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

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by

Thomas John Scott McMahon

In partial fulfillment of the requirements for the degree

of

Master of Science

Department of Entomology

THE UNIVERSITY OF MANITOBA

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

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

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ABSTRACT

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

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CHAPTER I: INTRODUCTION

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

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

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

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

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

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

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

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

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

CHAPTER II: LITERATURE REVIEW

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

Mosquito Oviposition:

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

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

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

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

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

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

Important container-breeding mosquitoes:

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

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

Species indigenous to North America:

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

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

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

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

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

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

<u>Species Introduced to North America:</u>

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

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

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

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

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

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

Mosquitoes and Tires

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

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

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

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

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

Berry and Craig (1984) surveyed scrap tires in Indiana specifically for *Oc. atropalpus*, but found ninety per cent of the larvae collected were *Cx. restuans*. They also measured the time between placing the tire in the environment, and subsequent colonization. They observed that *Cx. restuans* was the fastest colonizer, appearing in