 0.1563).

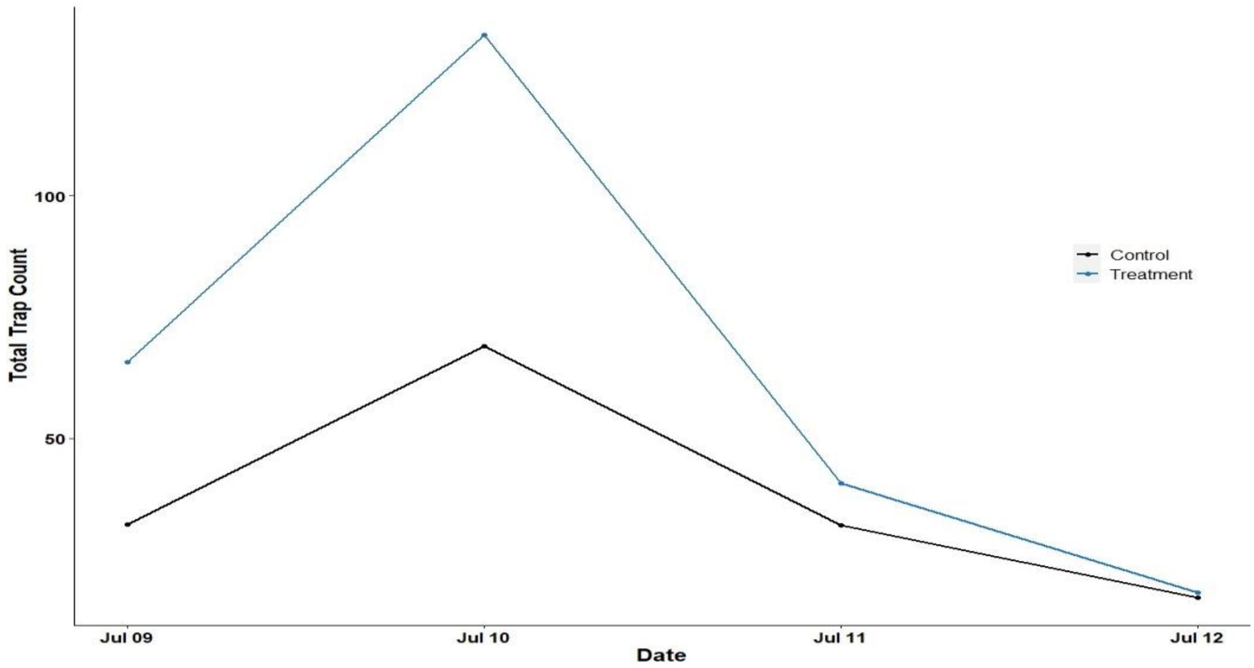


Figure 18. Average daily mosquito activity at trap locations treated with Malathion 95ULV® (blue) and in untreated control sites (black) when adulticide was applied on the evening of July 10, 2010.

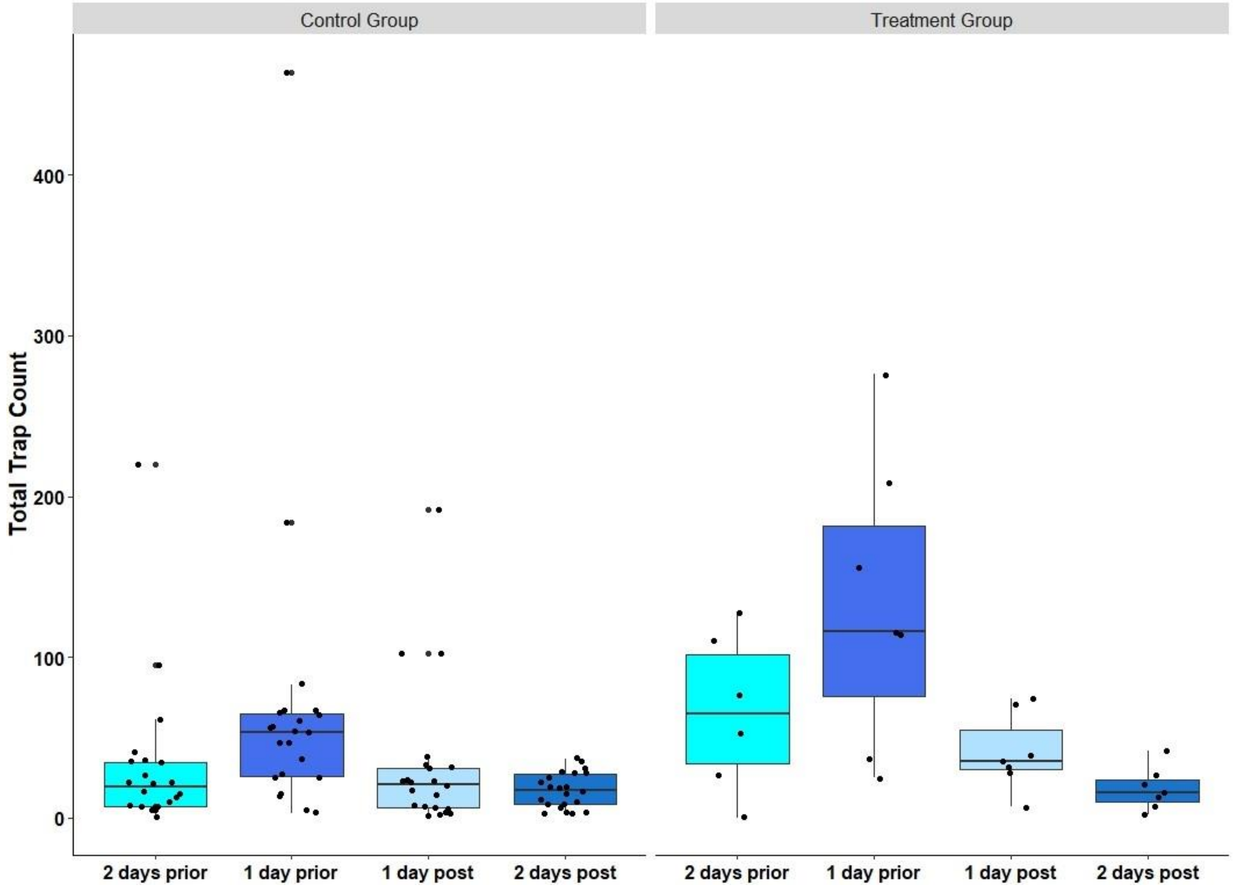


Figure 19. Daily changes in mosquito activity at trap locations in untreated (left) and treated (right) groups 2 days before and 2 days after treatment with Malathion 95ULV® on the evening of July 10, 2010. Trap collection data from individual trap locations are shown with black dots.

Treatment Evening: July 11, 2010

Figures 20 and 21 show the average daily and total daily mosquito activity in treatment and non-treatment locations for the treatment evening of July 11, 2010. T.aov shows that the post-spray count did not differ significantly between traps in untreated and treated locations after controlling for baseline variation (pre-treatment count) ($p = 0.0885$) (Table 7). Wilcoxon signed rank test of temporal changes in the treatment group shows that there are no significant daily changes in mosquito activity.

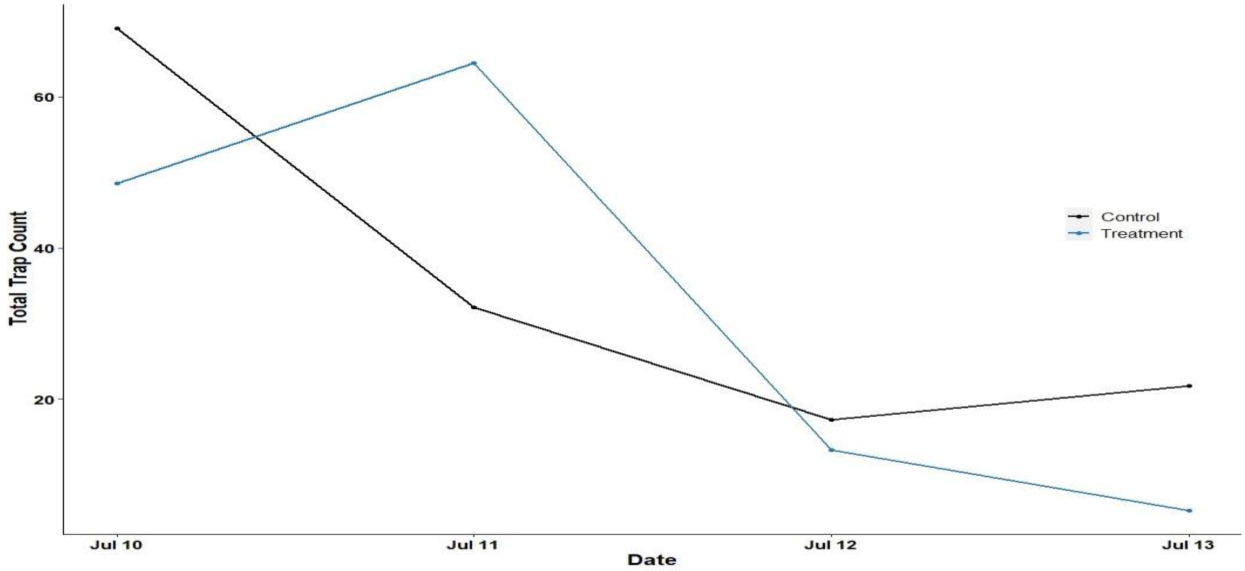


Figure 20. Average daily mosquito activity at trap locations treated with Malathion 95ULV® (blue) and in untreated control sites (black) when adulticide was applied on the evening of July 11, 2010.

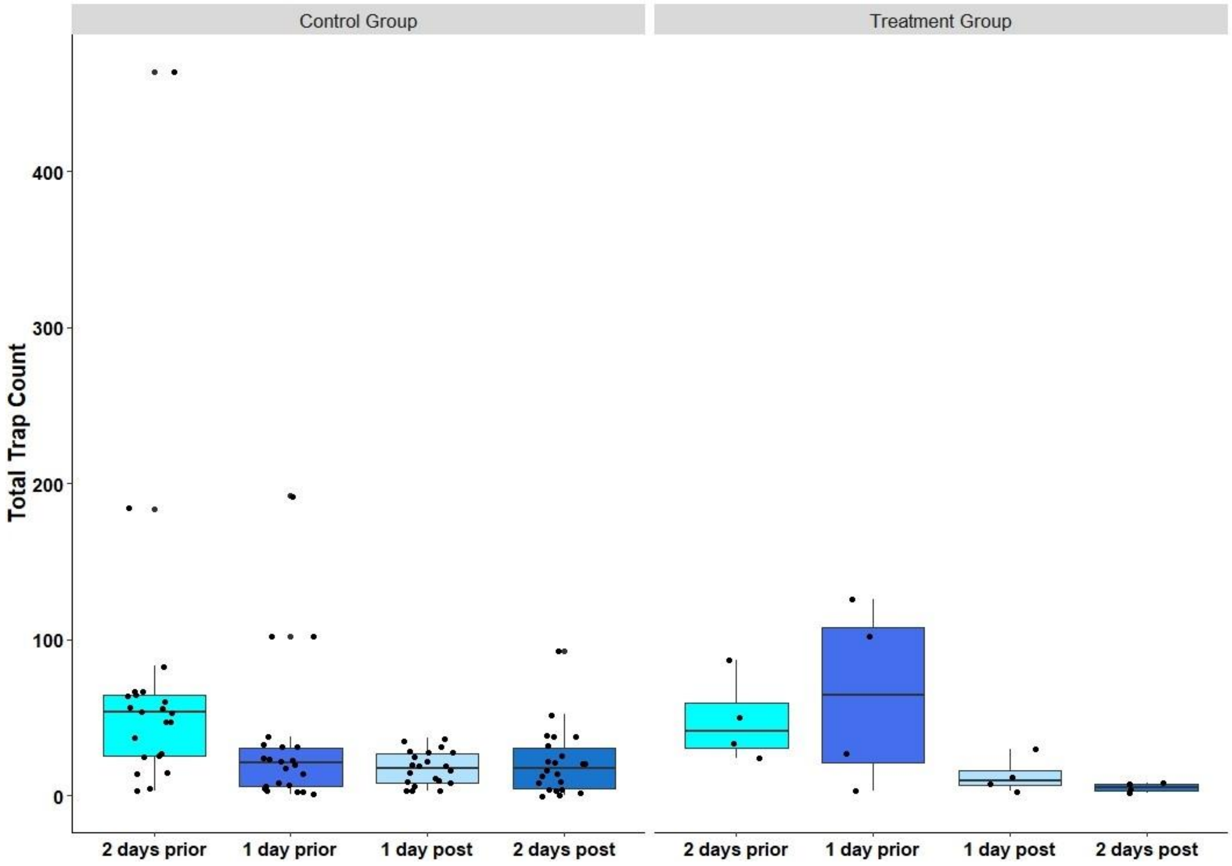


Figure 21. Daily changes in mosquito activity at trap locations in non-treatment (left) and treatment (right) groups 2 days before and 2 days after treatment with Malathion 95ULV® on the evening of July 11, 2010. Trap collection data from individual trap locations are shown with black dots.

2020: DeltaGard®20EW Treatments

Treatment Evening: July 14, 2020: Parks and Cemeteries

Figures 22 and 23 show the average daily and total daily mosquito activity in treatment and control locations for the treatment evening of July 14, 2010. T.aov shows that the post-spray count did not differ significantly between traps in untreated and treated locations after controlling for baseline variation (pre-treatment count) (p = 0.3682) (Table 7). Wilcoxon signed rank analysis of temporal changes in the treatment group shows that there were no significant daily changes in mosquito activity.

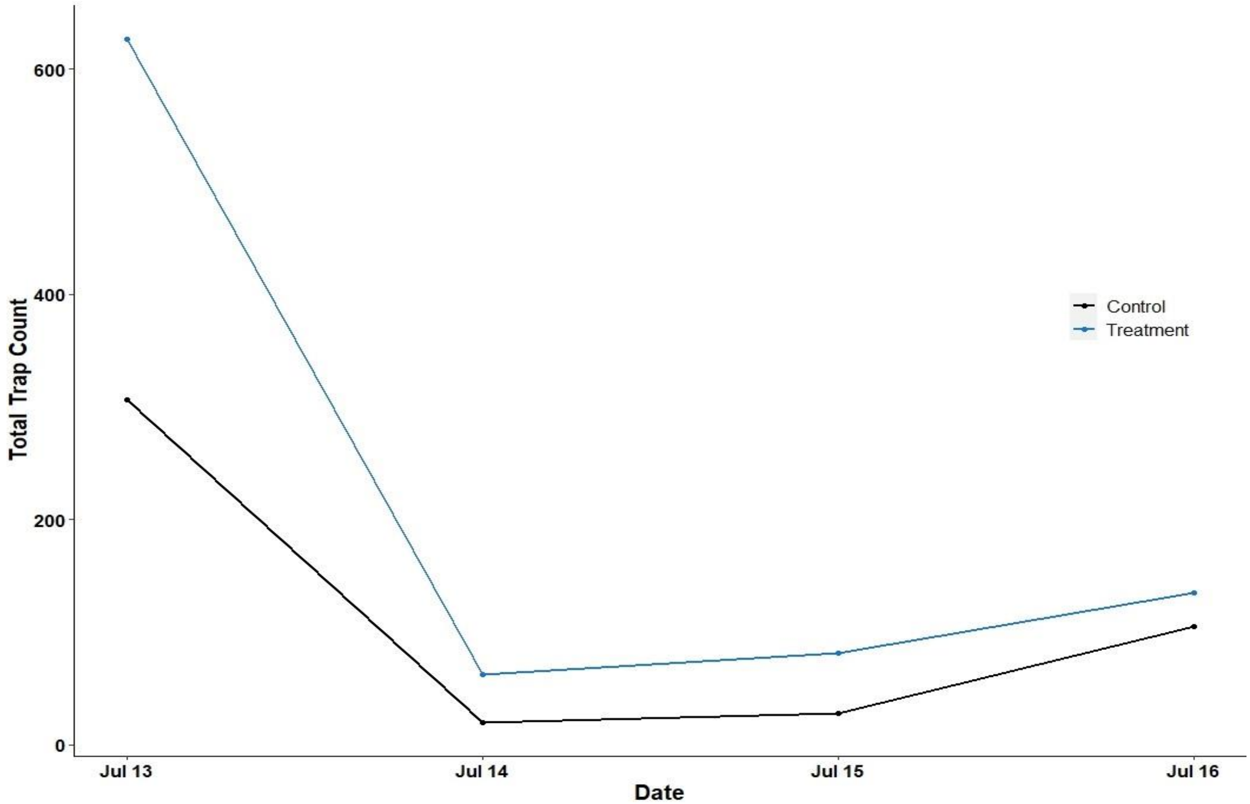


Figure 22. Average daily mosquito activity at trap locations treated with DeltaGard®20EW (blue) and in untreated control sites (black). Only parks and cemeteries were treated on the evening of July 14, 2020.

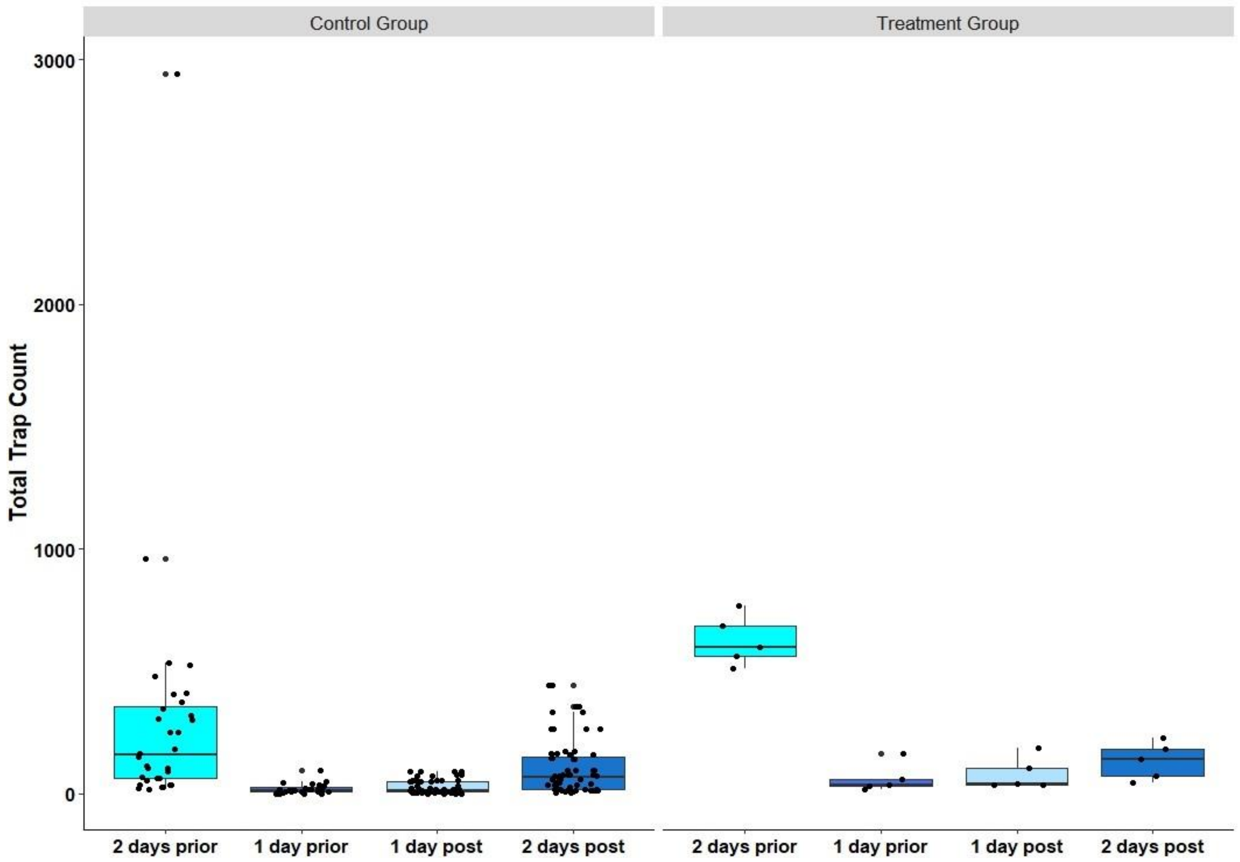


Figure 23. Daily changes in mosquito activity at trap locations in non-treatment (left) and treatment (right) groups 2 days before and 2 days after treatment with DeltaGard®20EW on the evening of July 14, 2020. Trap collection data from individual trap locations are shown with black dots. Only parks and cemeterieswere treated.

July 24-27: Rotational City-Wide Treatment

Treatment Evening: July 24, 2020

Figures 24 and 25 show the average daily and total daily mosquito activity in treatment and non-treatment locations for the treatment evening of July 24, 2020. T.aov shows that the post-spray count did not differ significantly between untreated and treated traps after controlling for baseline variation (pre-treatment count) (p = 0.4406) (Table 7). Wilcoxon signed rank analysis of temporal changes in the treatment group shows no significant daily changes in mosquito activity.

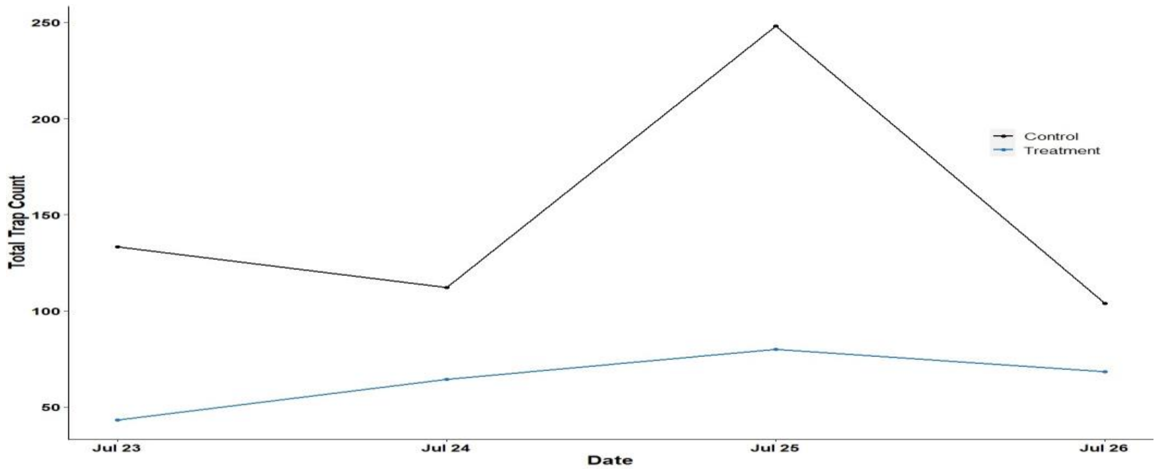


Figure 24. Average daily mosquito activity at trap locations treated with DeltaGard®20EW (blue) and in untreated control sites (black) when adulticide was applied on the evening of July 24, 2020.

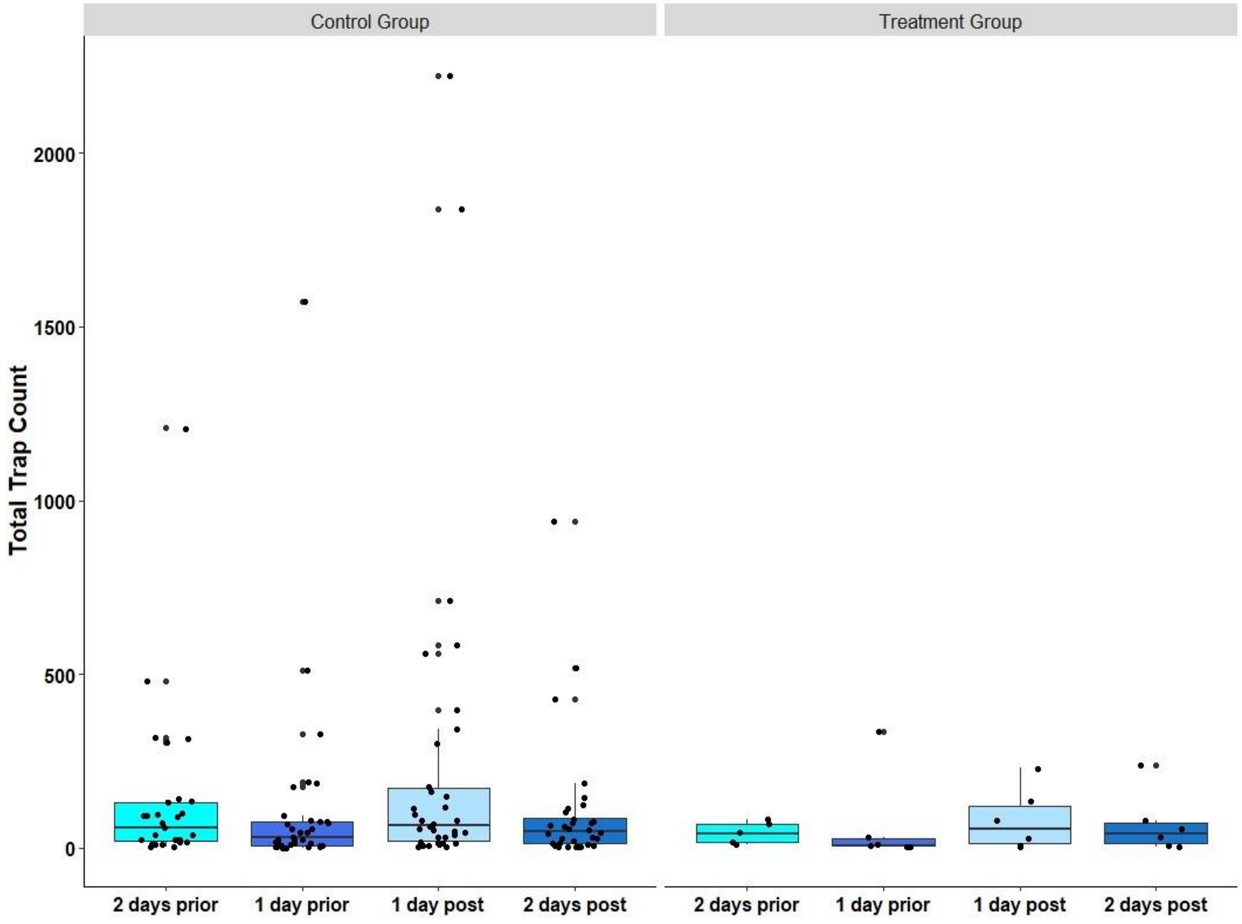


Figure 25. Daily changes in mosquito activity at trap locations in non-treatment (left) and treatment (right) groups 2 days before and 2 days after treatment with DeltaGard®20EW on the evening of July 24, 2020. Trap collection data from individual trap locations are shown with black dots.

Treatment Evening: July 25, 2020

Figures 26 and 27 show the average daily and total daily mosquito activity in treatment and non-treatment locations for the treatment evening of July 25, 2020. T.aov shows that the post-spray count did not differ significantly between untreated and treated traps after controlling for baseline (pre-treatment count) (p = 0.7164) (Table 7). Wilcoxon signed rank test of temporal changes in the treatment group shows a significant decrease in mosquito activity from July 24 to July 26 (p = 0.01782). All the other daily changes in mosquito activity were not significant.

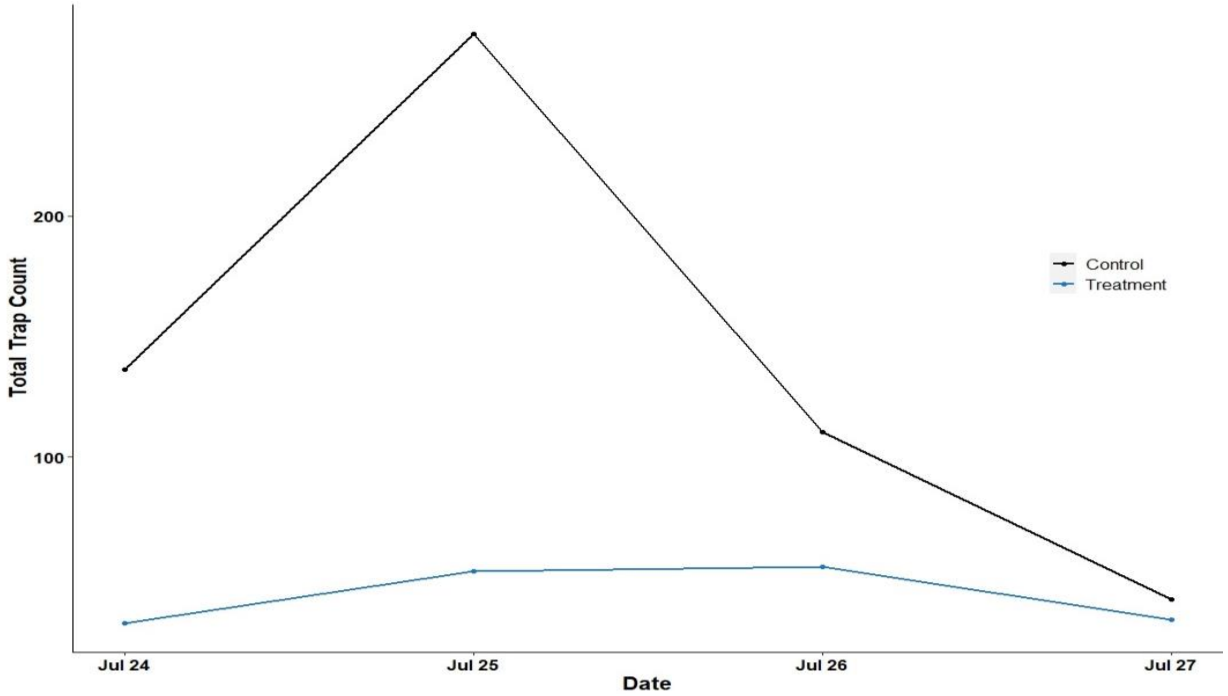


Figure 26. Average daily mosquito activity at trap locations treated with DeltaGard®20EW (blue) and in untreated control sites (black) when adulticide was applied on the evening of July 25, 2020.

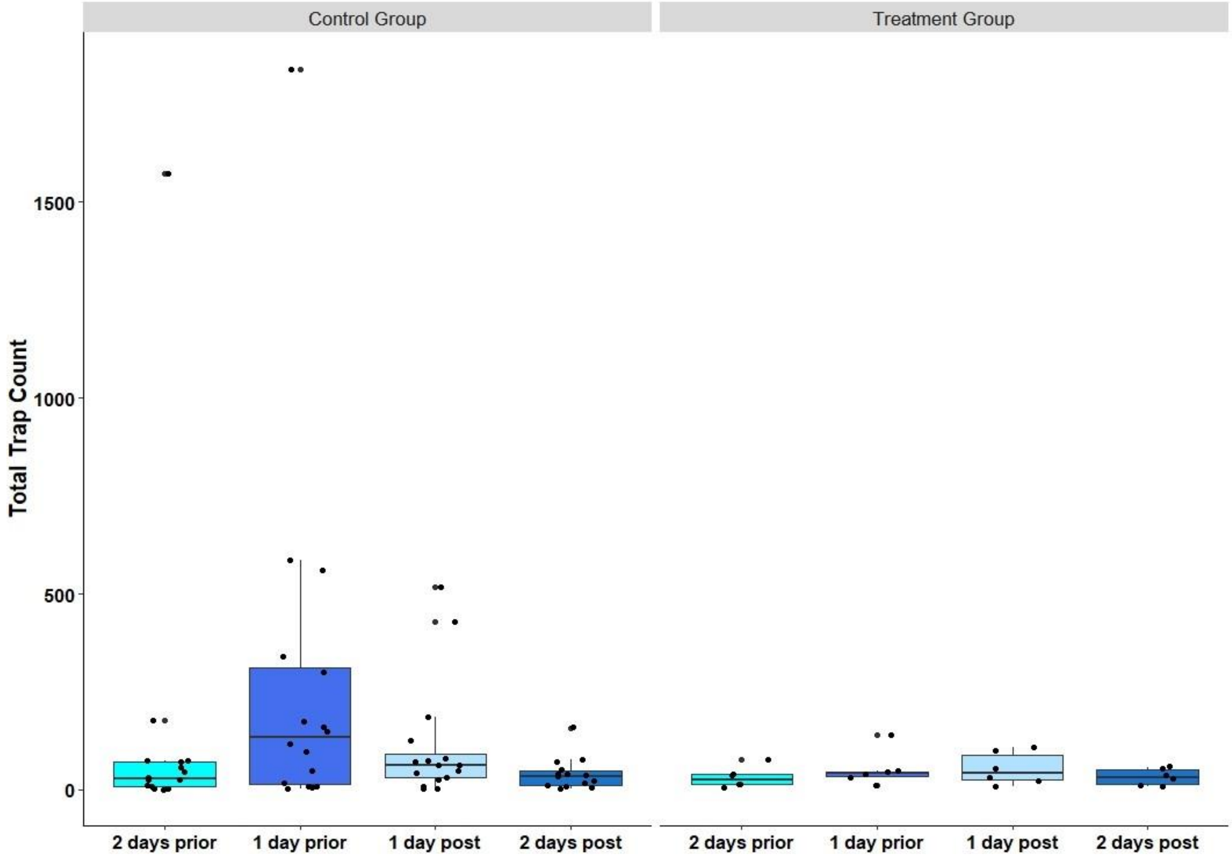


Figure 27. Daily changes in mosquito activity at trap locations in non-treatment (left) and treatment (right) groups 2 days before and 2 days after treatment with DeltaGard®20EW on the evening of July 25, 2020. Trap collection data from individual trap locations are shown with black dots.

Treatment Evening: July 26, 2020

Figures 28 and 29 show the average daily and total daily mosquito activity in treatment and non-treatment locations for the treatment evening of July 26, 2020. T.aov shows that the post-spray count did not differ significantly between untreated and treated traps after controlling for baseline (pre-treatment count) ($p = 0.2868$) (Table 7). Wilcoxon signed rank test of temporal changes in the treatment group shows that the changes in mosquito activity from July 26 to July 28 and from July 26 to 27 were not significant ($p = 0.0831$ and $p = 0.5013$ respectively). However, a significant decrease in mosquito activity was detected from July 25 to July 27 ($p = 0.0104$) as well as from July 25 to July 28 ($p = 0.0029$).

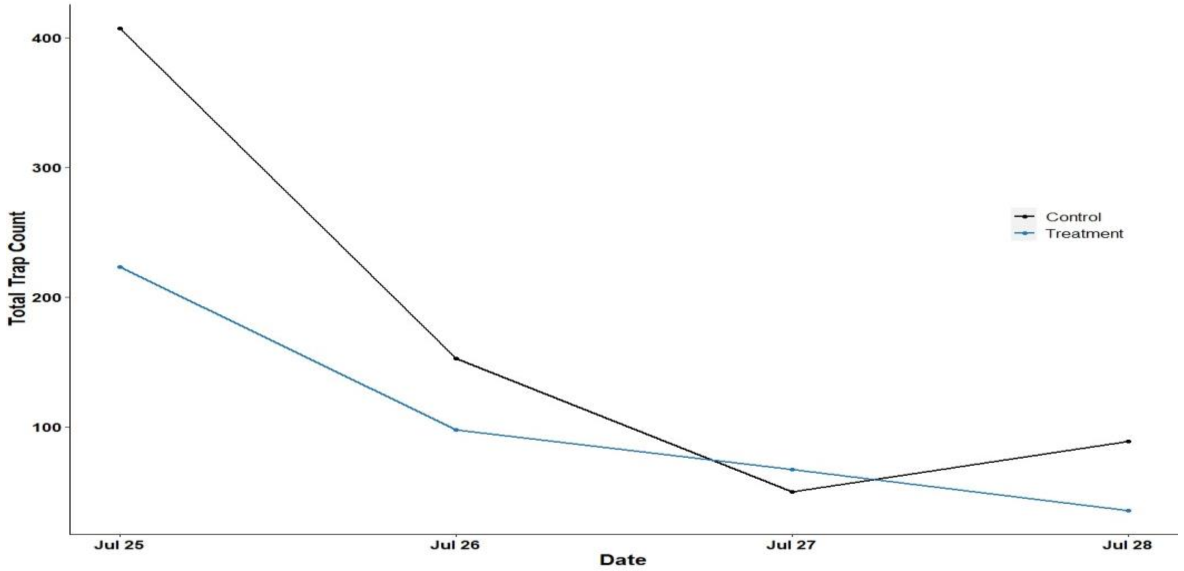


Figure 28. Average daily mosquito activity at trap locations treated with DeltaGard®20EW (blue) and in untreated control sites (black) when adulticide was applied on the evening of July 26, 2020.

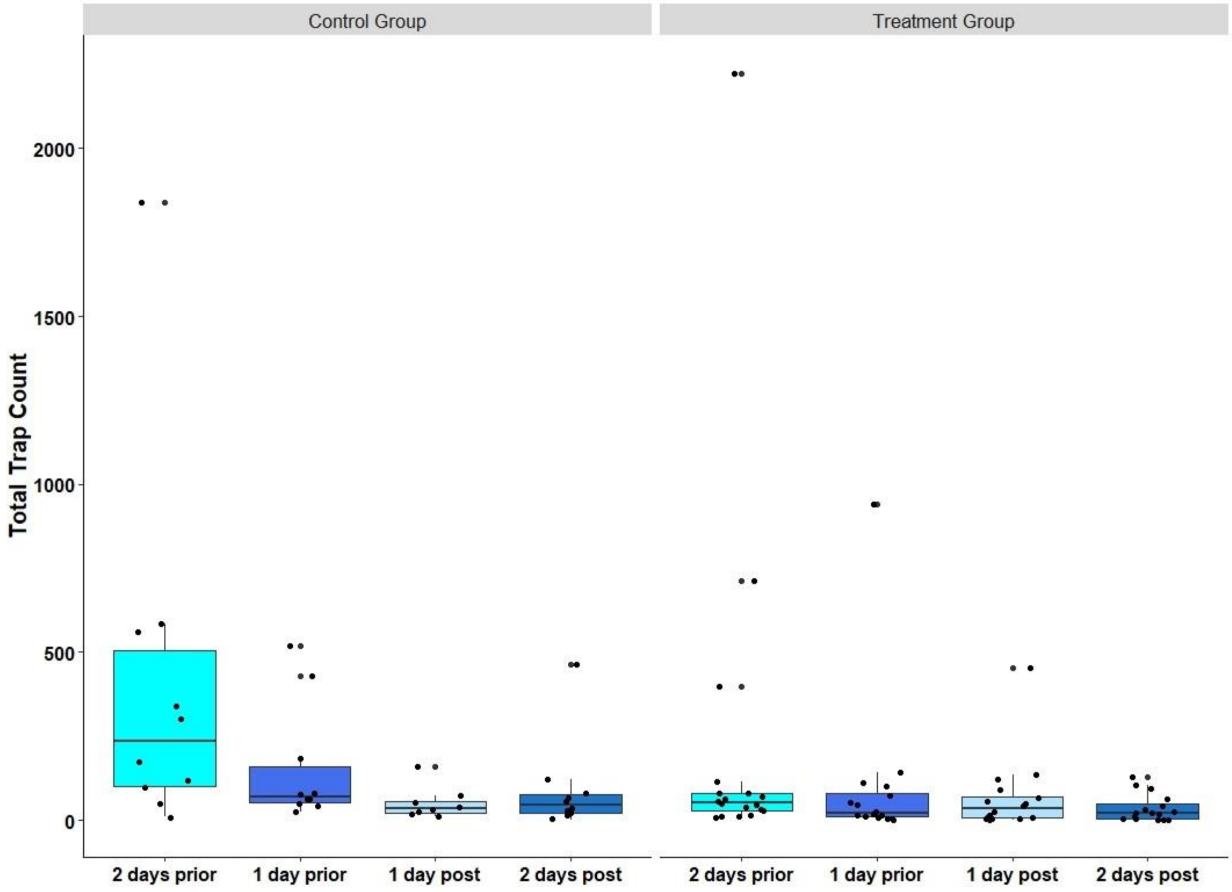


Figure 29. Daily changes in mosquito activity at trap locations in non-treatment (left) and treatment (right) groups 2 days before and 2 days after treatment with DeltaGard®20EW on the evening of July 26, 2020. Trap collection data from individual trap locations are shown with black dots.

Treatment Evening: July 27, 2020

Figures 30 and 31 show the average daily and total daily mosquito activity in treatment and non-treatment locations for the treatment evening of July 27, 2020. T.aov shows that the post-spray count did not differ significantly between untreated and treated traps after controlling for baseline (pre-treatment count) (p = 0.3930) (Table 7). Wilcoxon signed rank test of temporal changes in the treatment group shows that none of the daily changes in mosquito activity were significant.

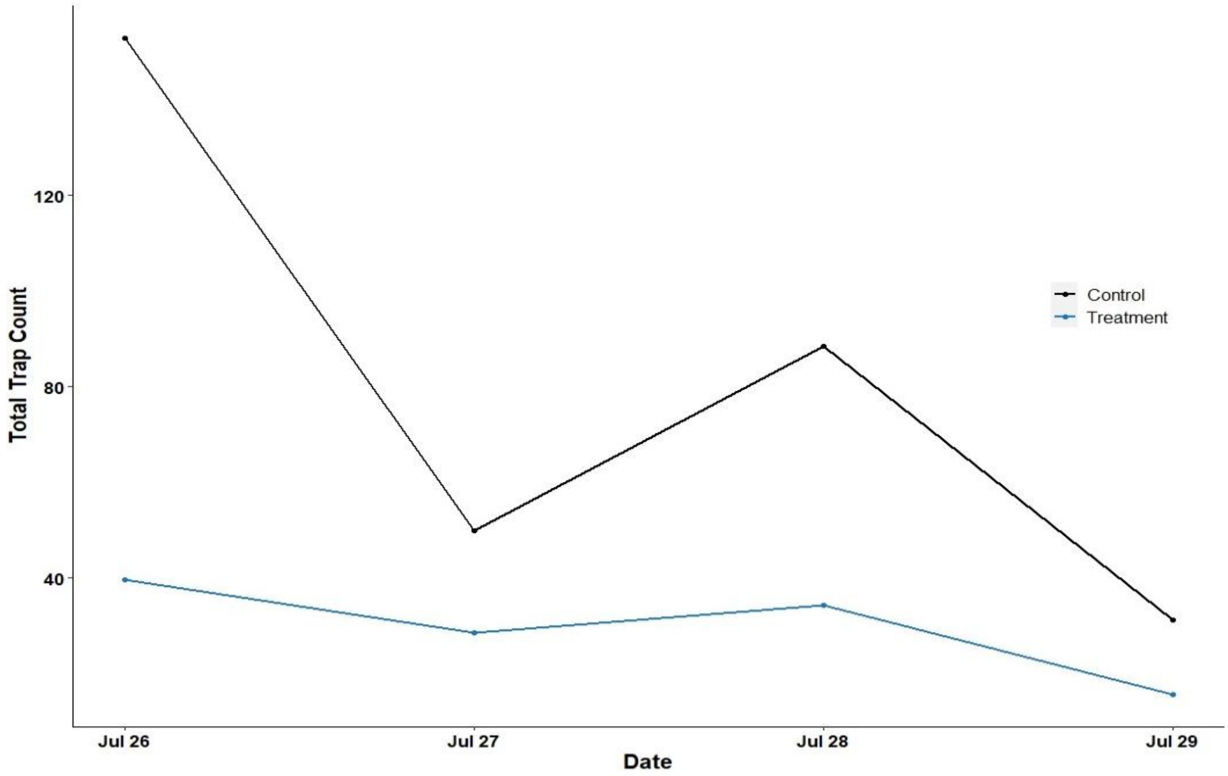


Figure 30. Average daily mosquito activity at trap locations treated with DeltaGard®20EW (blue) and in untreated control sites (black) when adulticide was applied on the evening of July 27, 2020.

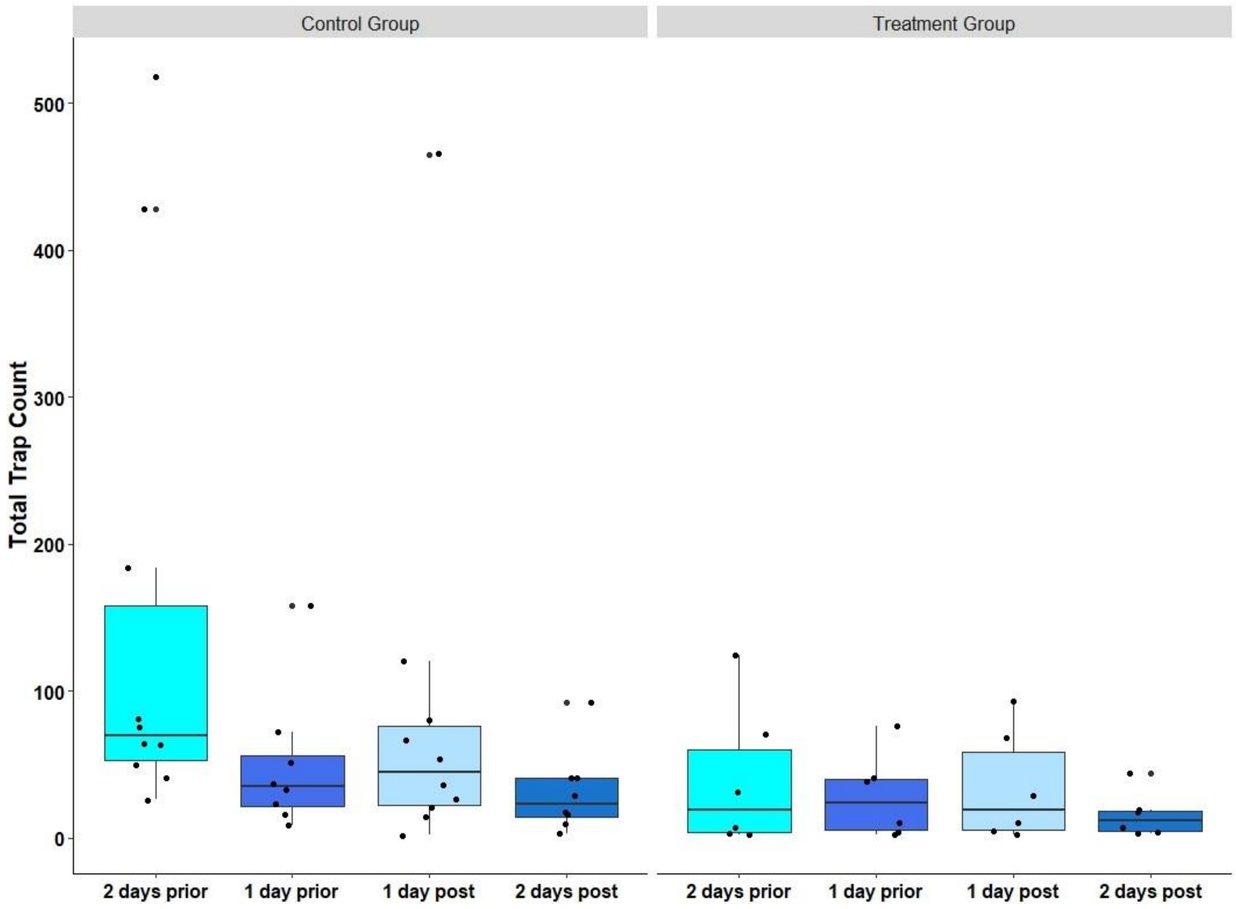


Figure 31. Daily changes in mosquito activity at trap locations in non-treatment (left) and treatment (right) groups 2 days before and 2 days after treatment with DeltaGard®20EW on the evening of July 27, 2020. Trap collection data from individual trap locations are shown with black dots.

The results of the T.aov analyses for adulticide treatment events in July 2010 (with Malathion 95ULV®) and 2020 (with DeltaGard®20EW) are summarized in Table 7. The model determined if there was a significant decrease in mosquito activity as monitored by traps located in adulticide treatment zones post-spray while controlling for baseline population (pre-treatment catches). None of these events show a significant change.

Table 7. The results of a T.aov analysis for each treatment event analyzed in July 2010 (Malathion 95ULV®) and 2020 (DeltaGard®20EW). The effect measured for significance ($p < 0.05$) was the post-treatment trap count (mosquito activity) and how it was affected by adulticide use after controlling for baseline population activity.

	2010: Malathion 95ULV® Applications	**2020: DeltaGard®20EW Applications**
Treatment Evening	**p-value**	**p-value**
July 10	0.4157	
July 11	0.0885	
July 14		0.3682
July 24		0.4406
July 25		0.7164
July 26		0.2868
July 27		0.3930

5.3 Wing Length Analysis

The average *Ae. vexans* wing length in Winnipeg, including all samples measured from 2019 mosquito trap collections containing 30+ *Ae. vexans*, was 3.98 micrometers. The wing lengths were distributed normally. When below average and above average wing lengths were tested for a significant difference in days to 90% depletion, the cohorts that had a mean wing length higher than the citywide average of 3.98 micrometers took significantly more days to 90% depletion than cohorts with a lower mean length ($p = 0.0480$). The dots shown in the violin plots in Figure 32 indicate the mean wing length of each cohort of 30+ *Ae. vexans*.

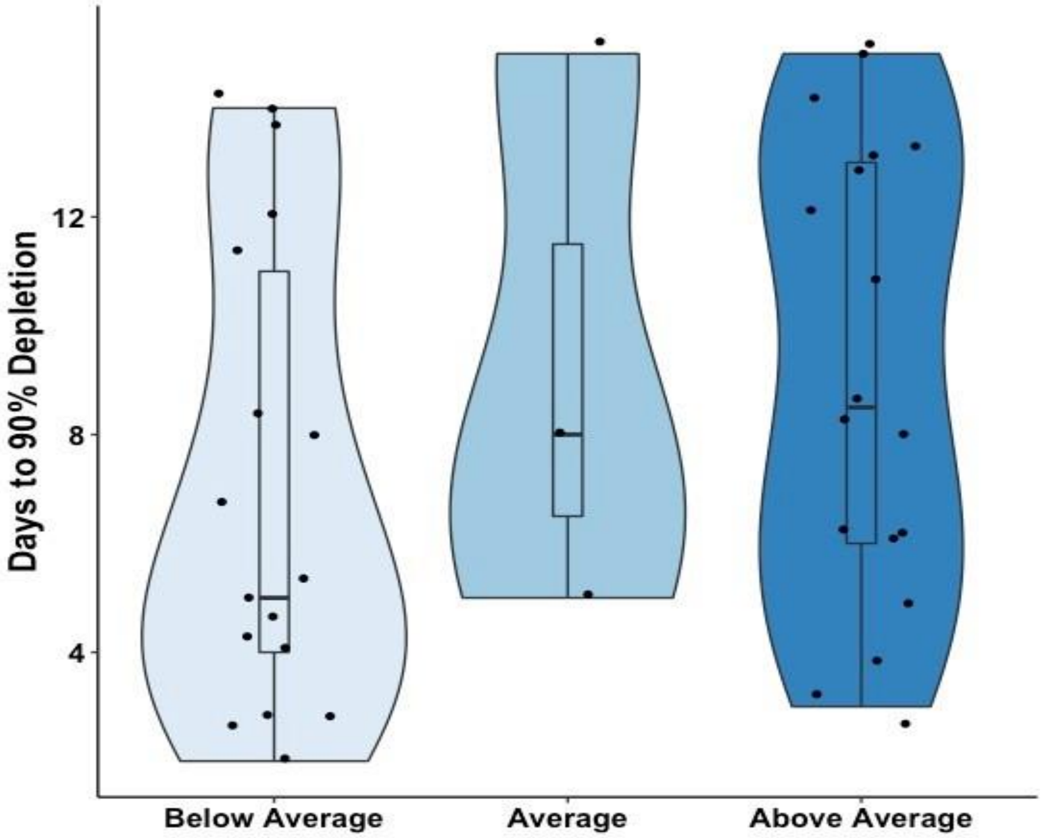


Figure 32. Average wing length in micrometers separated into three groups (below the overall citywide mean wing length of 3.98 micrometers, at the mean, or above the mean) for selected cohorts with 30+ *Ae. vexans* from 2019 trap collection data compared to the number of days to 90% depletion.

6.0 Discussion

The purpose of this study was to test DeltaGard®20EW for efficacy as a mosquito adulticide in Winnipeg, Manitoba. Although tested successfully in caged adult mosquitoes in 2015 (96% mortality), DeltaGard®20EW had to be tested operationally in Winnipeg's unique cityscape (Nawolsky and Wade 2016).

When Malathion 95ULV® was applied in July 2010 and when DeltaGard®20EW was applied in July 2020, no significant change in mosquito mortality was detected when comparing post-spray trap collections from traps in treated and untreated areas. When daily changes in mosquito activity were analyzed for significant increases and decreases, a significant decrease was detected the day after Malathion 95ULV® application. During the 2020 trials with DeltaGard®20EW application, no significant decreases in mosquito activity were detected. When different landscape features surrounding hot spot trap sites and non-hot spot trap sites were compared, the two groups were not significantly different.

There are several possible reasons for the general lack of significance in this study including gaps in floodwater mosquito biology knowledge, spatial considerations, and the challenges that come with designing statistical models to track mosquito activity changes and adulticide efficacy operationally.

Gaps in the Knowledge: *Ae. vexans* Biology

Gaps in floodwater mosquito biology, and specifically *Ae. vexans* biology need to be addressed. It is important to determine the egg drying period necessary after an oviposition event, the factors that contribute to instalment hatching, and the duration that eggs remain unhatched and viable in the soil. Quantitative sampling of the cumulative egg bank would be a useful contribution to understanding the degree to which future populations of floodwater mosquitoes are possible or likely after extended periods of drought. Knowing where the most eggs are concentrated would be a useful indicator of where the most adult mosquito activity may exist and persist. Once an adulticide program has commenced, these areas of the CoW can be prioritized, resulting in more efficient targeting of insecticide.

Although it is known that floodwater mosquitoes rely on rainfall/flooding events and appropriate temperatures to hatch (Wood, Dang and Ellis 1979), there were lower average mosquito trap counts in July 2010 (44) than there were in July 2020 (105) despite there being more rainfall (mm) in the former year. There was an average of 2.2mm daily in 2010 and an average of 1.3mm daily in 2020. A lower average trap count indicates less mosquito activity. Mosquito activity is generally thought to be positively correlated with humidity; however, the average humidity was also higher in July 2010 (78.4% versus 73.4% in July 2020) (Horsfall 1955; Wood, Dang and Ellis 1979; Day 2016).

There are a few possible explanations for the lower average trap counts in 2010. Fewer traps were used that year and so locations added in 2020 may harbor higher mosquito activity. Also, the 11 traps that I operated in 2020 were CDC traps, not NJLTs. The CDC traps operated in 2020 caught an average of 323 mosquitoes daily, whereas the NJLTs caught an average of 76 mosquitoes daily. Therefore, higher CDC trap counts may be skewing the results. Studies have found that the CO_2 lure in CDC traps act as a better lure for adult biting mosquitoes looking for animals that respire and provide blood meals (Slaff *et al*. 1983). CDC traps are generally thought to be a better indicator of mosquito activity (Slaff *et al*. 1983).

Meteorological variables may provide another possible explanation for the lower average trap counts in 2010. Higher levels of precipitation in 2010 would contribute to higher mosquito population, but not necessarily higher mosquito activity. PCA results for meteorological variables in July 2010 and 2020 are consistent with the hypothesis that temperature and precipitation had the smallest effect on adult mosquito activity if the average temperature remains above an activity threshold of approximately 15ºC (see Figure XXVIII in Appendix) (Wood, Dang and Ellis 1979). Given that floodwater mosquito population increases rely on rainfall events days or weeks before their emergence, higher amounts of precipitation on the day of a trap collection cannot necessarily predict mosquito activity on that day (Karki *et al*. 2016) except with respect to the humidity that recent rainfall may generate. The average wind speed in July 2010 was 30.0 kph versus 12.3 kph in July 2020. Higher wind speeds may have decreased adult mosquito flight and blood feeding attempts in 2010. Wind speed may be more predictive of mosquito *activity* levels, while precipitation may be more predictive of mosquito *population* counts. There may be high populations of adult mosquitoes without active movement and host-seeking if meteorological conditions are unconducive to those.

The AFA and other predictive models designed by CoW should consider the meteorological variables that predict activity instead of population size because that is what mosquito traps are measuring. Rainfall patterns surrounding emergences are currently considered in the CoW-ICB AFA because decisions to apply adulticide are dependent on rainfall that previously occurred to begin hatching mosquito eggs (CoW 2022). However, humidity and wind speed had the greatest effect on mosquito trap counts which indicate that they explain the most variation in adult mosquito activity. Other studies have found that precipitation weeks before trap collection, higher temperatures, and minimal wind speed increase mosquito activity (Karki *et al*. 2016; Drakou *et al.* 2020). These conditions tend to generate higher mosquito populations which will result in higher activity levels for the given set of meteorological conditions listed than lower populations.

The data used to perform this PCA were from a single weather station (Richardson Airport) in the CoW because it was the only station to consistently measure humidity, wind speed, temperature, and precipitation daily in July 2010 and July 2020. More accurate measurement of meteorological conditions directly at each individual trap location can be controlled for as a spatial covariate in future statistical modelling. Precise meteorological measurements may help to understand floodwater mosquito activity and population dynamics which are variable within the abatement area, thus improving adult mosquito control in Winnipeg by increasing knowledge of how these factors specifically affect *Ae. vexans* egg hatch, development, production of adult mosquitoes, and adult mosquito activity. By filling in these gaps in the literature, more effective mosquito control may be performed by targeting adulticide to specific locations that harbor more mosquito activity and by being able to predict when and where large adult mosquito emergences may occur in Winnipeg.

Spatial Considerations

Landscape features (i.e. trees, rivers, buildings, etc.) surrounding mosquito trap locations can alter the efficacy of an adulticide and can confound data collected from these traps, and so should be accounted for in analysis. The results of the Moran's I autocorrelation analysis indicate that traps that recorded the highest mosquito activity (and lowest mosquito activity) were clustered surrounding most adulticide treatment events in 2020. The only exception was the treatment event on July 6, 2020 when mosquito trap count data increased evenly citywide. However, this was not generally the case in 2010. Although some treatment events in 2010 displayed spatial clustering, not all of them did. A lack of spatial clustering surrounding treatment events mean that traps with significantly higher or lower counts are randomly dispersed in the environment (ESRI 2022). This can also occur if there are no traps with significantly higher or lower counts than their neighboring traps. Moran's I analysis did detect spatial clustering when the entire trap count data set from July 2010 and July 2020 was analyzed. Spatial autocorrelation indicated that further spatial analysis might yield insights with respect to local effects of adulticides.

First, I performed kriging analysis on the data to create interpolative surfaces which showed a decrease in mosquito activity in treatment areas after adulticide treatment (Figure 16 in Results). However, results of a leave-one-out cross validation test of these kriging outputs indicate that trap count values predicted from high measured values were under-estimated and over-estimated from low measured values. It is a property of kriging that tends to under-predict large values and over-predict small values (ESRI 2022). However, the prediction line was essentially horizontal, indicating that all predictions are nearly the same and that the data points are independent of each other (ESRI 2022). This outcome may be the result of an inadequate density oftrap locations set in Winnipeg and their inability to accurately measure adult mosquito activity throughout the city. For kriging, at least 30-50 data points are recommended, although in larger areas, similar to the size of Winnipeg, some authors have suggested that the minimum number of data points needed is as much as 100 (Webster and Oliver 1993) especially for data that exhibit a large amount of short range variability and that variograms computed on fewer than 50 data points are of little worth. Therefore, the kriging analysis based on the 53 trap locations operated in Winnipeg in 2020 is likely not sufficient.

Kriging also assumes the concept of randomness (i.e. traps dispersed without respect to one another), whereby the interpolated surface is hypothesized as one of many that mighthave been observed and all of which could generate the known data points (Ouma *et al*. 2012). The accuracy or power of the resulting 3D models is affected by the number of data points (traps) and their distribution (Alcaras *et al*. 2022). Several studies have been conducted on the influence of points density for interpolative surface generation (Bakula 2011; Stereńczak *et al*. 2016; Zhang*et al*. 2018). When testing kriging models, researchers found that a density equal to at least 1 point every 1000m^2 was sufficient to produce an accurate interpolative surface in areas characterized by a low level of variation in seabed morphology (i.e. elevation and water depth). However, this density had to be increased 10 times to produce an accurate model in areas with high variation in seabed morphology (Alcaras *et al*. 2022). Winnipeg's surface area is ~460km^2 and only 27 and 39 traps were set within this boundary in 2010 and 2020 respectively. Therefore, to accurately interpolate the entire surface of Winnipeg with these parameters, 460,000 traps would need to be operated. This is not possible due to limited resources and inefficiency. When taking a census of mosquito population dispersal, the design of the sampling may be affected by the spatial pattern of the organisms in question and a measure of the degree of clustering specific to the scale of the study area may need to be considered (Krebs 1998). Organisms that are clustered at a local scale may not appear clustered at a global scale and vice versa. Instead of considering mosquito population citywide, it may be useful to divide the city into strata with similar ground cover and landscape features (and possibly other variables) at a scale that may be helpful when comparing traps in locations treated with adulticide and traps located in untreated areas. Smaller stratified samples based on normalized difference vegetation index (NDVI) or land cover variables that determine the density of vegetation in different parts of Winnipeg should be created. Then, stratified random sampling can be performed by placing one trap every 1000m^2 in Winnipeg in these stratifications. In this way, traps with similar ground cover could be paired for comparison or specific landscape features could be accounted for in statistical modelling.

The placement of traps may also need to be randomized to produce the best interpolative surface. Kriging analysis is based on the assumptions that observation locations (trap placements in this study) are randomly distributed in space. This assumption is hard to meet because traps are placed based on locational criteria (ESRI 2022). The trap placement algorithm in Winnipeg may mean that mosquito trap counts in these collections overestimate true activity in the surrounding area because they are placed strategically in locations known to harbor high mosquito activity.

However, traps placed in habitats less conducive to mosquito activity may not catch enough mosquitoes to measure a significant change in activity. The sampling method used to measure mosquito activity may be flawed for these reasons. Mosquito trap counts are the major deciding factor for adulticide application by the CoW-ICB, but this may need to be reconsidered or the manner in which they are deployed changed. The current distributional pattern does not lend itself to a reasonable and reliable assessment of the efficacy of adulticiding efforts.

As mentioned, *Ae. vexans* congregate in riparian and well-vegetated/forested habitats (Wood, Dang and Ellis 1979; Day 2016; Balcaen 2020), even if they often host-seek in the open nearby. When analysis accounted for the distance to rivers and parks/open spaces and the number of trees surrounding traps, trap locations that were frequently hot spots tended to be closer to rivers and to have more trees within a 50km radius, probably due to higher humidity in these areas that is conducive to increased mosquito activity (Wood, Dang and Ellis 1979). No statistically significant difference between frequent hot spots and non-hot spots and their distance to rivers and number of trees within a 50km radius could be detected. A lack of significance in these results may be because "frequent" needs to be redefined to only include hot spots determined with 99% confidence or trap locations that were hot spots more than once in July 2010 and July 2020. Also, hot spot analysis may not have been the best method because of the limited number of traps operated in Winnipeg. More traps are needed to increase power to assess landscape features surrounding hot spots versus non-hot spots. Hot spot analysis takes neighboring observations into consideration when analyzing clusters of high values (ESRI 2022). If these neighboring traps are too far apart, hot spot analysis may not be precise enough to determine hot and cold spots.

Another possible explanation for the lack of significance in the proximity and density of landscape features analysis is that the open-source tree inventory used for this analysis does not include trees located outside of the city limits or in parks, and thus the estimates used were biased in an important way. Of the 34 trap locations that were hot spots at some point in July 2010 or July 2020, 16 were located outside of the city limits or in parks. This means that the trees surrounding almost half of the hot spots would not have accurate tree inventories associated with them. Therefore, these 16 traps were removed from the tree density analysis meaning that close to half of the data were excluded from the analysis, again limiting power. Traps located outside of city limits and in parks may be in densely forested areas or densely surrounded by trees and this information could contribute to the detection of significantly more trees surrounding hot spots than surrounding non-hot spots. Future studies should include the inventory of trees surrounding these traps to improve analysis.

Analysis comparing the distribution of landscape variables in hot spot and non-hot spot locations should be considered rather than Kruskal-Wallis analysis which compares the central tendency of the two groups. There was also a consistent proportion of mosquito trap sites that were hot spots between July 2010 and July 2020, however, traps were hot spots on more days in July 2020. It is possible that the higher wind speeds in 2010 contributed to fewer mosquito activity hot spots. It is also possible that CDC traps, shown in this study to collect more mosquitoes on average than NJLTs, may have been detected as hot spots more often. Of the CDC trap locations in 2020, 9 out of 11 were hot spots at some point in July and made up 39% of the total hot spots detected in that month.

Finally, the buffers used to analyze tree density surrounding trap locations were set arbitrarily. Changing these buffer sizes may change the results of the analysis. Buffer sizes could potentially be set to the approximate area of a swath of adulticide produced by ULV application to determine its efficacy (decreasing mosquito activity at a specific trap location) as it varies with surrounding vegetation density.

Ae. vexans mosquito populations tend to congregate near rivers and in densely vegetated areas in Winnipeg (Balcaen 2020). However, ULV application of DeltaGard®20EW may be less effective in their "hot spot" habitats because areas closer to rivers are encompassed by no-spray buffer zones to prevent aquatic contamination (CoW 2022). This means that mosquitoes resting in those areas are unlikely to encounter ULV aerosol droplets. Also, dense vegetation may provide a hiding place for mosquitoes because the ULV adulticide treatment may not penetrate as well as it would in more open spaces. The pesticide use label for DeltaGard®20EW states that it should be used at its maximum rate in highly vegetated areas which indicates that it is less effective at lower concentration in these conditions (CoW 2022). Bengoa *et al*. evaluated the efficacy of ULV truck-mounted equipment and obtained nearly 100% mortality in caged *Ae. albopictus* in an open area, but states that this impact would be lower in wild uncaged mosquitoes resting within vegetation (2014). When tested in Nice, France, deltamethrin ULV was found to be less effective against *Ae. albopictus* in more vegetated areas than *Ae. aegypti* in more open rural areas that lacked vegetation (Boubidi *et al*. 2016). The authors state that this may be because *Ae. albopictus* favor resting sites, particularly vegetation, that are devoid of air movement but the insecticide particles depend on the nuances of air movement to deliver them to the mosquito (Boubidi *et al*. 2016). Also, DeltaGard®20EW ULV treatment dispenses larger droplets (up to 50 microns) than those used for malathion ULV (up to 32 microns). Larger aerosol droplets may mean that DeltaGard®20EW is not as effective at penetrating dense vegetation and other barriers, thus ULV application of DeltaGard®20EW may be less effective in their "hot spot" habitats if those hot spots tend to be surrounded by more trees, houses, and fences that act as barriers to aerosol movement. These barriers should be considered in future analysis of DeltaGard®20EW efficacy to determine if density of trees (completely inventoried), houses, and fences prevent the adulticide from reaching mosquitoes that are active behind these barriers.

Apart from a potential lack of delivery to *Ae. vexans* resting sites, there are other potential limitations to the efficacy of ULV approaches, particularly in urban areas where walls, buildings, and fences in front yards may obstruct the drift of particles. Mortality in caged mosquitoes has little relation to mortality of resting or even free-flying mosquitoes, particularly at sites in vegetation or behind other obstacles. In studies of attempted control of *Ae. aegypti* in Venezuela, mortality was more than 90% in caged mosquitoes set in the open, but close to zero at typical indoor resting sites (Reiter 2014). Similarly, Mount reported 90% mortality of caged mosquitoes in an open field, but 34-67% in vegetation (1998) and Andis *et al*. observed 95.5% mortality in caged *Ae. aegypti* suspended in the open versus 89% in more sheltered locations (1987). Britch *et al*. evaluated the efficacy of truck-mounted ULV and thermal fogging and found that there is a 100-fold greater chance that a droplet will contact a mosquito in a sentinel cage in a thermal fog application versus a ULV application (2010). Thermal fogging penetrates vegetation better because it produces smaller particles than ULV which can move past barriers more easily. Boubidi *et al*. concluded that there is no documented evidence that ULV treatments have ever had a discernible impact on transmission of dengue or chikungunya anywhere in the world (2016). They concluded that, in the event of outbreaks of disease, truck mounted ULV is unlikely to have significant impact on transmission but that, despite being highly labor-intensive, thermal or ULV aerosols dispensed from portable sprayers rather than truck-mounted equipment are the method of choice (Boubidi *et al*. 2016). Clearly this is not practical on any large scale because it would require an increase in budget, time, and personnel, but may still be useful in the event of potential "hot-spots" of local transmission because portable application could be targeted in these locations.

It is possible that ULV application may result in residual effects of DeltaGard®20EW if droplets that do not evaporate land on vegetation. However, the small amount of active ingredient applied with spray (0.5-1.5g/ha) and the tendency for ULV droplets to evaporate when they do not contact flying adult mosquitoes may mean that not enough deltamethrin lands on surfaces and vegetation to detect this potential residual effect. Malathion 95ULV® which has a much shorter half-life than DeltaGard®20EW in environmental conditions meaning that the latter is more likely to have residual effects (Krieger 2010). DeltaGard®20EW is used as a barrier spray and to treat bed nets, which indicates some degree of binding to substances on which mosquitoes might rest (Ehiri *et al*. 2004; Bengoa *et al*. 2013). This means that there is a chance that mosquitoes will encounter treated vegetation during resting periods. However, deltamethrin is also an effective repellent for *Ae. aegypti* and *Ae. albopictus* (Chattopadhyay *et al.* 2013; Bibbs and Kaufman 2017; Bowman *et al*. 2018). *Ae. vexans* may also be repelled by residual deltamethrin, avoiding chronic residual toxicological effects by avoiding vegetation that has been sprayed. Further studies should determine the residual and repellency effects of DeltaGard®20EW on *Ae. vexans*.

The location and number of traps, the specific meteorological conditions at trap locations, the landscape features surrounding trap locations, and the residual and repellency effect of DeltaGard®20EW should be considered in future when designing a sampling plan and analytical model to evaluate the effectiveness of DeltaGard®20EW in Winnipeg, Manitoba for adult mosquito control.

Designing a Statistical Model

A non-parametric version of an ANCOVA model called T.aov was used to compare post-spray collections in treatment and control locations while controlling for baseline variation in mosquito activity (pre-treatment collections) for Malathion 95ULV® application in July 2010 and DeltaGard®20EW application in July 2020. A lack of significance between the untreated and treated groups post-spray for all the analyzed trials suggests that the observed change in trap catches in treatment areas may have been due to natural background mosquito population fluctuations.

For the July 2010 trials, the results of the Wilcoxon signed rank test only determined a significant decrease in mosquito activity in the treatment group during the July 10th trial. The mosquitoes collected for the two-days post-spray were significantly decreased by Malathion 95ULV® application, suggesting it to be effective. During the July 11, 2010 trial, there was no significant reduction in mosquitoes, however, the untreated group from the July 11th trial increased for the two-days post-spray period whereas the trap counts in treated areas did not. Given the natural fluctuation in mosquito activity indices, Malathion 95ULV® may have decreased mosquito activity for at least two days after it was applied, but the data available are insufficient to conclude this. Malathion 95ULV® has a faster killing effect than deltamethrin (Krieger 2010). It is possible that Malathion 95ULV® droplets, being smaller than those produced for DeltaGard®20EW ULV application, may be able to penetrate vegetation more effectively to target *Ae. vexans* while they are resting (Rathburn and Dukes 1989; Mount 1998; Andis *et al*. 1987). The smaller droplets also remain suspended in the air for longer and are more likely to contact flying mosquitoes the night of treatment (Brill and Morrison 2013).

For the July 2020 trials, Wilcoxon signed rank analysis detected no significant decreases in mosquito activity during adulticide application trials. It is possible that tracking more than two-days post-spray may be useful because DeltaGard®20EW, being a type II pyrethroid, has a slow-kill effect (Sawicki and Thain 1962; Lund and Narahashi 1983).

Specific to the parks and cemeteries trial on the evening of July 14th, 2020, with DeltaGard®20EW, no significant effect was detected. The treatment zones were much smaller than they would be when an entire IMA is treated, so mosquitoes are more likely to migrate in and out of these treatment areas in short time periods. *Ae. vexans* have been found to migrate up to 8 km in their lifetime (Balcaen 2020). This may skew results if mosquitoes migrate in and get caught in traps post-spray. Parks also tend to have more dense vegetation that can act as places of refuge for mosquitoes from adulticide droplets. Rivers also represent zones where adulticide cannot be applied, giving mosquitoes even more refuge when they congregate in riparian habitats. Also, there were only five traps located in treatment areas for this trial, potentially limiting the power of the analysis (Suresh and Chandrashekara 2012).

The general lack of measurable efficacy for DeltaGard®20EW demonstrated in this study may be due to the difficulty in designing a statistical model to accurately detect changes in uncaged wild mosquito activity with too-few sampling points distributed throughout the study area. The nature and cause of background activity fluctuations are difficult to determine. These changes may be due to natural background activity, other phenomena (i.e., landscape and meteorological), or adulticide application. The lack of true non-treatment experimental controls available, the pre-spray population being too low for a significant change in mosquito activity to be detected, the location and number of traps (described previously), and the specific meteorological conditions at trap locations (described previously) may all contribute to inadequate statistical power.

It was difficult to separate treated areas from untreated areas in the 2020 trials because the entire city was sprayed in rotation within a four-day time window. Collections from traps outside of the city were not necessarily good references because those locations likely vary in a significantly ecological way from urban trap locations. For example, farmland, forests, and a lack of urban features characterize areas outside of Winnipeg. Future work should include specific treated and untreated locations with similar surrounding landscapes to control for these potentially confounding factors. It should measure meteorological variables directly at the trap locations to include as a covariate in a non-parametric ANCOVA or similar analysis. In both years, average precipitation was less than normal. Dry conditions may have produced too few mosquitoes to measure statistically significant changes in activity.

DeltaGard®20EW is a slow-kill insecticide that has been observed to generate less mosquito activity two-days post treatment (Sawicki and Thain 1962; Lund and Narahashi 1983). Malathion 95ULV® application produced more significant next-day changes in treated populations, at least sometimes. This analytical result may be due to the availability of better-quality reference collections (clearer experimental control traps), and/or malathion being applied at greater levels of active ingredient and with smaller droplet sizes that are more likely to penetrate vegetation and remain suspended in the air to contact flying mosquitoes, thus making it an operationally more effective mosquito adulticide than DeltaGard®20EW when applied with ULV technology.

Wing Length Analysis

Prioritization of certain locations of the city for adulticide use may be useful and proactive if some parts of the city produce larger mosquitoes that live longer because a greater duration of relief from nuisance is theoretically possible. Various studies provide evidence of the positive correlation between adult mosquito body size and longevity (Packer and Corbet 1989; Yeap *et al.* 2011; Guitiérrez *et al*. 2020). However, there is some dispute of this claim (Barreaux *et al*. 2018; Yan *et al*. 2021). The evidence for and against the positive correlation between mosquito body size and longevity is discussed in the previous literature review.

The lack of significance when comparing *Ae. vexans* wing length and days to 90% depletion in three groups (mean cohort wing length above the citywide *Ae. vexans* wing length average, at the citywide average, and below the citywide average) may be due to the undetectable significant difference between mean cohort wing length at and above the citywide average (Figure 32 in Results). Cohorts of *Ae. vexans* with mean wing lengths below the citywide average took significantly fewer days to reach 90% depletion than cohorts of mosquitoes with a mean wing lengthabove the citywide average. This indicates that mosquito body size may be correlated with longevity in *Ae. vexans* in Winnipeg, Manitoba.

This analytical approach rests on the assumption that samples of measured mosquitoes represent a cohort or cohorts which could be followed via trap catches for periods of time, but, background population dynamics in wild mosquitoes are notoriously variable, and, it's possible that cohorts differentially fell victim to predation or weather changes. Previous nutritional availability for different samples may have further confounded analysis. A proportional hazards model may also be applied to the data to check for temporal changes in cohort longevity. These factors might usefully be incorporated in future modelling.

Conclusion

When DeltaGard®20EW efficacy was tested operationally in Winnipeg, no significant change in mosquito mortality was detected when comparing traps in treated and untreated areas. This indicates that any changes may be due to natural background fluctuations in mosquito activity and population. When daily changes in mosquito activity were tested during the 2020 DeltaGard®20EW trials, no significant decreases were detected surrounding adulticiding events.

The lack of significance in the DeltaGard®20EW trials may be due to issues with modelling like a lack of untreated (experimental control) trap locations, a starting population that was too low to detect significant changes, a lack of specific knowledge about the cumulative egg bank, too few trap locations, traps being placed strategically instead of randomly, and a lack of meteorological and landscape data specific to trap locations. Future studies should assign specific pairs of treated and untreated trap locations with similar surrounding landscape features (i.e., trees, building density, river proximity, etc.). Years with higher mosquito populations than in 2020 should also be used to test DeltaGard®20EW efficacy operationally. Future experiments should use more traps located more randomly so that traps aren’t only placed in strategic areas known to harbor more mosquito activity. Often, these locations are within or close to the buffer zones surrounding rivers or are surrounding by dense vegetation which may hinder adulticide efficacy. Further development of the non-parametric ANCOVA model should include meteorological data specific to trap locations as a covariate.

Measuring adulticide efficacy in wild mosquitoes and creating models to analyze changes in their activity is challenging. Future studies need to have specific untreated and treated traps that are paired using stratified random sampling that separates the city according to an NDVI or land cover classification. Certain strata can then be selected and 1 trap can be placed per $1000m^2$ to better analyze changes in mosquito activity. Specific meteorological variables surrounding these traps should be controlled for in the model used for analysis with an emphasis on wind speed and humidity because they are better predictors of mosquito activity than temperature and precipitation.

Wing lengths were used as a proxy for adult mosquito body size. A significant correlation between body size and longevity was detected. However, it is hard to determine background population dynamics in wild mosquitoes and to know if the same cohort is being monitored. Nutritional availability and temperature of water for developing larvae and mosquito predation must also be considered.

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8.0 Appendix

Table I. All layers were defined with NAD 1983 UTM 14 Transverse Mercator. Daily spatial autocorrelation output for NJLT mosquito trap locations and their corresponding trap count is listed for the month of July 2010. Moran's I was used to determine spatial autocorrelation. When the entire month of July 2010 was analyzed together using Moran's I, significant clustering was revealed (p = 0.00). Adulticide treatment evenings (with Malathion 95ULV®) are indicated by a * beside the date. Spatial autocorrelation was only detected on July 25, 27, 28, and 29. Significant clustering of trap count data was only detected during the treatment events from July 27-29.

Date (2010)	Autocorrelation	Moran's I	Z-score	P-value	Variance	Expected I
Jul 1	Random	-0.091218	-0.498941	0.617821	0.013459	-0.033333
Jul 2*	Random	-0.012503	0.179275	0.857722	0.010935	-0.031250
Jul 3*	Random	-0.144185	-1.082149	0.279186	0.010891	-0.031250
Jul 4*	Random	0.017742	0.531651	0.594967	0.008845	-0.032258
Jul 5*	Random	0.061557	0.805873	0.420316	0.013865	-0.033333
Jul 6	Random	-0.010905	0.247407	0.804594	0.007449	-0.032258
Jul 7	Random	0.006916	0.661727	0.508146	0.003327	-0.031250
Jul 8	Random	-0.064368	-0.394876	0.692934	0.007034	-0.031250
Jul 9	Random	-0.052038	-0.190336	0.849046	0.010799	-0.032258
Jul 10*	Random	0.025280	0.599335	0.548950	0.008897	-0.031250
Jul 11*	Random	-0.023935	0.071307	0.943154	0.010524	-0.031250
Jul 12	Random	-0.140444	-1.006019	0.314407	0.011781	-0.031250
Jul 13	Random	-0.034519	-0.031237	0.975080	0.010953	-0.031250
Jul 14	Random	-0.046197	-0.139943	0.888705	0.011407	-0.031250
Jul 15	Random	-0.187740	-1.478326	0.139350	0.011207	-0.031250
Jul 16	Random	-0.014158	0.190758	0.848715	0.008029	-0.031250
Jul 17	Random	-0.068975	-0.353005	0.724085	0.011421	-0.031250
Jul 18	Random	-0.072068	-0.468156	0.639673	0.007602	-0.031250
Jul 19	Random	0.070239	0.941679	0.346357	0.011615	-0.031250
Jul 20	Random	-0.019672	0.113866	0.909344	0.010338	-0.031250
Jul 21	Random	-0.008861	0.220673	0.825347	0.010294	-0.031250
Jul 22	Random	0.045735	0.696415	0.486169	0.012542	-0.032258
Jul 23	Random	-0.064109	-0.346997	0.728593	0.008967	-0.031250
Jul 24	Random	0.029574	0.570423	0.568391	0.011750	-0.032258
Jul 25	Clustered	0.191057	1.947334	0.051495	0.013151	-0.032258
Jul 26	Random	0.130227	1.596718	0.110329	0.010355	-0.032258
Jul 27*	Clustered	0.314165	3.346795	0.000818	0.010652	-0.031250
Jul 28*	Clustered	0.123305	1.844752	0.065074	0.007019	-0.031250
Jul 29*	Clustered	0.249690	2.665068	0.007697	0.011112	-0.031250
Jul 30	Random	-0.006652	0.228884	0.818959	0.011550	-0.031250
Jul 31	Random	0.054979	1.036916	0.299775	0.007078	-0.032258

Table II. All layers were defined with NAD 1983 UTM 14 Transverse Mercator. Daily spatial autocorrelation output for NJLT and CDC mosquito trap locations and their corresponding trap count is listed for the month of July 2020. Moran's I was used to determine spatial autocorrelation. When the entire month of July 2020 was analyzed together using Moran's I, significant clustering was revealed (p = 0.00). Adulticide treatment evenings (with DeltaGard®20EW) are indicated by a * beside the date. Spatial autocorrelation was detected on July 3, 14-26, and 28-31. Significant clustering was generally detected around treatment events except on the 6th of July.

Date (2020)	Autocorrelation	Moran's I	Z-score	P-value	Variance	Expected I
June 30	Random	0.000291	0.283069	0.777124	0.009832	-0.027778
Jul 1	Random	0.023711	0.501679	0.615893	0.011213	-0.029412
Jul 2	Random	-0.050052	-0.212543	0.831683	0.010983	-0.027778
Jul 3*	Clustered	0.988875	7.902265	0.000000	0.016034	-0.011765
Jul 4	Random	-0.082118	-0.439509	0.660293	0.017601	-0.023810
Jul 5	Random	-0.099520	-0.751052	0.452621	0.009676	-0.025641
Jul 6*	Random	0.066896	1.015434	0.309899	0.008190	-0.025000
Jul 7	Random	0.020233	0.244952	0.806493	0.034099	-0.025000
Jul 8	Random	-0.008759	0.077341	0.938352	0.040845	-0.024390
Jul 9	Random	0.166122	0.258071	0.796352	0.544966	-0.024390
Jul 10	Random	0.086675	0.963766	0.335163	0.014299	-0.028571
Jul 11	Random	0.065686	1.098713	0.271893	0.007236	-0.027778
Jul 12	Random	0.065686	1.098713	0.271893	0.007236	-0.027778
Jul 13	Random	0.065686	1.098713	0.271893	0.007236	-0.027778
Jul 14*	Clustered	0.281766	2.843138	0.004467	0.012048	-0.030303
Jul 15	Clustered	0.665754	4.045335	0.000052	0.028294	-0.014706
Jul 16	Clustered	1.041809	6.097143	0.000000	0.030026	-0.014706
Jul 17	Clustered	1.042243	6.107564	0.000000	0.029948	-0.014706
Jul 18	Clustered	0.989674	6.944736	0.000000	0.020818	-0.012346
Jul 19	Clustered	0.989674	6.944736	0.000000	0.020818	-0.012346
Jul 20	Clustered	0.989674	6.944736	0.000000	0.020818	-0.012346
Jul 21	Clustered	0.99297	6.849301	0.000000	0.021542	-0.012987
Jul 22	Clustered	1.003387	5.956507	0.000000	0.029178	-0.014085
Jul 23*	Clustered	1.161599	7.383942	0.000000	0.0025378	-0.014706
Jul 24*	Clustered	1.752562	8.953160	0.000000	0.039037	-0.016393
Jul 25*	Clustered	1.450275	5.260230	0.000000	0.077931	-0.018182
Jul 26*	Clustered	1.083496	4.021171	0.000058	0.075153	-0.018868
Jul 27*	Random	0.228589	0.0858560	0.390583	0.084110	-0.020408
Jul 28	Clustered	1.631103	7.489418	0.000000	0.048440	-0.017241
Jul 29	Clustered	1.181063	5.350189	0.000000	0.050140	-0.016949
Jul 30	Clustered	1.064316	7.399543	0.000000	0.021210	-0.013333
Jul 31	Clustered	0.991151	6.560777	0.000000	0.023395	-0.012346

Table III. All layers were defined with NAD 1983 UTM 14 Transverse Mercator. Daily hot spot analysis output for NJLT mosquito trap locations and their corresponding trap count is listed for the months of July 2010 and July 2020. Getis-Ord Gi* statistic was used to determine hot spots. The number of times a trap location was detected as a hot spot on any day in July 2010 or July 2020 at each confidence level are listed by trap number.

Hot Spots	90% confidence	95% confidence	99% confidence
Trap (July 2010)			
11		4	
15	1	1	
17	1	1	4
18	1		
19	1	1	
20		1	
26			1
27	1		1
29	2	3	3
30		1	
35	1	5	7
39			1
41		1	1
42			3
43	1	2	
44	1	3	10
Trap (July 2020)			
1			2
2		3	5
3		1	1
4		1	3
5		1	2
7		3	3
8	1		
10		3	5
11		1	3
15		1	
20		6	1
25		1	2
30		2	1
34	1		2
37	1	1	3
39			1
40	1	1	1
43		1	
44		2	6
52	1		
54	1	2	2
56	1		
61		1	2
63	1	4	8
64			1

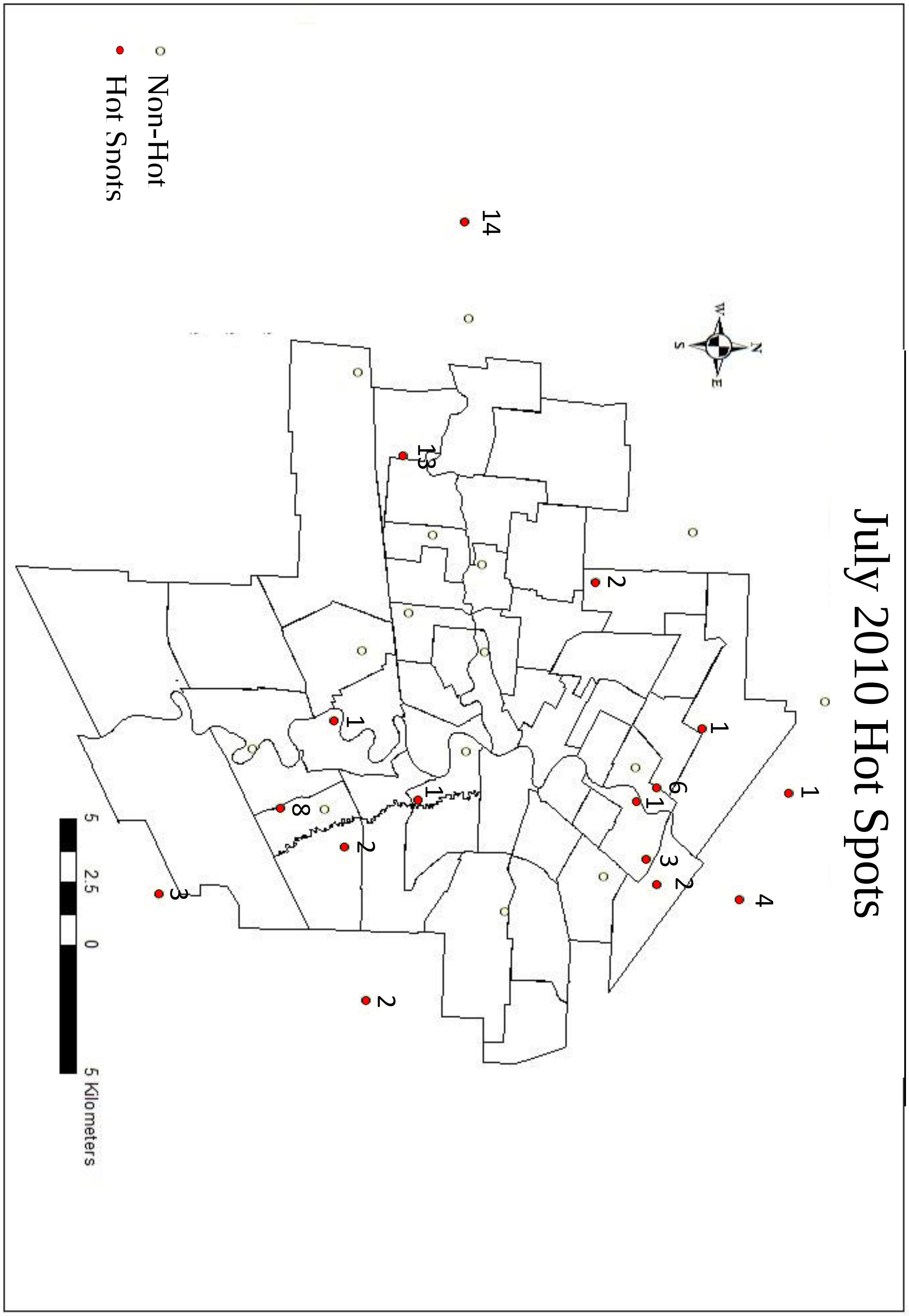


Figure I. NJLT mosquito trap locations and their corresponding trap count data were analyzed daily using the Getis-Ord Gi* statistic to determine hot spots in July 2010. Red circles indicate mosquito trap locations that were hot spots at some point in July 2010 and yellow circles indicate trap locations that were never hot spots during that month. The number of times each location was a hot spot on different days in July 2010 is indicated above each hot spot.

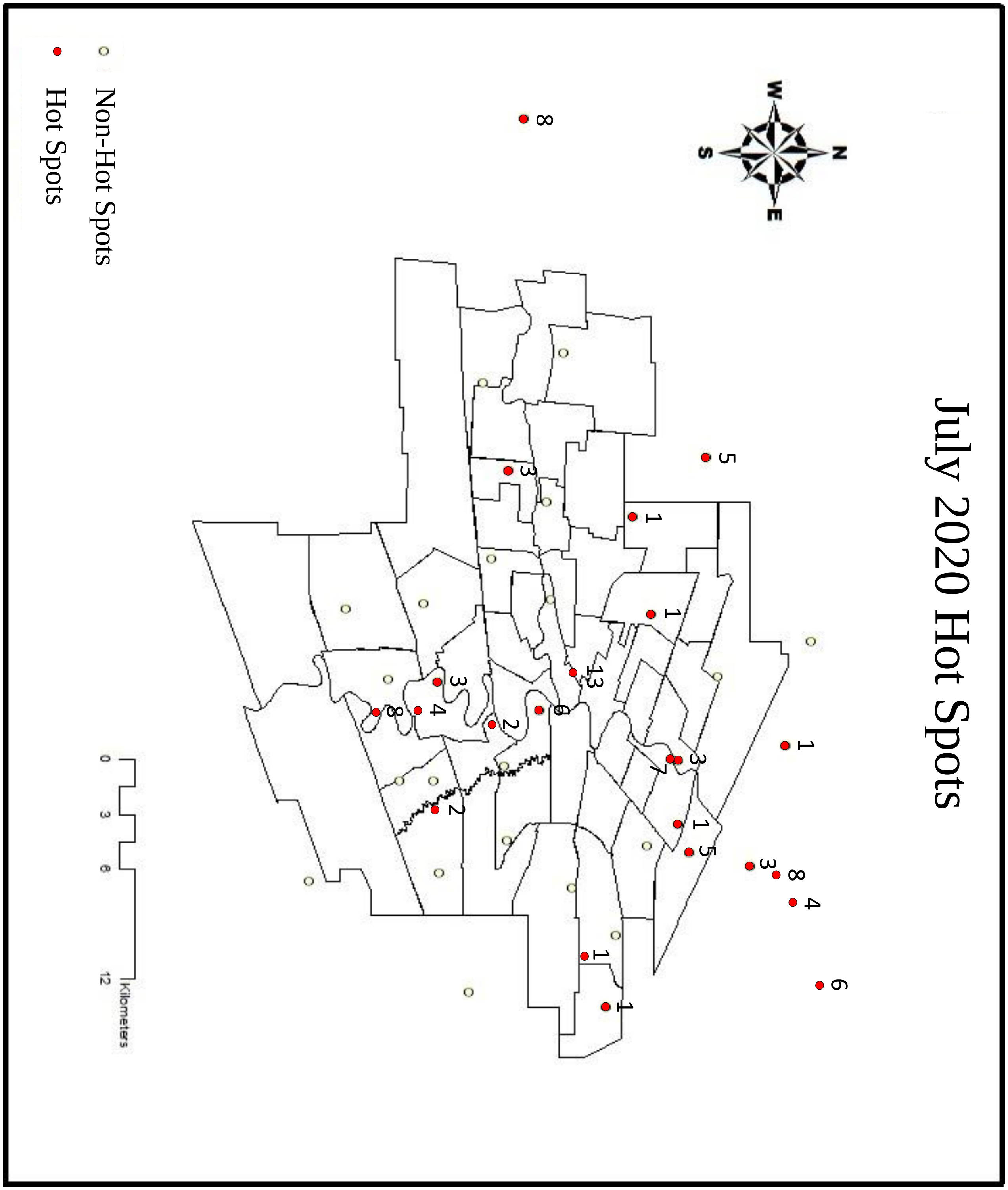


Figure II. NJTL and CDC mosquito trap locations and their corresponding trap count data were analyzed daily using the Getis-Ord Gi* statistic to determine hot spots in July 2020. Red circles indicate mosquito trap locations that were hot spots at some point in July 2010 and yellow circles indicate trap locations that were never hot spots during that month. The number of times each location was a hot spot on different days in July 2020 is indicated above each hot spot.

Table IV. Multiring buffers were made 50m, 100m, and 150m from each trap location. Within these buffers, the number of trees and parks/open spaces was counted. Near tables and joins were used to determine the closest river and closest park/open space to each trap location. The number of times the trap locations were hot spots in July 2010 and 2020 combined is included in red font. There were no cold spots in either year. Any missing trap numbers indicate traps that were from another year and were not included in this analysis. Traps that were hot spots at some point in either July 2010 or 2020 tend to be closer to rivers and to have more trees surrounding them as seen in Figures 13-15 in Results. However, these differences between hot spots and non-hot spots were not found to be significant

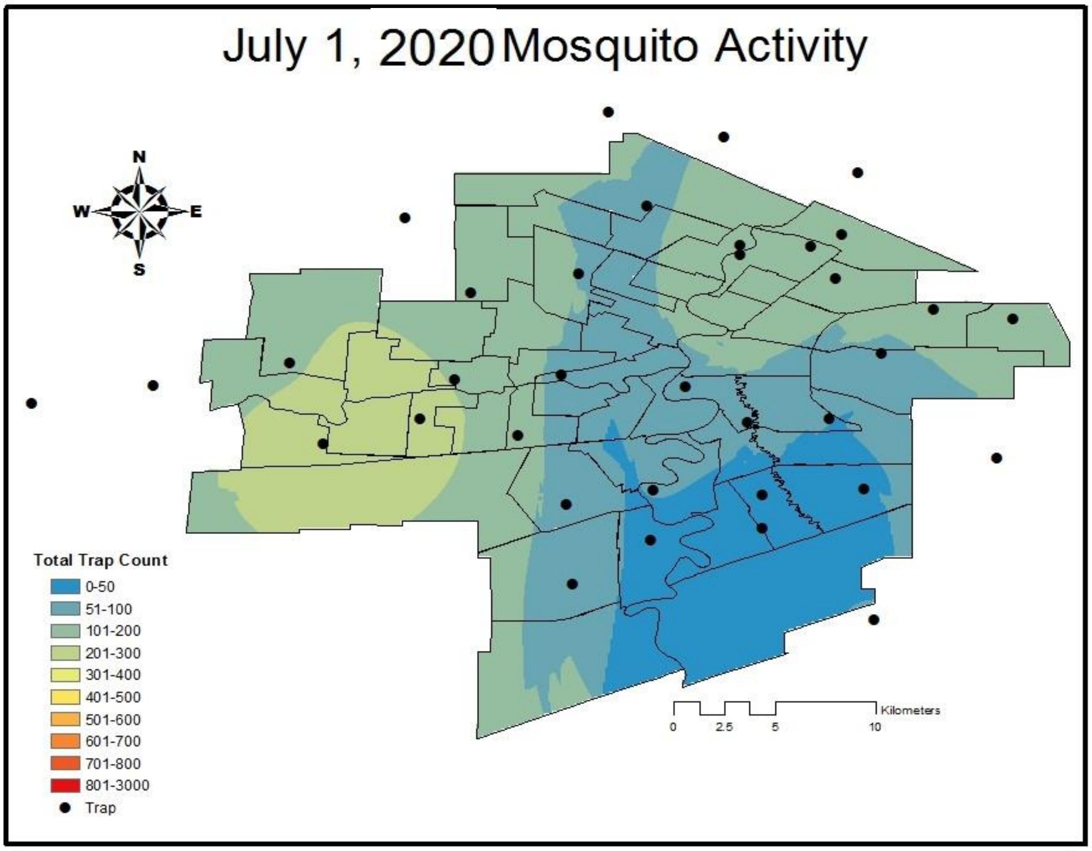
Trap	Trees 50m	Trees 100m	Trees 150m	Closest River	Distance to Closest River	Closest Park/Open Space (OS)	Distance to Closest Park/OS	Parks 50m	Parks 100m	Parks 150m	Number of Hot Spots
1	5	32	83	Seine	297.63	Shorehill Demetrioff Park	103.38	0	0	1	2
2	22	50	134	Red River	169.66	King's Park	0	1	1	1	8
3	2	10	28	Red River	326.83	Carey Park	204.64	0	0	0	2
4	4	15	38	Red River	559.63	St. Germain Park	52.89	0	1	2	4
5	18	61	132	Red River	522.71	Norwood C.C	156.83	0	0	0	3
6	28	85	170	Red River	6925.56	Yale Avenue Playground	163.80	0	0	0	0
7	0	0	0	Red River	3456.21	North Perimeter Park	6263.96	0	0	0	6
8	17	85	192	Red River	7001.56	Yale Avenue Playground	237.51	0	0	0	1
9	14	69	180	Red River	7540.89	Kern Park	80.85	0	2	2	0
10	0	0	0	Red River	311.07	North Perimeter Park	2432.28	0	0	0	8
11	0	0	0	Red River	717.81	North Perimeter Park	3177.46	0	0	0	8
13	13	46	92	Assiniboine River	491.90	Linwood Lot	151.32	0	0	0	0
14	17	63	132	Assiniboine River	293.30	William Marshall Park	409.98	0	0	0	0
15	0	0	0	Omand’s Creek	119.13	Omand's Creek Park North	546.37	0	0	0	3

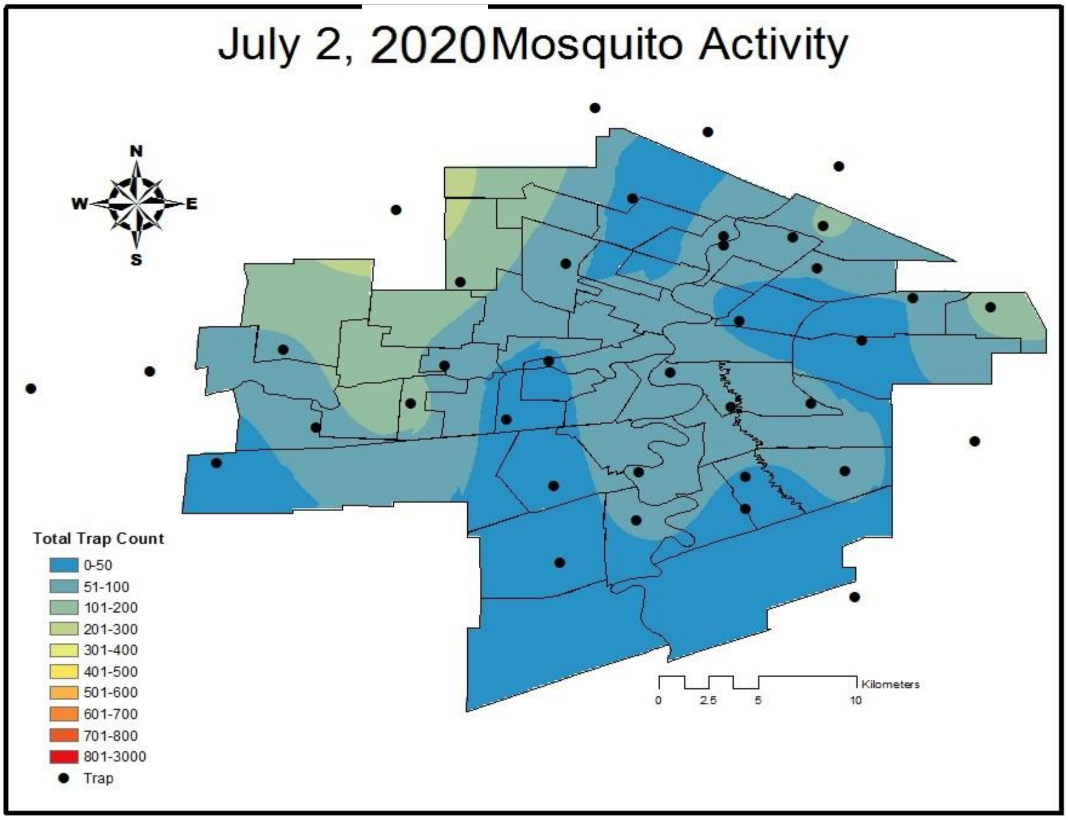
16	3	53	130	Red River	738.51	Bleak House	52.85	0	1	1	0
17	25	140	294	Red River	798.33	Kildonan Park	57.21	0	1	1	6
18	0	0	0	Red River	3527.67	Daylan Marshall Gate Park	349.02	0	0	0	1
19	14	43	148	Red River	1864.35	Bunn's Creek Centennial Park	56.02	1	1	1	2
20	91	274	497	Red River	134.13	Fraser's Grove Park	0	1	1	1	8
21	3	25	53	Red River	3077.13	John De Graff Park	186.98	0	0	0	0
22	18	18	18	Red River	916.36	Abdo and Samira El Tassi Park	347.48	0	0	0	0
23	0	0	0	Red River	388.49	North Perimeter Park	3939.99	0	0	0	0
24	5	60	112	Riviere Seine River	4607.04	Helene Marsh Park	224.17	0	0	0	0
25	16	62	138	Red River	609.80	Coronation Park	241.24	0	0	0	3
26	40	111	243	Riviere Seine River	176.40	King George Park	76.67	0	1	1	1
27	15	49	103	Seine River	859.78	Island Shore Park	58.88	0	1	1	2
28	13	35	85	Seine River	641.38	Brentford Park	59.01	1	1	0	0
29	0	17	46	Seine River	1322.02	John Forsyth Park	234.40	0	0	0	8
30	91	32	719	Red River	325.63	St. Vital Park	0	1	1	1	4
31	3	79	152	Red River	173.20	King's Park	0	1	1	1	0
32	30	67	110	Red River	2445.17	Van Walleghem Park	217.32	0	0	0	0
33	10	59	139	Assiniboine River	2237.25	Boulton-Mathers Retention Pond	114.43	0	0	3	0
34	0	0	0	Assiniboine River	1336.64	Assiniboine Forest	0	1	1	1	3

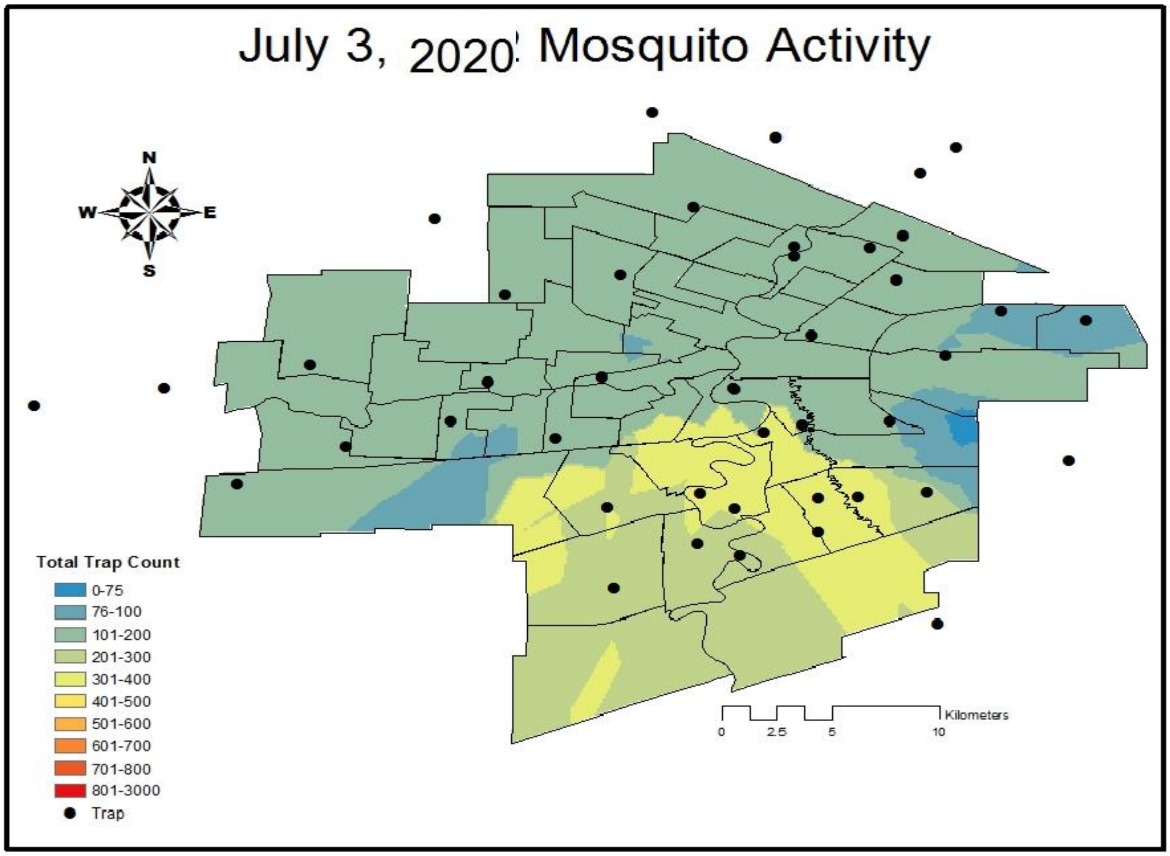
35	1	6	11	Assiniboine River	1052.86	Marcy Beaucage Park/Roblin Park C.C	294.30	0	0	0	13
36	0	0	0	Assiniboine River	3388.04	Ridgewood West Path South	1043.90	0	0	0	0
37	0	0	0	Omand’s Creek	1584.63	Little Mountain Park	0	1	1	1	5
38	0	0	0	Red River	7518.92	Amber Trails Buffer	3715.75	0	0	0	0
39	0	0	0	Red River	3897.65	Orion Crescent Walk	3304.70	0	0	0	2
40	0	0	0	Red River	741.157	North Perimeter Park	1444.63	0	0	0	3
41	0	0	0	Seine River	6609.82	Sage Creek-Bishop Grandin Buffer	2797.64	0	0	0	2
42	0	0	0	Seine River	724.61	Sioux Riverbank	2678.84	0	0	0	3
43	9	68	156	Red River	1405.13	Paufeld Park	52.24	0	1	1	4
44	0	0	0	Assiniboine River	160.30	John Blumberg Park	4133.51	0	0	0	22
45	0	0	0	Assiniboine River	352.63	John Blumberg Park	369.98	0	0	0	0
46	11	58	123	Red River	2517.75	Van Walleghem Park	394.76	0	0	0	0
47	14	117	204	Assiniboine River	195.12	Aubrey Playground	54.33	0	2	3	0
51	7	15	25	Red River	2378.25	Linden Ridge Park	436.62	0	0	0	0
52	12	34	77	Omand’s Creek	2369.44	Mokriy Ecological Reserve	204.41	0	0	0	1
54	51	160	333	Red River	1754.43	Bunn's Creek Centennial Park	1.35	0	0	0	5
55	3	26	122	Red River	2100.47	Ken Oblik Parkway	71.45	0	1	1	0
56	10	52	100	Assiniboine River	1795.46	Assiniboia West Rec. Assoc.-Morgan Site	168.49	0	0	0	1
57	3	11	22	Riviere	2294.59	Lomond Park	180.25	0	0	0	0

				Seine River							
61	30	251	676	Red River	278.23	Kildonan Park	0	1	1	1	3
62	18	81	183	Red River	6458.39	Morley R. Kare Park	44.42	1	1	1	0
63	0	0	0	Assiniboine River	977.12	Air Canada Window Park	97.97	0	0	0	13
64	10	23	74	Red River	8733.60	Transcona Trail-E	17.06	1	1	2	1
65	1	26	55	Red River	905.79	South Winnipeg C.C-Richmond Kings Site	795.56	0	0	0	0
66	0	13	32	Seine River	2282.02	Wood Sage Park	7.80	1	1	2	0

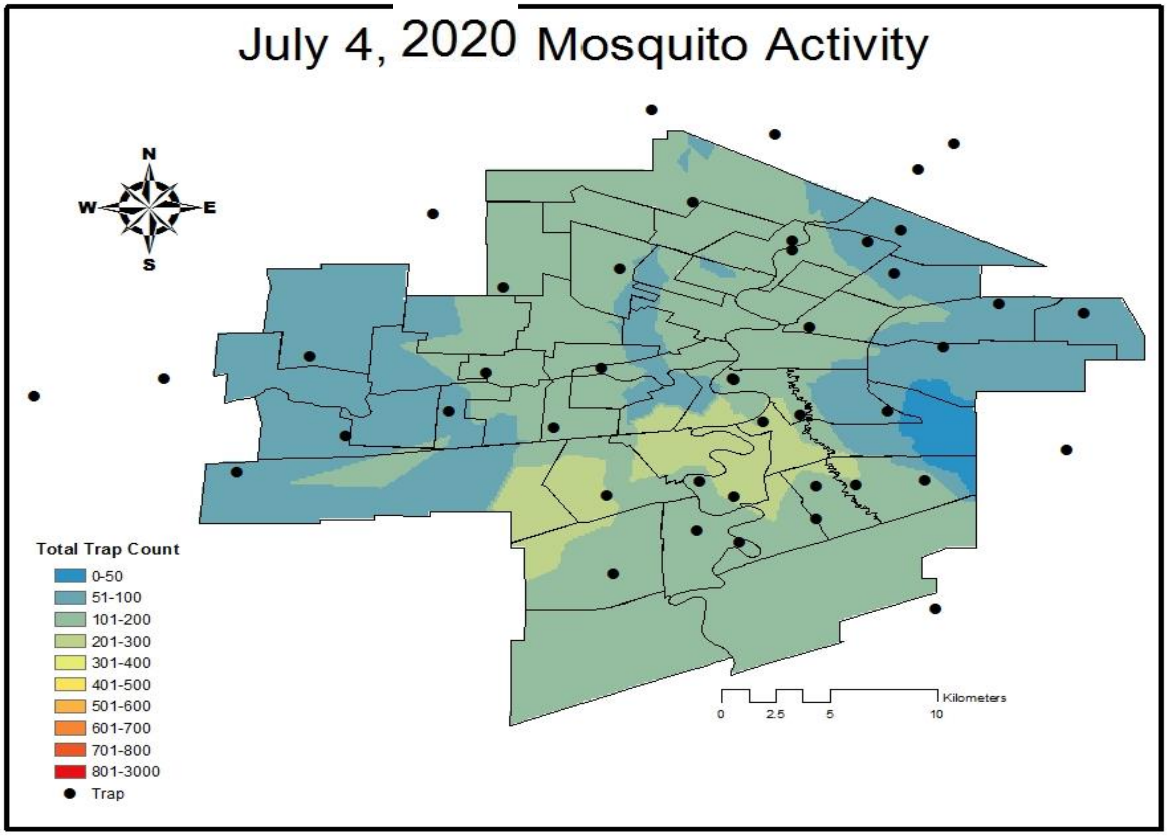
July 2020 Daily Kriging Surfaces



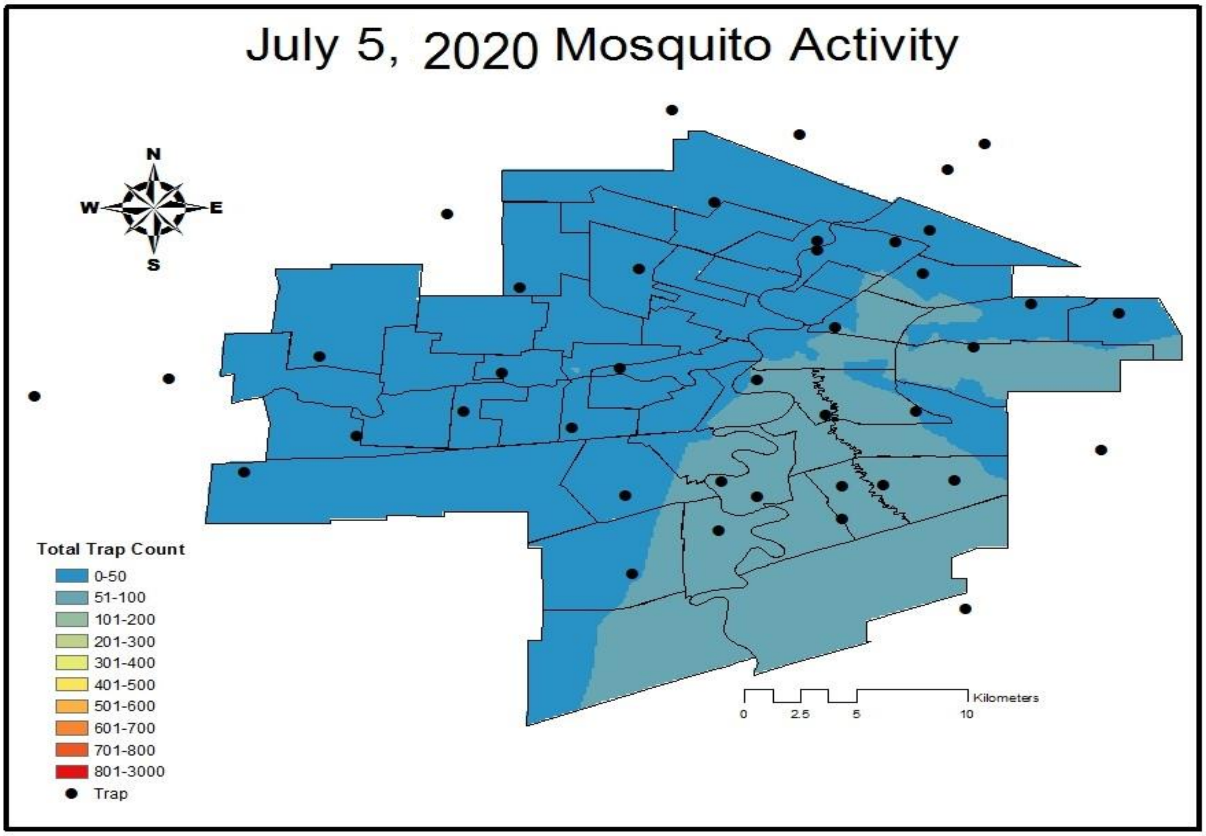


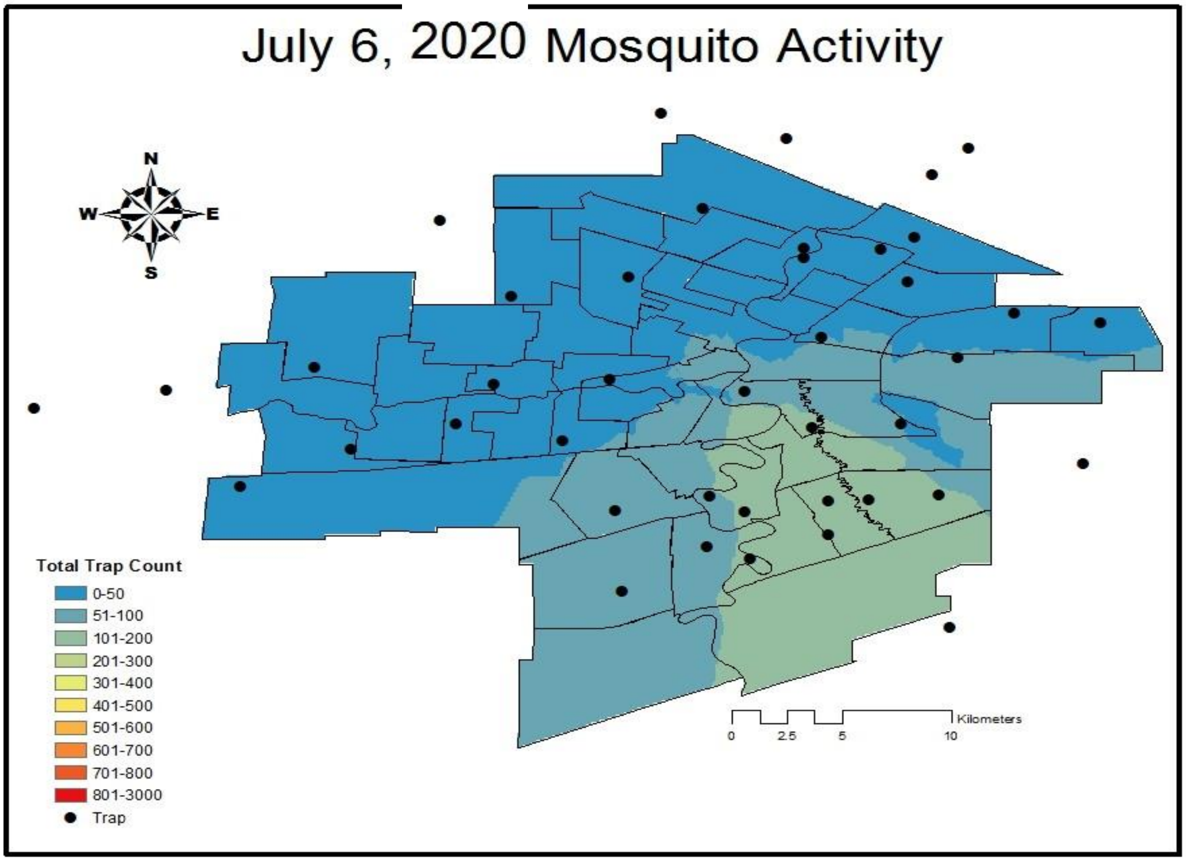


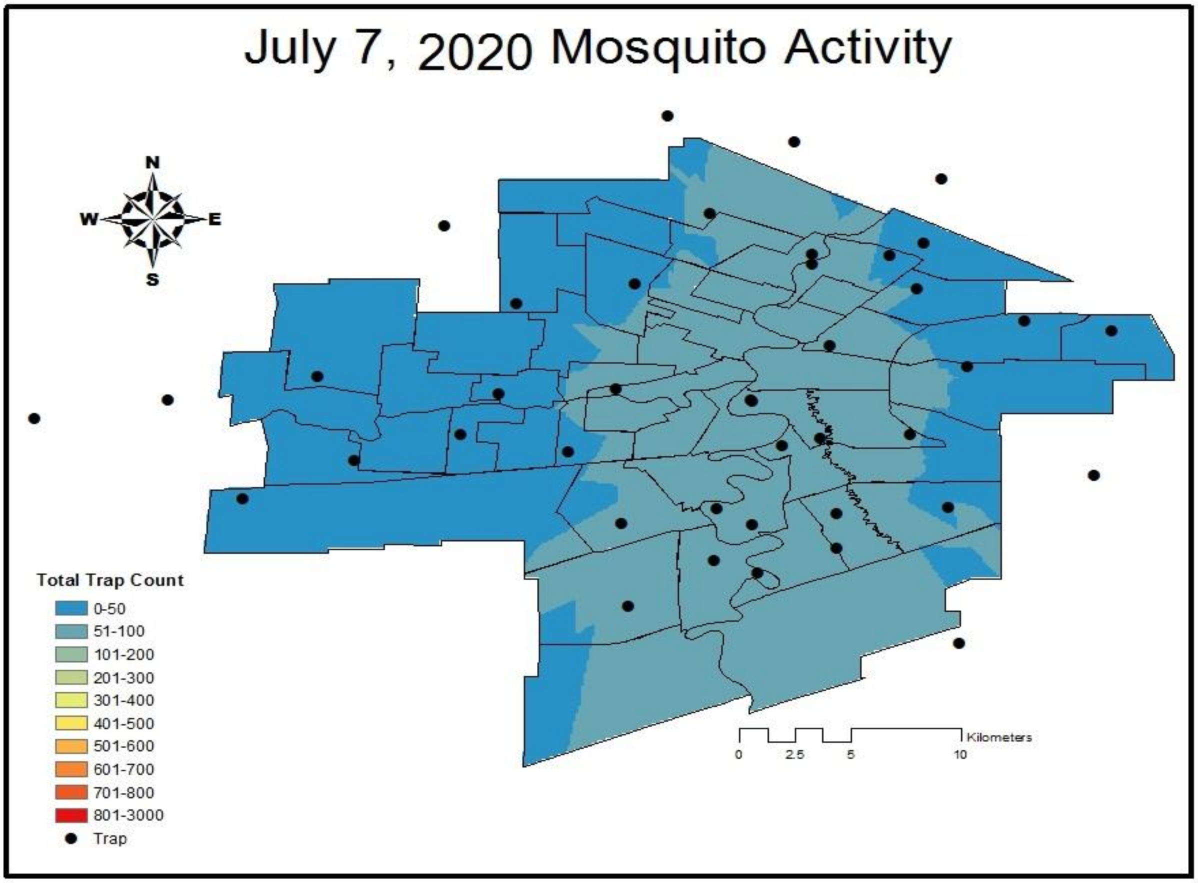
July 3, 2020 Mosquito Activity

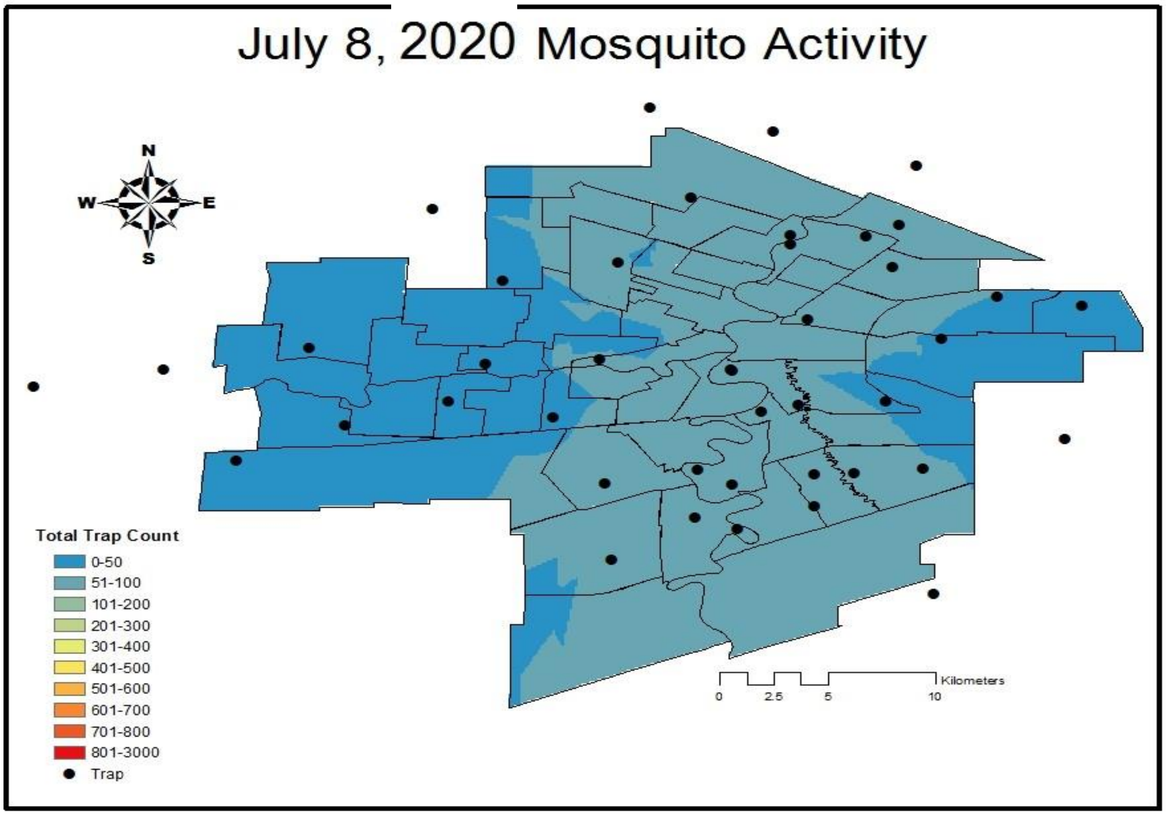


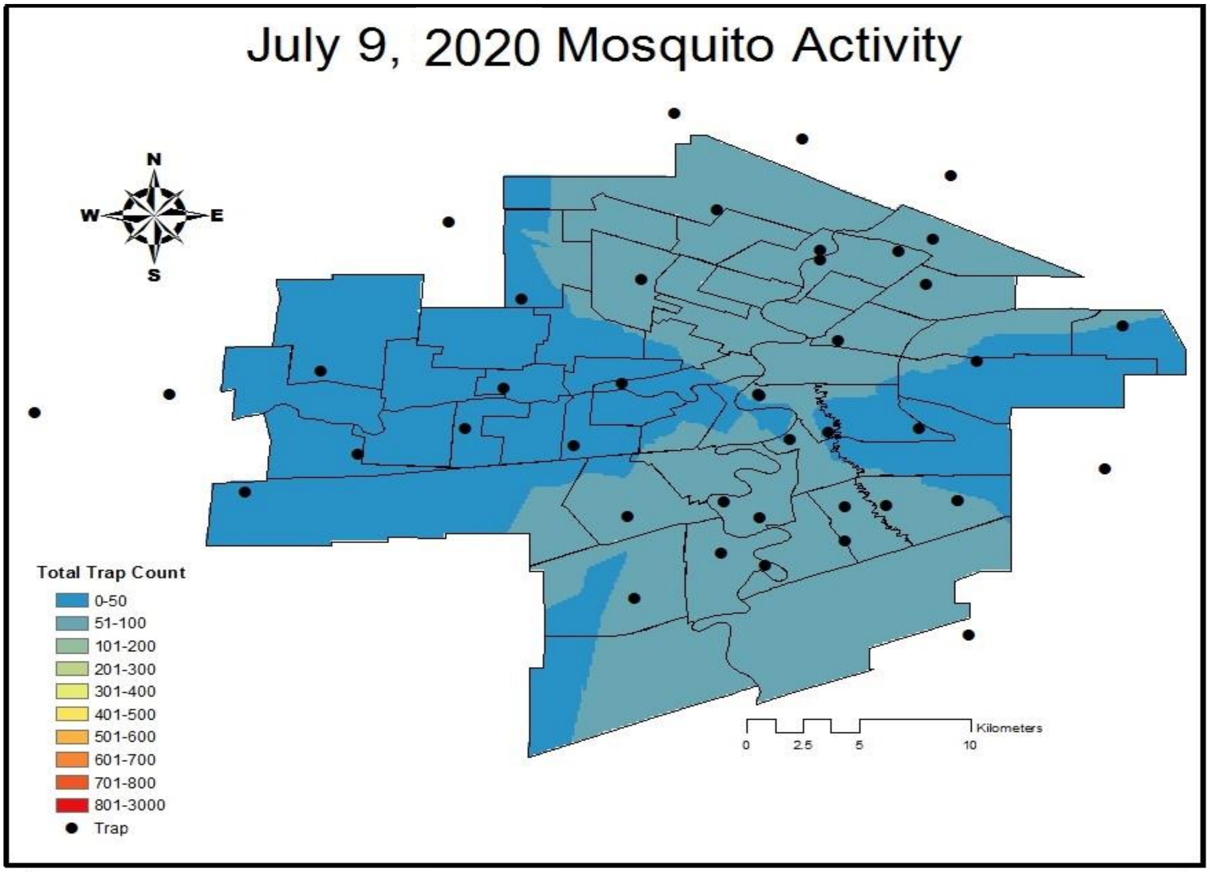
July 4, 2020 Mosquito Activity


July 5, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers

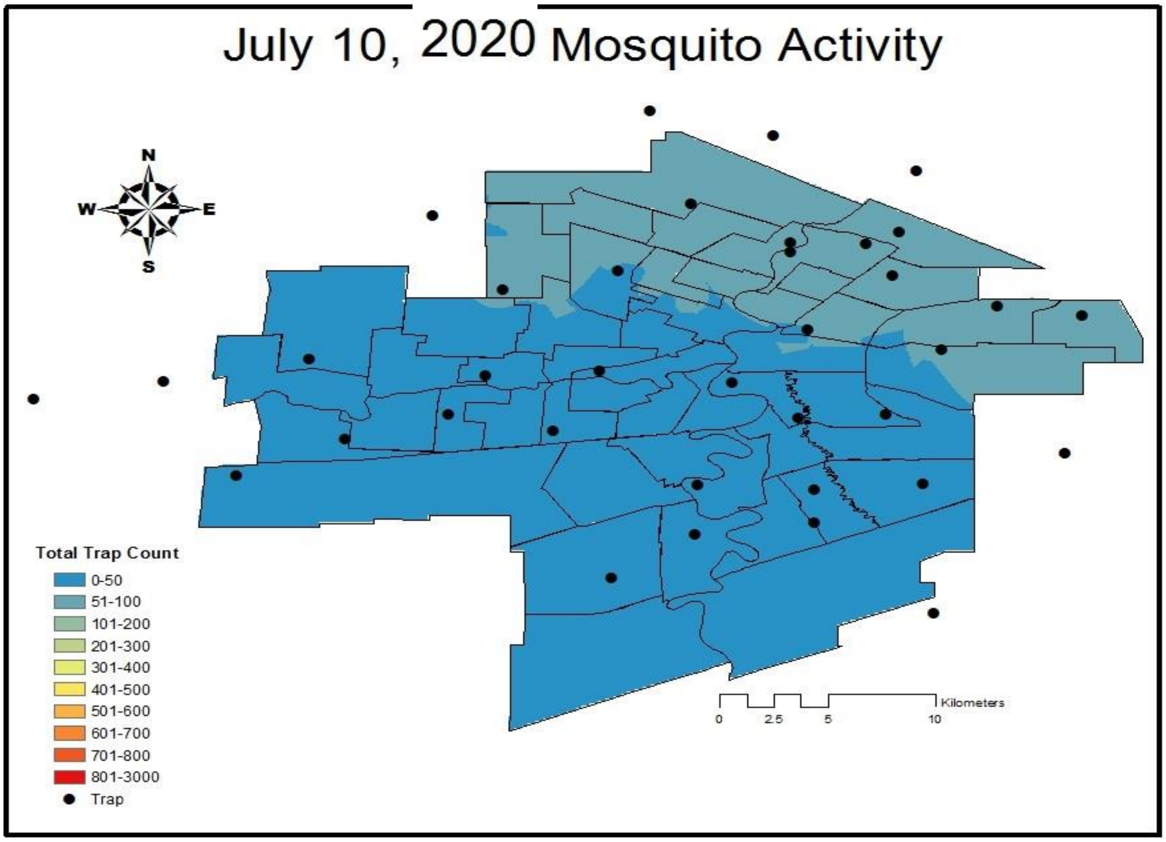

July 6, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers


July 7, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers

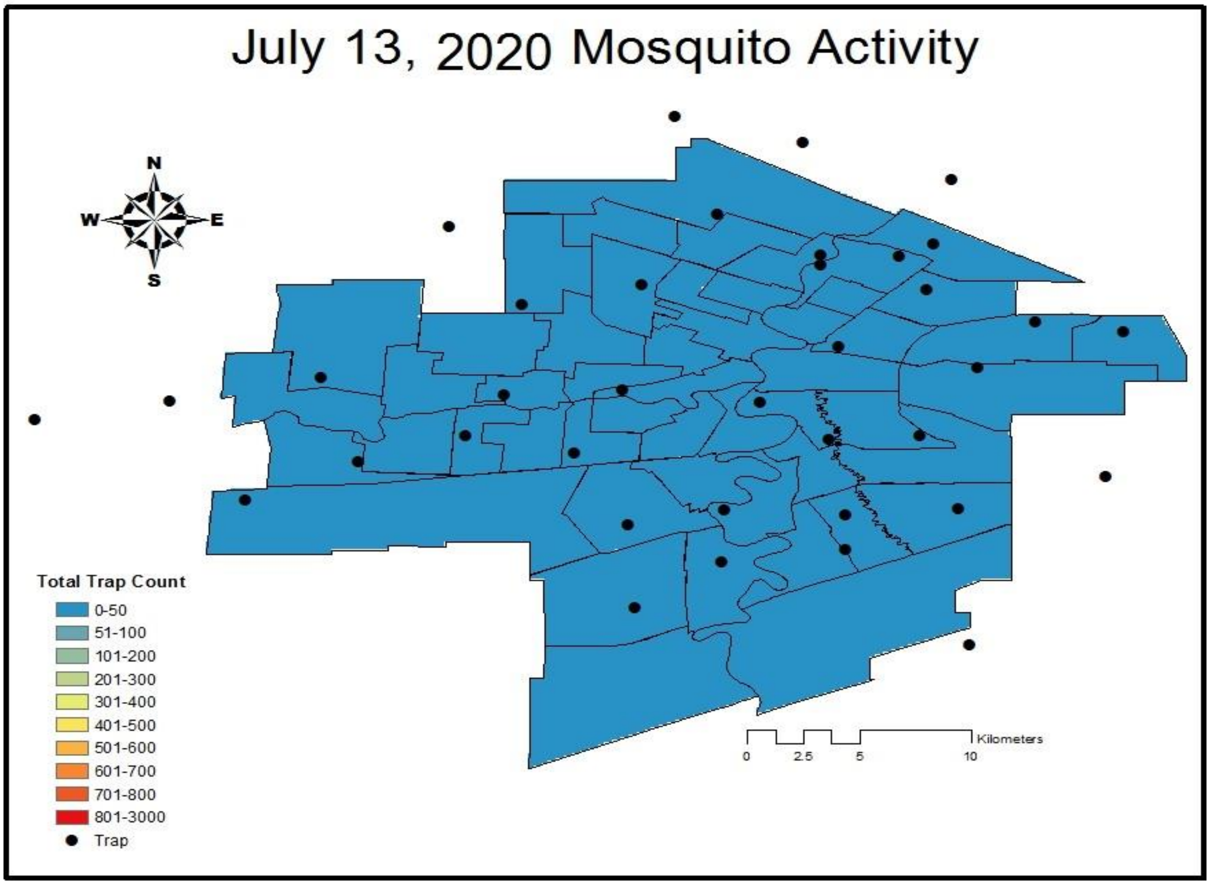

July 8, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers

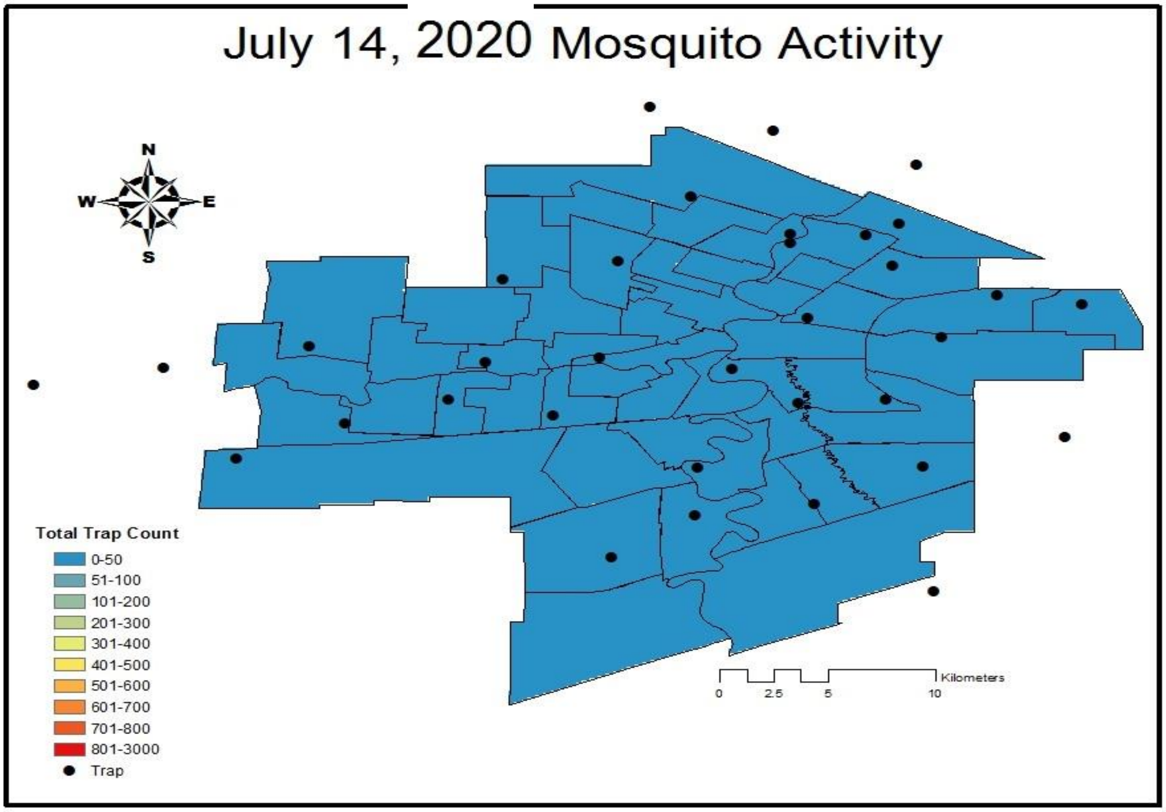


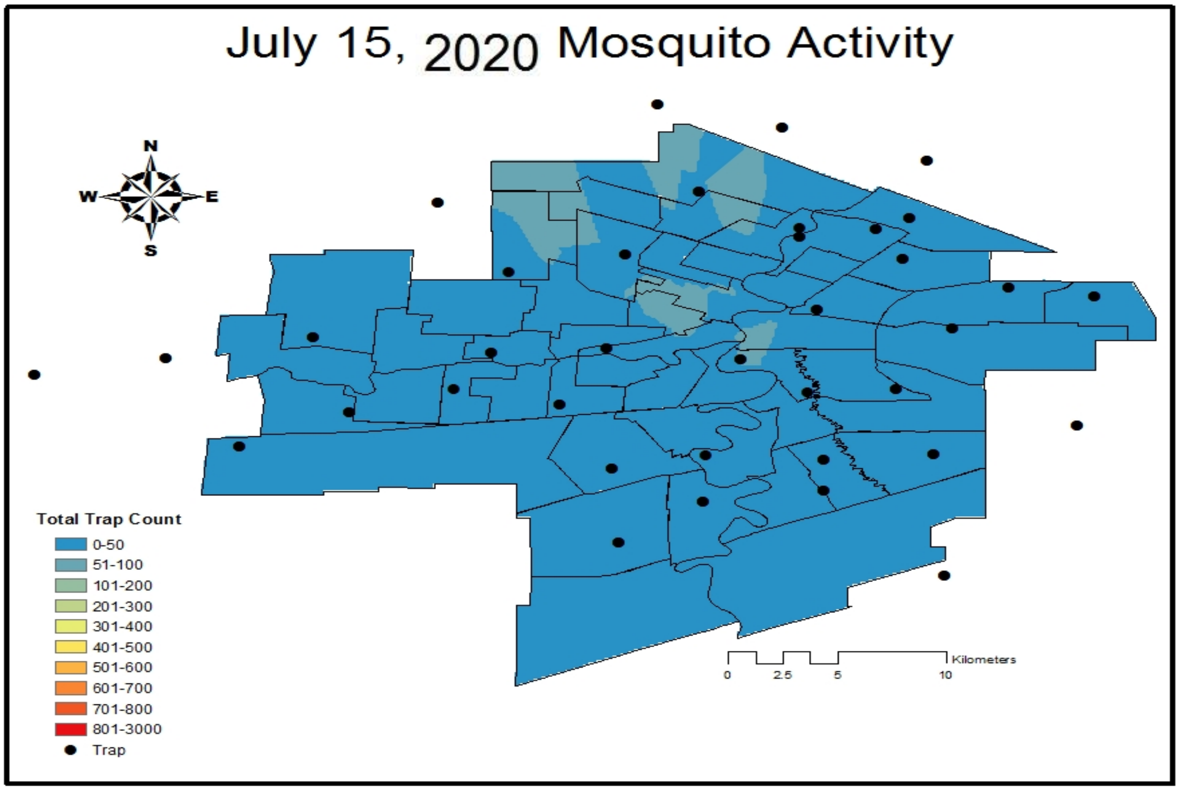
July 9, 2020 Mosquito Activity



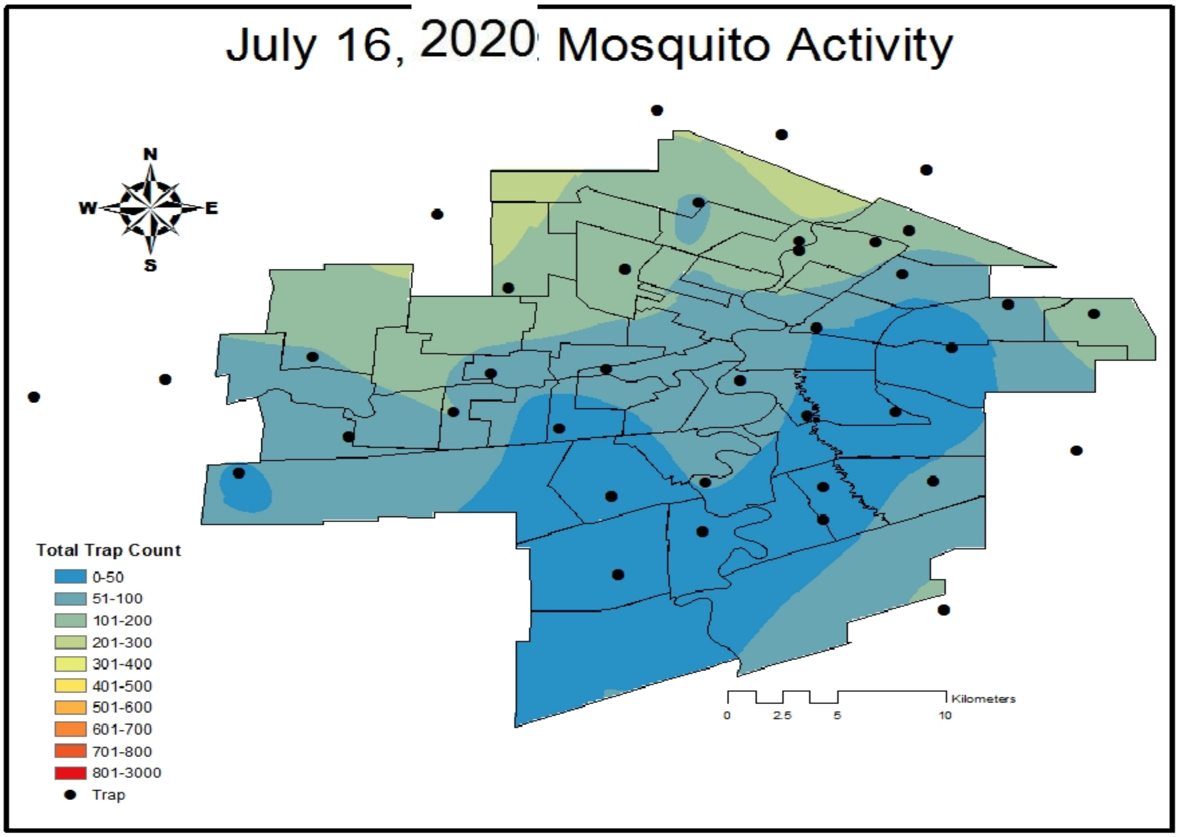
July 10, 2020 Mosquito Activity


July 13, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers

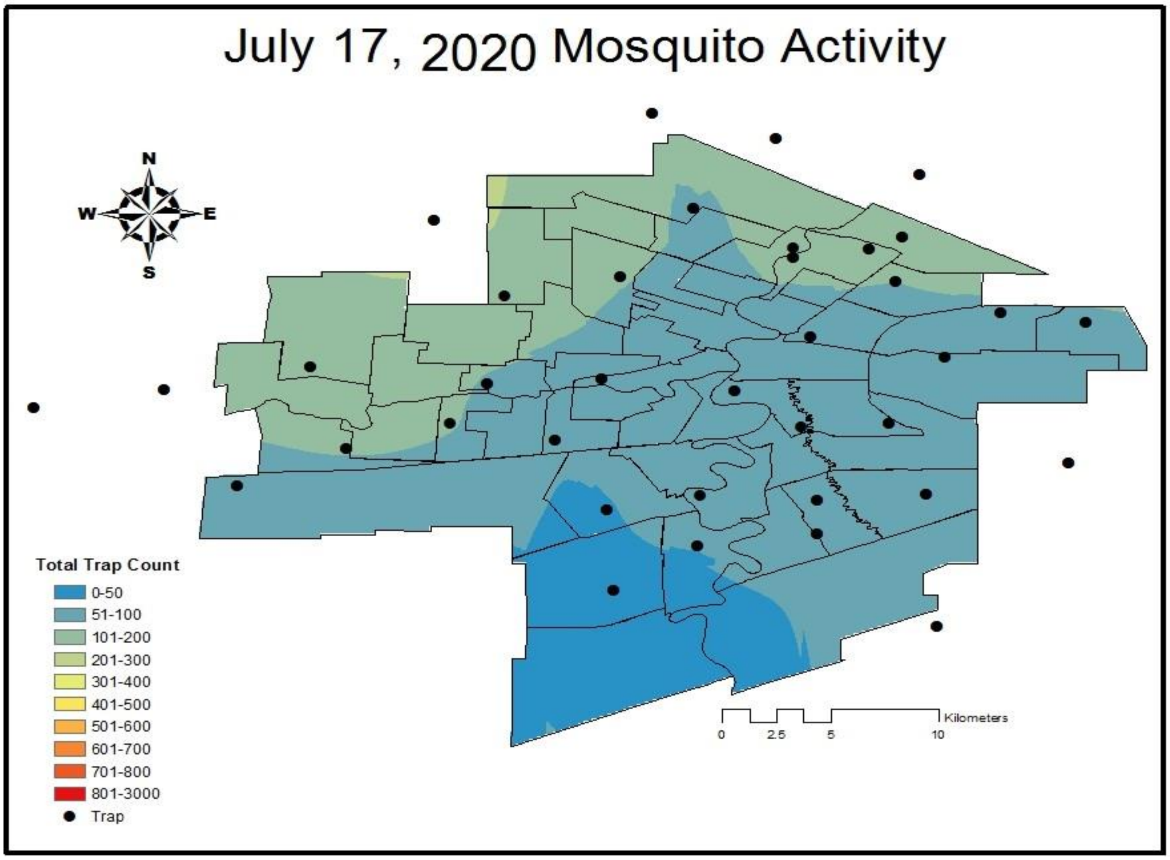

July 14, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers

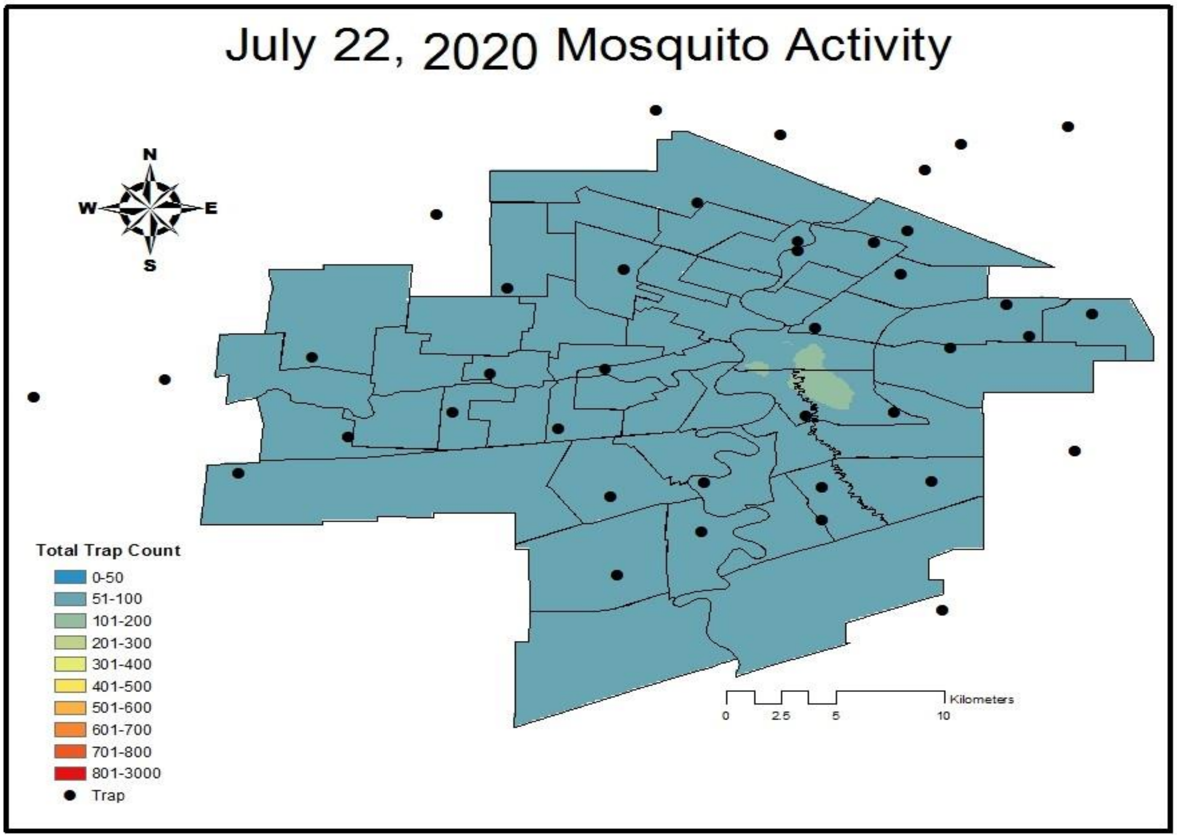


July 15, 2020 Mosquito Activity



July 16, 2020 Mosquito Activity


July 17, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers


July 22, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
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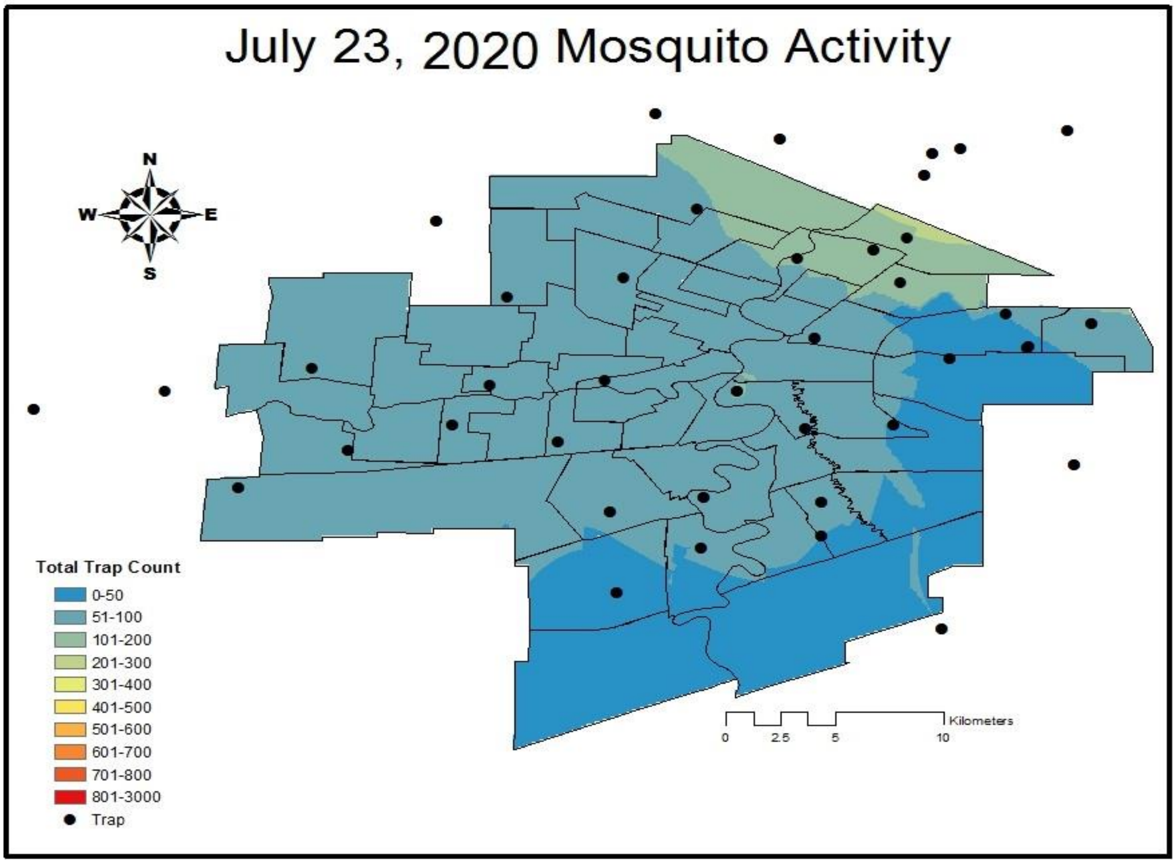
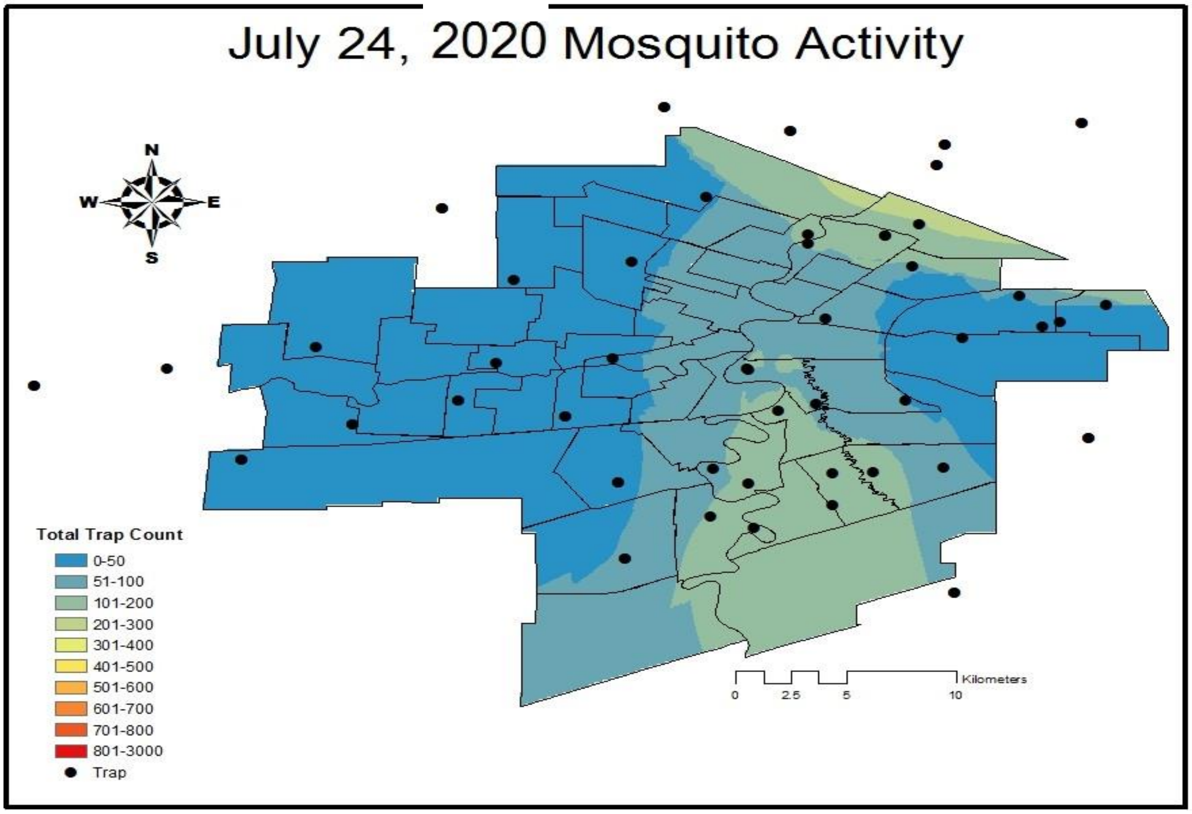
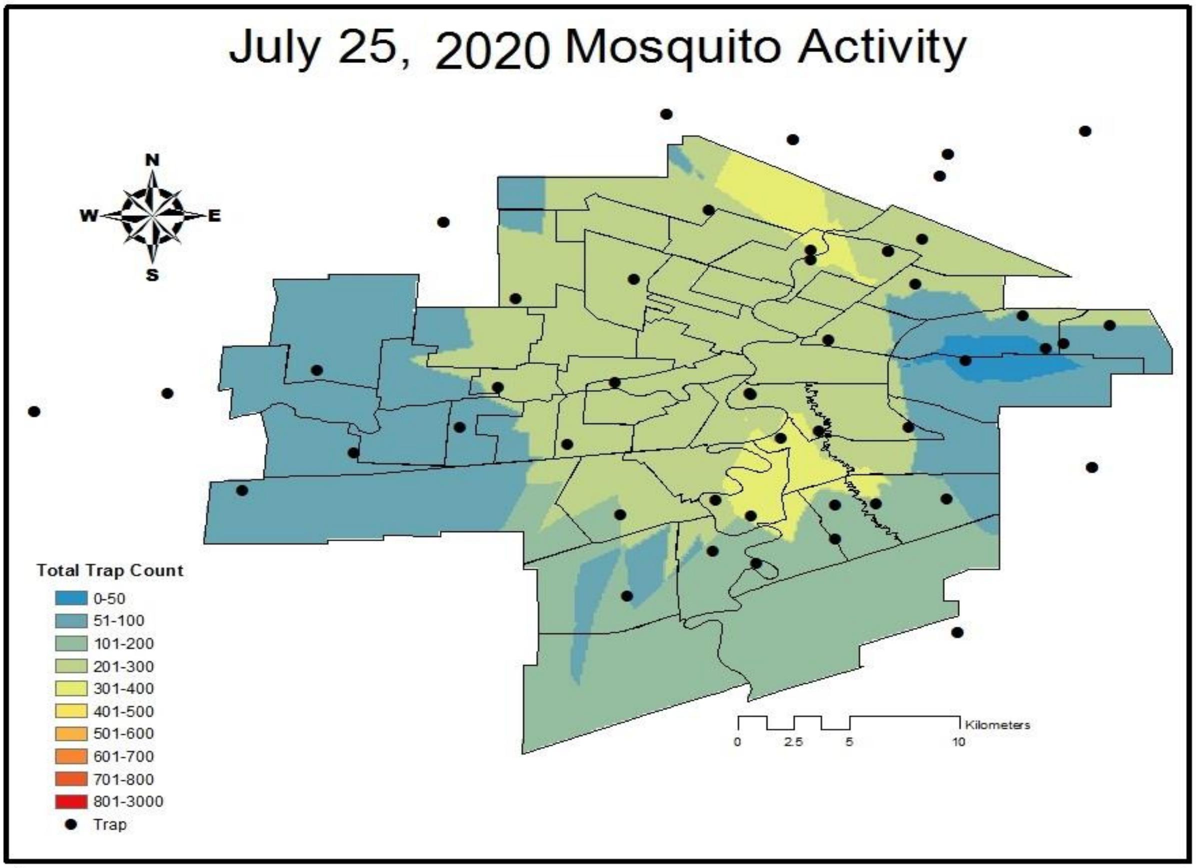
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers



July 23, 2020 Mosquito Activity



July 24, 2020 Mosquito Activity


July 25, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
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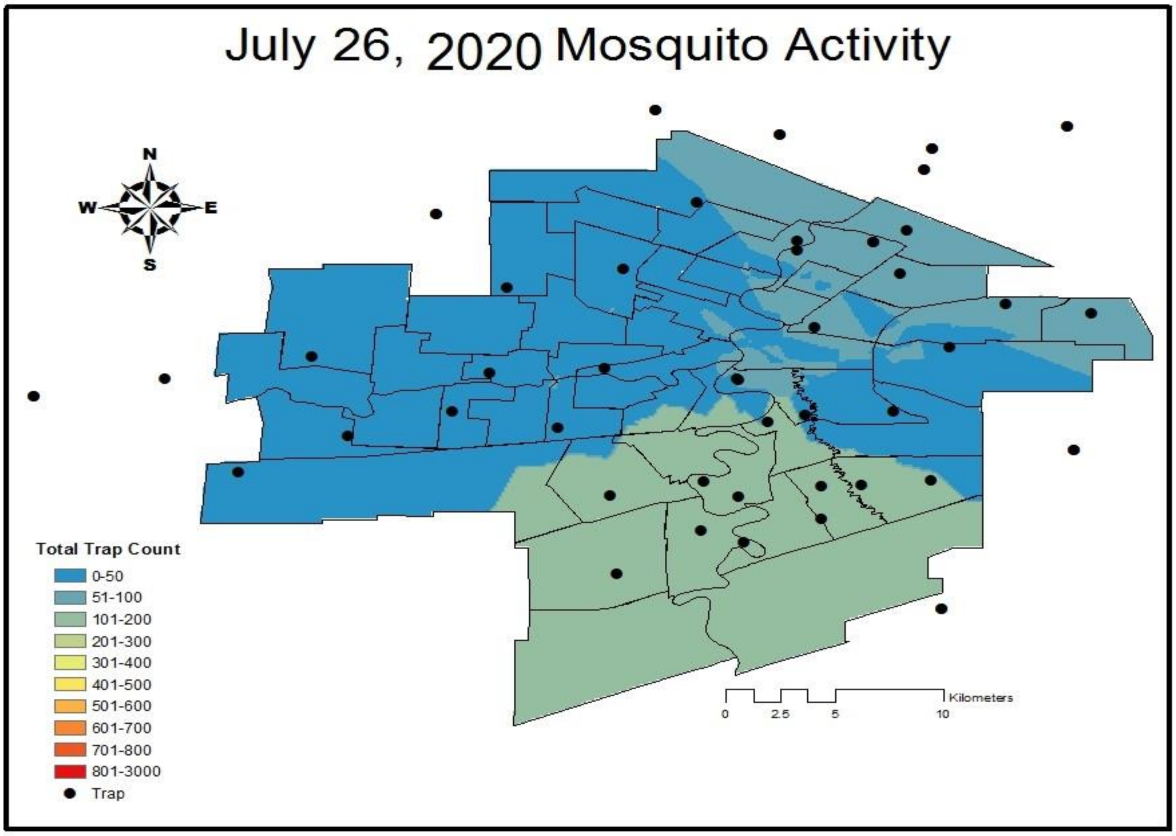
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers


July 26, 2020 Mosquito Activity
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W
E
S
Total Trap Count
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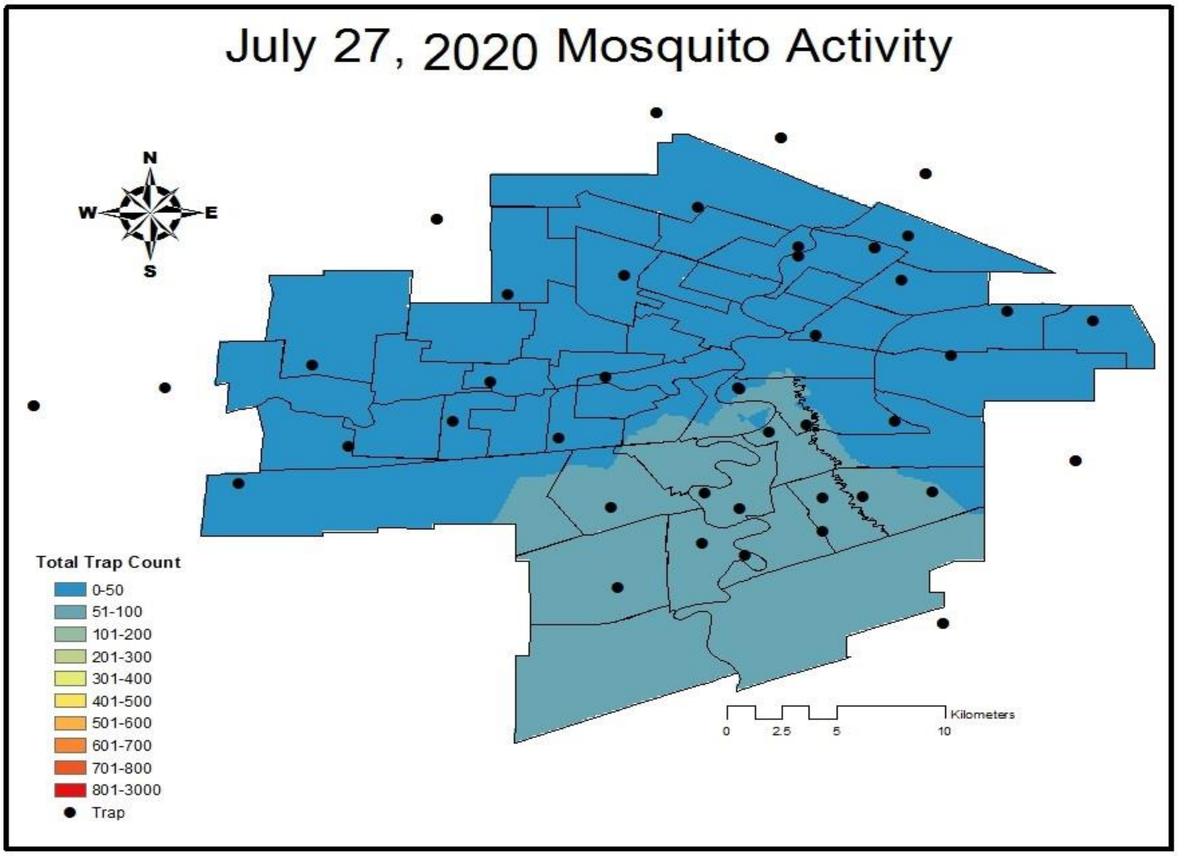
401-500
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers


July 27, 2020 Mosquito Activity
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W
E
S
Total Trap Count
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51-100
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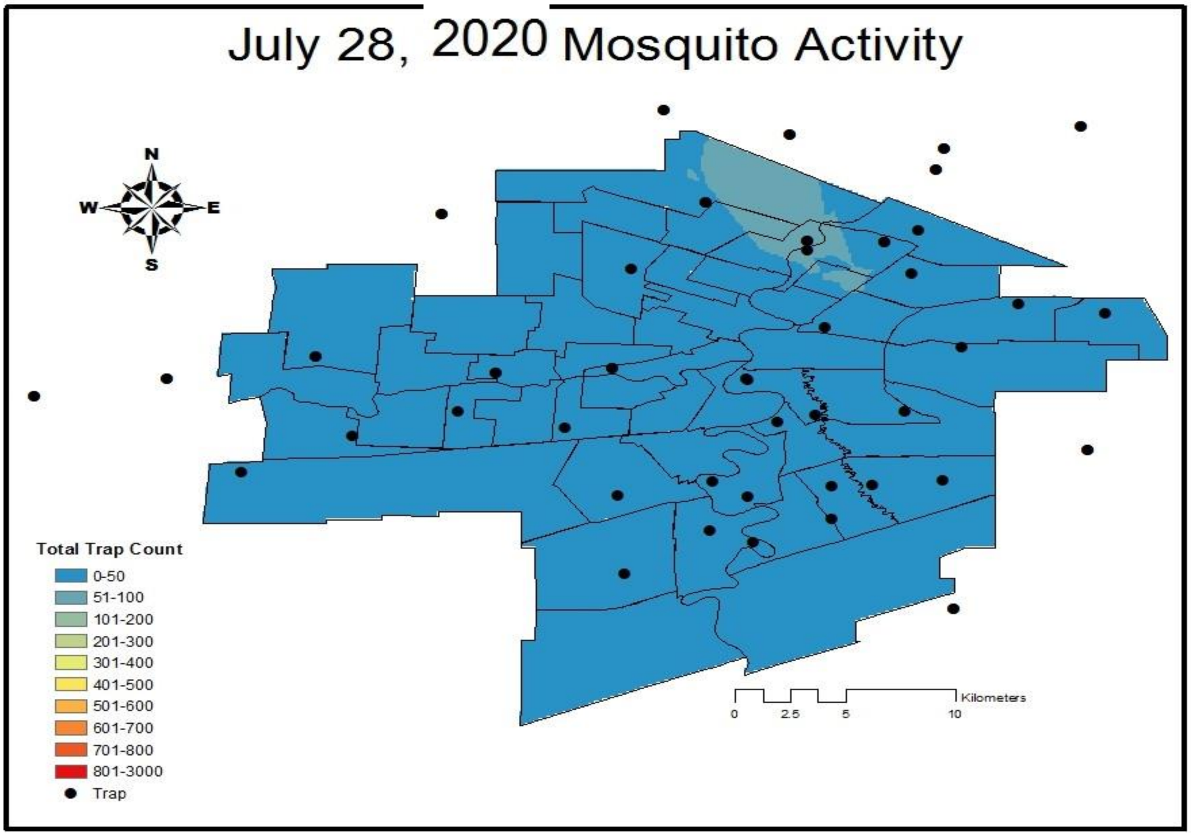
Trap
0
2.5
5
10
Kilometers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W
E
S
Total Trap Count
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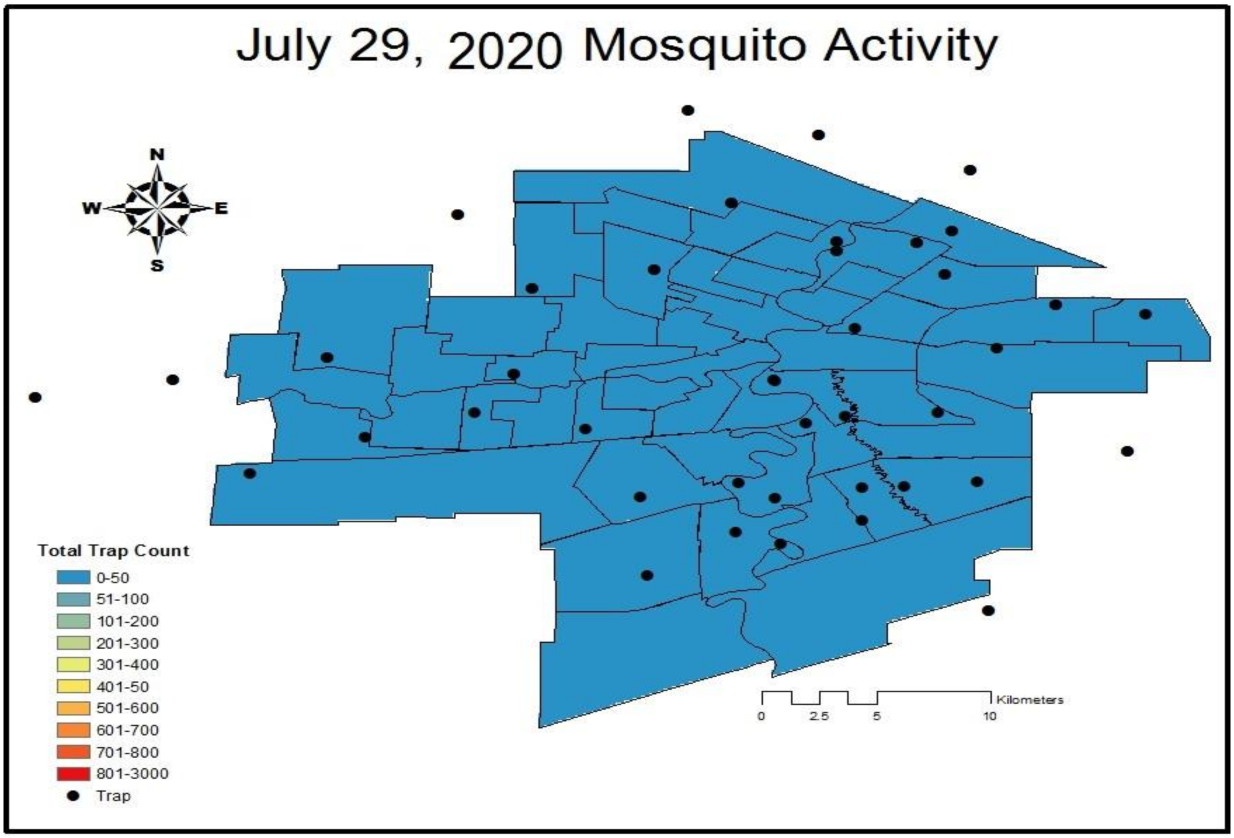
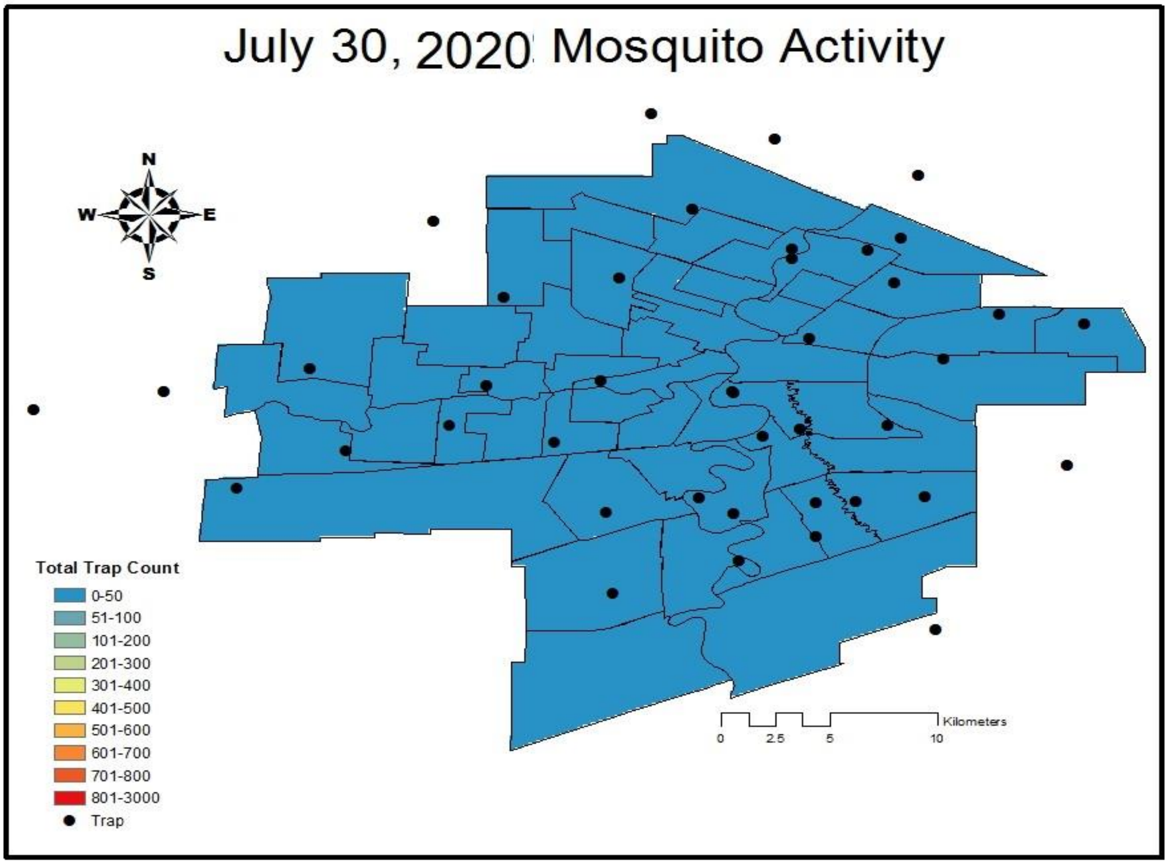
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers


July 29, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-50
501-600
601-700
701-800
801-3000
Trap
0
2.5
5
10
Kilometers


July 30, 2020 Mosquito Activity
N
W
E
S
Total Trap Count
0-50
51-100
101-200
201-300
301-400
401-500
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601-700
701-800
801-3000
Trap
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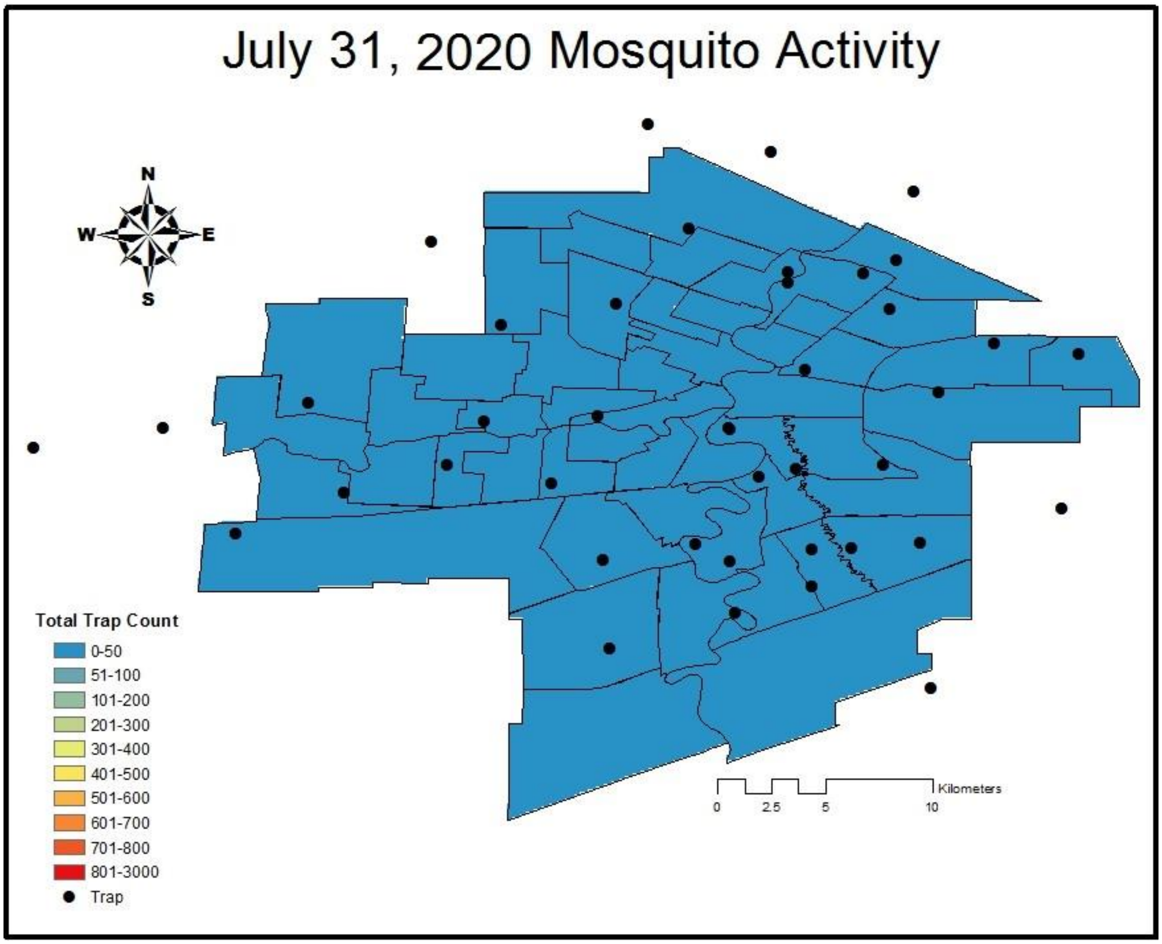
2.5
5
10
Kilometers



Figures III – XXVII. Kriging interpolative surfaces based on trap locations (shown with black dots) and their corresponding trap count values daily in July 2020. See kriging analysis (Section 5.2) for cross-validation results. The results indicated inconsistencies between expected and observed results. Locations in this year were treated with DeltaGard®20EW in different locations depending on the date. Treatment patterns can be found in Figures 10 and 11 in Methods. Higher mosquito activity is detected near parks and cemeteries before they are treated on July 3 and 6. This trend is not detected surrounding the July 14 treatment event where mosquito activity appears to be low citywide. For the rotational citywide treatment, mosquito activity appears to decrease after treatment.

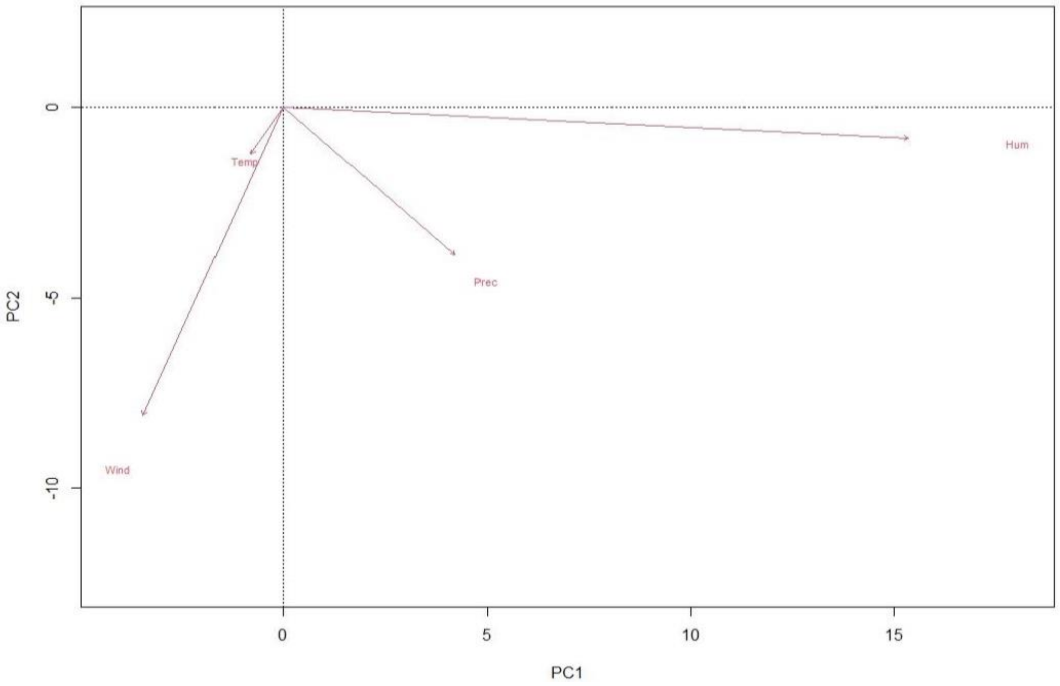


Figure XXVIII. PCA output for meteorological variables from July 2010 and July 2020. The axesindicate the principal component (PC) 1 which explains the largest possible variation in the dataset, and PC2 which explains most of the variation left.