Abandoned Tire Health Risk Survey/Analysis:

Potential Mosquito Vectors of Pathogenic Agents

ATI TRP 99-08: Final Research Report

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Section One: Background

While there has been a long-standing recognition that some action on the part of state government was necessary to deal with the problem of abandoned tires, there was little consensus on how a waste tire remediation program could be administered effectively. The high cost of such a program was revealed in 1992, when John Ranson, West Virginia Secretary of Commerce, noted the state was facing a multi-million dollar price tag for dealing with abandoned tire piles (Chas. Gaz., 11 Nov '92). Mr. Ranson's cost estimate became a reality with the clean up of Shorty's Tire Dump near Dolly Sods in Grant County. This site received considerable media attention because of its size (estimated at 10 million tires) and clean-up cost of $7.3 million (Chas. Gaz., 2 Apr. '95). Adding impetus to the development of a waste tire cleanup program in West Virginia was the fact that other states–most notably Texas, Illinois and Louisiana–have been operating tire clean up programs, supported by a fee on each new tire sold, for 10 years or more (Long, 1996).

Concern over waste tire piles falls into two broad categories: 1) environmental degradation; and 2) public health. The waste tire problem was finally addressed in the 2000 legislative session with the introduction of Senate Bill No. 427, as requested by then Gov. Underwood. Legislative findings in Article 24, Section 17-24-1 of that bill were (in part):

> *"The accumulation of waste tires has also become a significant* environmental and public health hazard to the state, and the location and number of waste tires are directly related to the efficiency of travel, by citizens, visitors and of commerce, along public highways in West Virginia. In particular, the legislature recognizes that waste tires are widespread in location and in number throughout the state; waste tires physically touch and concern public highways, including,but

not limited to state roads, county roads, park roads, secondary routes and orphan roads, all of which interferes with the efficiency of public high ways; and further that the existence of waste tires along and near public highways is sometimes accompanied by other hazards and, in turn, adversely impacts the proper maintenance and efficiency of public highways for citizens."

"The Legislature also recognizes and declares that waste tires are a public nuisance and hazard; that waste tires serve as harborage and breeding places for rodents, mosquitoes, fleas, ticks and other insects and pests injurious to the public health,safety and general welfare; that waste tires collected in large piles pose an excessive risk to public health, safety and welfare from disease or fire; that the environmental, economic and societal damage resulting from fires in waste tire piles can be avoided by removing the piles; and that tire pile fires cause extensive pollution of the air and surface and ground water for miles downwind and down stream from the fire."

"Therefore, in view of these findings the Legislature declares it to be the public policy of the state of West Virginia to eliminate the present danger resulting from discarded or abandoned waste tires and to eliminate the visual pollution resulting from waste tire piles,and that in order to provide for the public health, safety and welfare, quality of life, and to reverse the adverse impacts to the proper maintenance and efficiency of public highways, it is necessary to enact legislation to those ends by providing expeditious means and methods for effecting the disposal of waste tires."

Senate Bill No. 427, giving authority to The Department of Transportation's Division of Highways (WVDOH) to administer funds and write the regulations for a waste tire remediation program, was passed March 11, 2000, and signed by Gov. Underwood April 4, 2000. The effective date of S.B. 427 was 90 days from passage, June 9, 2000.

Mr. Russ Rader, manager of the waste tire program, was instrumental in drafting the regulations for the "Waste Tire

Remediation/Environmental Clean Up" under authority of: W. Va. Code §§17-23-2 and 3 and 17-24, et seq. Clean up priorities established by these regulations are:

a. health issues – vector, rodent, water;
b. potential for ground water contamination;
c. potential for stream pollution;
d. potential for air quality problems and/or susceptibility of waste tire pile to fire;
e. proximity to population centers;
f. potential number of population affected;
g. number of waste tires in pile; and
h. other special conditions which justify immediate action.

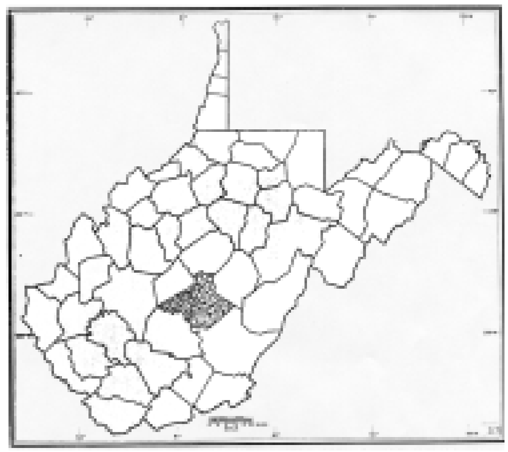
Clearly, S.B. 427 gives high priority to vector control at tire dumpsites. The present research project is designed to evaluate mosquito vector populations at waste tire sites in Nicholas County with the hope that data recorded for the target county can be extrapolated to other areas of West Virginia.

There are 27 species of mosquitoes indigenous to the state (Butler and Amrine, 1980; Joy, et al., 1994), and several of these species are known vectors (i.e., carriers) of pathogenic viruses (Joy and Hildreth-Whitehair, 2000; Nasci et al., 2000). Thus, the primary goal of this study is to identify those mosquito species (whether potential vectors of pathogens, or not) found in tire habitats of Nicholas County, and to evaluate the prevalence (i.e., frequency of occurrence) of each species. To achieve this goal, an attempt will be made to record all tire dump sites in the county and examine tires at as many of those sites as possible throughout the mosquito-breeding period of April through October of 2001 (and possibly 2002). It should be understood that S.B. 427 defines a tire dumpsite as an

area containing more than 100 waste tires which are not mounted on wheels. While this is an adequate working definition for the purpose of licensing or granting operation permits to salvage yards, it inadequately addresses public health concerns because accumulations of but a few tires may serve as excellent mosquito breeding sites, especially if found near human habitations. As a result, in this study, any accumulation of tires, however small, will be considered as potential sites for mosquito borne pathogen transmission.

Section A

An account of significant progress (findings, events, trends, etc.) made during the reporting period.



Left: Fig. 1 Map of WV; Nicholas Co. darkened

A total of 431 waste tire pile sites was identified in Nicholas Co., West Virginia (Fig. 1) during the course of the first year of this proposed 2-year study (Table 1). Only 261 of those sites were physically examined for the presence of mosquito larvae, however, because some accumulations of waste tires were unsuitable as mosquito larval habitats (i.e., tires were in sheltered enclosures, packed with mud and gravel for creek bank protection or sealed with rims); or permission to examine tires could not readily be obtained from the property owner (i.e., owners were often absent).

Below: Table 1. Number of waste tire pile sites identified by category (see Tables 2 through 5 for category descriptions). N_T = total number of sites identified; N_N = number of sites not examined; N_E = number of different sites examined for mosquito larvae; N_V = number of visits to those N_E sites; N_P = number of N_V sites positive for one or more species of mosquito larvae; and N% = percentage of N_V sites positive for mosquito larvae.

Cat	N_T	N_N	N_E	N_V	N_P	N%
1	197	83[1]	114	269	233	86.6
2	130	12[2]	118	303	268	88.4
3	104	75[3]	29	39	13	33.3
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Tot's	431	170	261[4]	611	514	84.1

[1] Permission to examine site(s) could not be obtained from landowner.
[2] Difficulty in gaining access to site.
[3] Tires at site(s) unsuitable as mosquito larval habitat(s) (i.e., tires were under shelter; possessed rims, etc.).
[4] Many of these sites were visited on more than one occasion (but none were visited more than once in a given month) so the number of site visits (column N_V) exceeds the number of sites examined.

Table 2 Categories of Tire Accumulations

CATEGORY 1
High potential for mosquito transmission of pathogens.

CATEGORY 2
Moderate potential for mosquito transmission of pathogens

CATEGORY 3
Low potential for mosquito transmission of pathogens

Above: Figure 2a: Example of Category 1 (peri-domestic) sites where tires are found near areas of human habitation.

Each of the 261 examined sites was placed into one of three **categories (Table 2 above). Descriptions for those categories are:**

Category 1 – peri-domestic sites (areas of human habitation or human visitation such as shopping areas and sports venues) (Table 3; Fig. 2);.

Category 2 – woodland sites not near areas of human habitation or activity (Table 4; Fig. 3); or

Category 3 – sites where tires were unsuitable for mosquito breeding and subsequent larval development (Table 5; Fig. 4).

Above: Figures 2b This Nicholas Co. sport venue [a popular motorcycle and ATV race track] is an excellent example of a Category 1 site where people are brought into close contact with tires that harbor mosquitoes

Dates of collection (throughout the anticipated breeding season of March through November), grid coordinates (latitude/longitude), elevation, and number of tires at each examined site were entered into a spreadsheet format. Those data are being prepared for mapping onto an Arcview software program and/or photo ortho-quad maps.

Many of the 261 examined sites were visited on more than one occasion resulting in a total of 611 collection visits to the 261 sites (Table 1). No site was visited more than once in any given

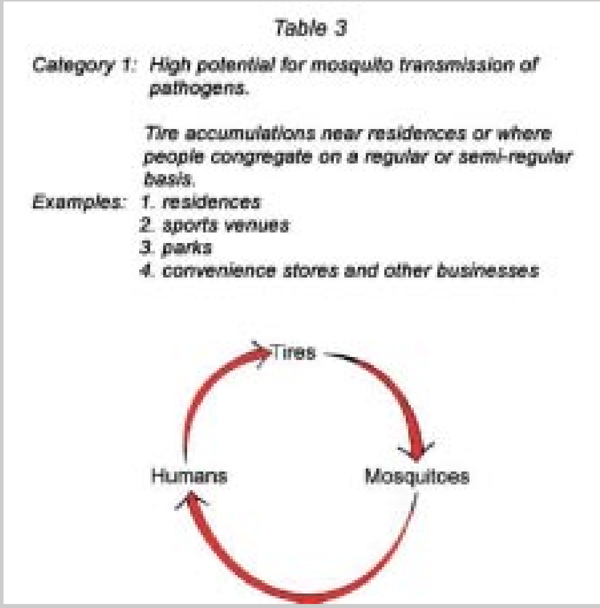


Table 3

Category 1: High potential for mosquito transmission of pathogens.

Tire accumulations near residences or where people congregate on a regular or semi-regular basis.

Examples:
1. *residences*
2. *sports venues*
3. *parks*
4. *convenience stores and other businesses*

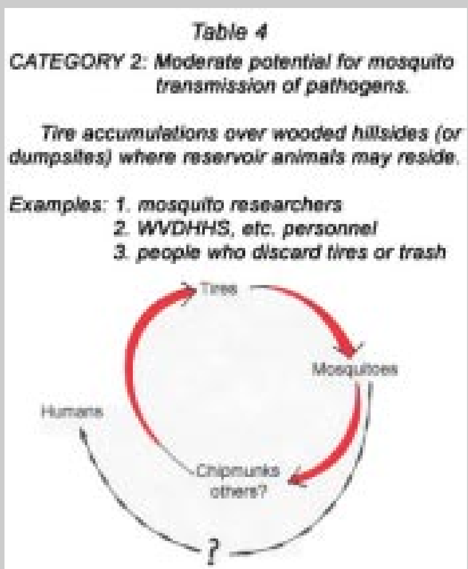


Table 4

CATEGORY 2: Moderate potential for mosquito transmission of pathogens.

Tire accumulations over wooded hillsides (or dumpsites) where reservoir animals may reside.

Examples:
1. *mosquito researchers*
2. *WVDHHS, etc. personnel*
3. *people who discard tires or trash*

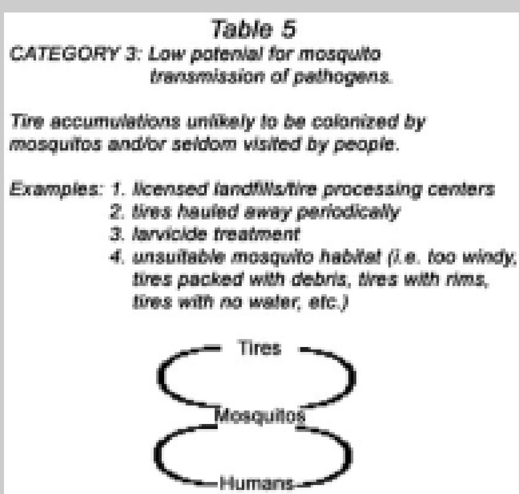


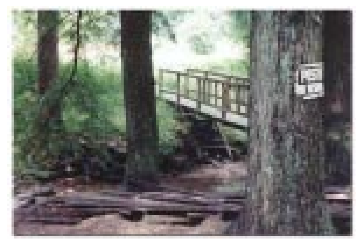
Table 5

CATEGORY 3: Low potenial for mosquito transmission of pathogens.

Tire accumulations unlikely to be colonized by mosquitos and/or seldom visited by people.

Examples:
1. *licensed landfills/tire processing centers*
2. *tires hauled away periodically*
3. *larvicide treatment*
4. *unsuitable mosquito habitat (i.e. too windy, tires packed with debris, tires with rims, tires with no water, etc.)*

Above: Figures 3 Examples of Category 2 sites where accumulation of tires is not found near areas of human habitation.



Above: Figures 4 Examples of Category 2 sites where tires are generally unsuitable for mosquito breeding/larval colonization habitats. Tires in bottom photo were packed with mud for stream bank protection, whereas tires in top photo all had rims Even though tires in top photo were close to residences, this was considered a Category 3 site because of the unsuitability for mosquito larval colonization.

month, and relatively few Category 3 sites were examined because they were, by definition, considered unsuitable habitats.

Collections and compilation of data in this manner allowed investigators to:

1) identify those mosquito species that utilize tires as breeding sites and subsequent larval development habitats on a month to month basis;
2) determine the monthly frequency (i.e., the likelihood of encountering) for potential mosquito vectors of pathogenic agents;
3) determine the monthly frequency of those mosquitoes not generally associated with pathogenic agents; and
4) evaluate which of those mosquito species are peri-domestic and which ones are not.

In summary, 269, 303 and 39 visits were made to Category 1, Category 2 and Category 3 sites, respectively, with 86.6% (233 of 269), 88.4% (268 of 303) and 33.3% (13 of 39) of the visits in those respective

Table 6. Four-letter designator codes for each mosquito species. These codes are used in document tables to conserve space.

Designator Code	*Scientific Name*
aeal	*Aedes albopictus* (Skuse)
aeve	*Aedes vexans* (Meigen)
anba	*Anopheles barberi* Coquillett
anpu	*Anopheles punctipennis* (Say)
anqu	*Anopheles quadrimaculatus* Say
cpip	*Culex pipiens* Linnaeus
cres	*Culex restuans* Theobald
cter	*Culex territans* Walker
ocat	*Ochlerotatus atropalpus* (Coquillett)
octr	*Ochlerotatus triseriatus* (Say)
osig	*Orthopodomyia signifera* (Coquillett)
trut	*Toxorhynchites r. septentrionalis* (Dyar &

Table 7. Total number of site visits by category (all months combined). Number of site visits positive for each species shown in boxes below mosquito species designator codes (i.e. octr was found in 161 of the 269 Category 1 site visits).

Cat	No. Sites/ Visits[1]	octr	cres	cter	ocat	trut	aeal	anpu	anba	cpip	osig	anqu	aeve
1	114/ 269	161	125	84	46	29	30	30	6	17	1	0	0
2	118/ 303	208	94	67	41	41	18	16	16	2	9	2	1
3	29/ 39	7	6	5	2	1	1	0	1	0	0	0	0
Tot	261/ 611	376	225	156	89	71	49	46	23	19	10	2	1

[1] N_E / N_V of Table 1.

categories testing positive for one or more species of mosquitoes (Table 1).

A total of 12 species of mosquitoes was collected and identified (Darsie and Ward, 1981) from tires at waste tire sites in Nicholas County during the course of this investigation (March through November, 2001). Each species was assigned a 4-letter designator code (Table 6) to conserve space in report tables. The number of site visits testing positive for each mosquito species, by site category, and the percent frequency of visits testing positive, are shown in Tables 7 and 8, respectively. Monthly summaries of frequencies of occurrence for each species, by site category, are shown in Tables 9 through 17; with the monthly distribution of species by category appearing in Table 18 and Fig. 5. *(Tables 9 through 17 are located in the appendix.)*

Clearly, *Ochlerotatus triseriatus*, the vector of LaCrosse encephalitis, was the most commonly encountered mosquito species in each of the three site categories (Table 8), and in every collection month with the exception of November (Fig. 5). This species is considered to be the only one with a high frequency (> 50% of all sites examined) of occurrence (all three site categories combined). The finding of *Oc. triseriatus* in high frequencies was not surprising as this species is considered the most abundant tree hole breeder in North America (Jenkins and Carpenter, 1946; Means, 1979; Wilmot et al. 1992). These data also conform to previous findings of *Oc. triseriatus* in West Virginia (Butler and Amrine, 1980; Joy et al., 1994; Joy and Hildreth-Whitehair, 2000).

Species occurring at moderate frequencies (> 25% to 50%) were *Culex restuans* and *Cx. territans*. Species occurring at low frequencies (5% to 25%) were *Oc. atropalpus*, *Toxorhynchites rutilus*, *Aedes albopictus* and *Anopheles punctipennis*. Rare species (those in fewer

Table 8. Frequencies of occurrence (number of site visits/total number site visits) in percentages for each mosquito species by site category (i.e., octr was found 59.9% of the 269 Category 1 site visits).

Cat	No. Visits	octr	cres	cter	ocat	trut	aeal	anpu	anba	cpip	osig	an qu	ae ve
1	269	59.9	46.5	31.2	17.1	10.8	11.1	11.1	2.2	6.3	0.4	0	0
2	303	68.6	31.0	22.1	13.5	13.5	5.9	5.3	5.3	.7	3.0	.7	.3
3	39	17.9	15.4	12.8	5.1	2.6	2.6	0	2.6	0	0	0	0
Tot	611	61.5	36.8	25.5	14.6	11.6	8.0	7.5	3.8	3.1	1.6	.3	.2

Peridomestic species (Cat 1 selected over Cat 2)

Species	X^2 value
Culex restuans	13.116*
Culex territans	5.633*
Ochlerotatus atropalpis	1.144
Aedes albopictus	4.380*
Anopheles punctipennis	5.874*
Culex pipiens	NC

Non-peridomestic species (Cat 2 selected over Cat 1)

Species	X^2 value
Ochlerotatus triseriatus	4.439*
Toxorhynchites rutilus	0.764
Anopheles barberi	2.807
Orthopodomyia signifera	NC
Anopheles quadrimaculatus	NC
Aedes vexans	NC

The null hypothesis (Ho) is that the frequency of occurrence at Category 1 sites equals that at Category 2 sites. Asterisks indicate rejection of Ho at the $P <$ 0.05 level. NC = not calculated because of too few observations in one, or both, cells. Chi square values reflect Yates's correction for continuity."

than 5% of all sites examined) were *An. barberi*, *Cx. pipiens* (the suspected vector of West Nile virus), *Orthopodomyia signifera*, *Ae. vexans* and *An. quadrimaculatus*.

Aedes vexans and *An. quadrimaculatus* do not normally breed in tires (Means, 1987), thus the finding of these two species is considered accidental. *Anopheles barberi* and *Or. signifera* are treehole breeders (Means, 1987), thus their occurrence in tires was expected. The fact that they were infrequently encountered is likely a reflection of their low populations in West Virginia. *Culex pipiens* breeds in a wide variety of habitats (Ross, 1947; Breeland et al., 1961), but this species is more likely to be found in open habitats (e.g., marshes, drainage ditches). As a result, the frequency of occurrence given for this species in tires is probably not a true estimate of its actual presence in the target county or in the state.

Larval Presence by Month of Collection

Tires at all site categories were examined every month from March through November 2001. No mosquito larvae were collected in March. Mosquito larvae were first encountered in April, but only three

species were found in the 37 site visit examinations for that month, and two of those species occurred at low (11.1%) frequencies (Fig. 5). We were confident that April represented the beginning of the breeding (and larval development) season for two reasons: 1) only three species were found; and 2) most of the larvae were quite small (i.e., 2nd or 3rd stadia), having not had time to develop fully to the 4th, and final, stadia (i.e., larval stage).

May was a transitional month, with five species of larvae identified from field collections. Two of those species, *Oc. triseriatus* and *Culex restuans*, occurred at relatively high frequencies (Table 18; Fig. 5). Additional species appeared each month as the summer went on with 7, 10, 11 and 11 species being identified for the months of June, July, August and September, respectively. The number of identified species declined somewhat, to 9 and 10, for the months of October and November, respectively, although the frequencies of occurrence for each of the species except *Cx. territans* were noticeably lower than in previous months (Fig. 5). Although our data do not reflect this trend, the numbers of larvae observed in field collections from May through September were generally high (often too numerous to count), whereas the numbers of individual larvae (sometimes just one or two per tire) dropped precipitously in October and November, clearly indicating the end of the breeding season. In addition, adults were commonly active in all months except March, October and November.

As reported previously, *Oc. triseriatus* was the most commonly encountered mosquito species. This dominance was seen in every monthly collection; i.e., the calculated frequency of occurrence for this species was higher (all site categories combined) than any other spe-

cies for every month (except November where the frequency was equal to that of *Cx. restuans*) throughout the study period (Fig. 5).

In addition, the appearance of *Oc. triseriatus* early in the season (April) was explosive, with nearly 84% of the examined sites being positive for this species. Frequencies of *Oc. triseriatus* remained high (> 68%) every month until a dramatic decline in September; this decline continued through November. *Ochlerotatus triseriatus* is a concern of public health professionals because not only do you have a mosquito species known as a highly competent vector of LaCrosse encephalitis, but that species is the most common one in the study area (and probably the state), as well. Clearly, continued surveillance efforts are recommended for this vector species.

The second most common species, *Culex restuans*, exhibited a very different pattern of larval colonization, with a high frequency (> 60%) first appearing in May followed by a steady decline each month until the end of the study (Fig. 5). The viruses of eastern and western encephalitis have been isolated from wild caught *Cx. restuans* females. *Culex restuans* feeds primarily on birds, but Means (1987) notes that because this species is known to feed on mammals, including humans, it should be considered a potential vector of encephalitis viruses from birds to mammals.

Culex territans, a moderately frequent species like *Cx. restuans* (Table 8), exhibited nearly the opposite larval colonization pattern, with steadily increasing frequencies of occurrence from May through September (Fig. 5). This species, even though common, only takes blood meals from amphibians, and as such is of no public health, or veterinary, importance.

In general, the remaining nine species increased in frequency of occurrence with each passing month until September (Fig. 5). Still, those species are characterized by low overall frequencies, and thus are unlikely candidates to monitor for public health purposes. *Culex pipiens*, for example, is suspected as an important vector for West Nile virus, but with frequencies of occurrence seldom exceeding 5% in any given month, this species may not be established well enough in tire piles in the state to justify its monitoring in such habitats. Monitoring *Cx. pipiens* larval populations in other types of habitats (e.g., marshes, roadside ditches, municipal drainage systems) – especially after heavy precipitation events – may, however, be prudent.

Another species, *Aedes albopictus*, is of some concern to public health officials because it is known to vector certain viruses to humans, such as dengue fever, which is now endemic to Florida. Then too, it is an introduced species (the Asian tiger mosquito), and public health professionals are uncertain about its ability to become well-established in West Virginia. Exotic species often out compete native ones (the introduced Asian clam and zebra mussel immediately come to mind as zoological examples) when introduced into new habitats. And since *Oc. albopictus* larvae use the same type of habitats as our native *Oc. triseriatus* (and others), one idea that has been advanced is *Ae. albopictus* could threaten indigenous *Ochlerotatus* populations. That might be a desirable outcome if a vector species were replaced by a non-vector one, but *Ae. albopictus* is characterized as both an aggressive feeder on humans and a highly competent vector of certain viral pathogens. In other words, replacing the native *Oc. triseriatus* with the exotic *Ae. albopictus* has an element of uncertainty to it, and could result – as with the clams – in a situation less desirable than the present one. Interestingly, data indicate *Ae. albopictus* may not compete successfully with *Oc. triseriatus* because the former species appears to be a late season breeder, whereas the native species appears early in April and is found in high frequencies throughout the breeding season. One might also cite evidence that the Asian tiger mosquito has been present in West Virginia for at least five years now (Joy first found it in Williamson, W.Va., in 1996), but the species could not yet be described as one that has dispersed significantly throughout the state (or even throughout Nicholas County).

Larval Presence by Site Category

The 2 X 2 Chi square contingency test was used to determine if certain mosquito species were more frequently found in peri-domestic (i.e., Category 1) or forested (i.e., Category 2) sites. In Table 8 one can see larvae of six species – *Culex restuans*, *Cx. territans, Oc. atropalpis, Ae. albopictus, Anopheles punctipennis* and *Cx. pipiens* – were more often collected from tires in areas where people congregate (i.e., peri-domestic sites; see Table 3). The higher frequency in Category 1 sites was statistically significant (at the $P < 0.05$ level shown in Table 8) for *Cx. restuans, Cx. territans, Ae. albopictus* and *An. punctipennis*. There was no significant difference in site category selection for *Oc. atropalpis. Culex pipiens* appears to favor peri-domestic sites to a high degree, but too few collections were made at Category 2 sites to develop valid statistical inferences (Table 8).

Six additional species – *Oc. triseriatus, Toxorhynchites rutilus, An. barberi, Orthopodomyia signifera, An. quadrimaculatus* and *Ae.*

vexans – were collected more often in Category 2, or forested sites. The higher frequency in Category 2 sites was, however, statistically significant only for *Oc. triseriatus* (Table 8). The fact that the mosquito vector of LaCrosse encephalitis is significantly less likely to be found in peri-domestic situations is interesting. This encouraging bit of epidemiological evidence is, however, blunted by the fact that *Oc. triseriatus* is the most common mosquito found associated with tires in both peri-domestic and forested situations. As a result, continued efforts to clean up waste tire piles in areas of human activity, coupled with ongoing mosquito surveillance efforts, are to be encouraged.

It was mentioned in the previous section that there was some concern about the introduced Asian Tiger mosquito competing, temporally, with the native tree hole breeder, *Oc. triseriatus*. Little temporal competition is evident (Fig. 5), and with *Ae. albopictus* larvae being found more frequently in peri-domestic situations (rather than forested habitats like *Oc. triseriatus*) it would appear that spatial competition may be lacking, as well.

B. Description of technical and/ or cost problems encountered.

No significant problems were encountered, although actual expenses for lodging and meals were considerably lower than estimated.

C. Outline of work planned for next reporting period; second year, 2002, of proposed study.

During the first year of this proposed two-year study, we developed a significant database outlining the frequency of occurrence for 12 species of mosquitoes by tire dumpsite characteristics and month of collection. In the first quarter of 2002, we plan to complete site mapping by transposing latitude/longitude information from our Excel database onto a GIS mapping system (ArcView or photo ortho quads). We also plan to complete our first year data analyses by evaluating the presence of mosquito species as a function of site elevation and number of tires at each site examined.

In the second year, we also propose to add two new components to this investigation: 1) the analysis of mosquitoes for the presence of LaCrosse encephalitis virus; and 2) the determination of larval (and pupal, if possible) densities throughout the mosquito breeding and larval development season.

1. Viral assessments.

Ochlerotatus triseriatus is the known vector for LaCrosse encephalitis, a viral disease that primarily strikes children under the age of 15 years. It is also evident that West Virginia reports more cases of LaCrosse encephalitis than any other state (Nasci, et al., 2000), with 20% to 40% of those cases, in any given year, appearing in Nicholas County, alone. This disease appears to be spreading from its Nicholas Co. focal point, because Fayette and Raleigh counties are now reporting incidence rates comparable to those seen in Nicholas County (Dr. James McJunkin, Charleston Area Medical Center, pers. comm.).

From an epidemiological standpoint, there are three questions to be addressed.

1. What is the number and distribution of LaCrosse encephalitis cases in the human population?
2. What is the frequency of occurrence and distribution of the mosquito vector, *Ochlerotatus triseriatus*?
3. What is the frequency of occurrence of the etiologic agent (i.e., the virus) in the mosquito vector population?

We have a considerable amount of information on the first two questions. Data covering several years are available from the WVDHHS, Division of Disease Surveillance and Control, to answer the first question, and our work over the past year provides ample information about the monthly frequency and distribution of *Oc. triseriatus* in Nicholas Co. tire piles. There have been no attempts to address the third question, however, and this will be the primary focus of our second year's investigation.

We plan to examine pools of *Oc. triseriatus* larvae from Category 1 (peri-domestic) and Category 2 (woodland) sites each month (April through October 2002) for the LaCrosse encephalitis virus using the protocol developed by Wasieloski et al. (1994). We will also examine pools of *Oc. triseriatus* larvae from a county (e.g., Cabell and/or Wayne) where LaCrosse is rarely diagnosed, to serve as a control. The goal is to determine if LaCrosse is more prevalent in sites around human habitation, or if the virus is more prevalent in sites where reservoir hosts (i.e., ground squirrels) reside. The null hypothesis will be that LaCrosse prevalence at Category 1 sites equals that at Category 2 sites. Such information is essential to the understanding of LaCrosse encephalitis transmission in nature.

2. Larval (pupal?) densities.

Casual field observations from the past year indicated high larval densities of *Oc. triseriatus* in the months of April through August, with noticeable declines in density from September through November. Unfortunately, we did not track these larval, or pupal, population densities in 2001. Clearly, the potential for viral transmission by adult mosquitoes depends, in part, upon the number of emergent adults. Large numbers of larvae and/or pupae would, in theory, produce more adults and increase potential viral transmission. Because of density-dependent factors some larval-positive containers may produce few adults, whereas pupal densities are highly correlated with subsequent adult densities (Focks, 1999).

To examine the relationship between larval densities and subsequent production of adults, we will establish ten test sites (five at Category 1, and five at Category 2 sites): each site with one tire having a density of ten larvae per liter of water, a second tire with100 *Oc. triseriatus* larvae per liter of water and a third tire with 250 larvae per liter of water. Each of the tires will be covered with aluminum screening and all adults will be counted (and sexed) upon emergence to determine the percent emergence as a function of larval density. These tests will be run at sites early (May), mid- (July) and late (September) in the breeding season.

We will also count all *Oc. triseriatus* larvae (and pupae) from five tires at each of ten sites (five Category 1, and five Category 2 sites) each month from April through October. The mean number of larvae (and pupae) per liter of water will be calculated for each month to determine larval/pupal population densities throughout the breeding season.

Appendices:

Appendix A: References cited in Section A

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Appendix B: Additional activities related to annual progress

Additional activities relating to grant activities fall into one of three categories: 1) consultations; 2) research presentations; and 3) ancillary research.

1. Consultations (in chronological order)

1-a. Met with Mr. Russ Rader (WV Division of Highways) in Charleston, WV, (17 Jan) to discuss SB 427 and the resulting DOH “waste tire” regulations (which were written by Mr. Rader). I was informed the incoming administration was going to oversee waste tire dumps on a regional rather than statewide basis.

1-b. Dr. Danae Bixler, M.D. (WV Health and Human Services, Division of Disease Surveillance and Control) visited our medical entomology and GIS laboratories 12 Feb to discuss the establishment of a mosquito surveillance program in West Virginia. Primary topics of discussion were how to identify those mosquitoes indigenous to the state and how to integrate mosquito collection databases with GIS software programs operated by Dr. James Brumfield (biological and physical sciences).

1-c. Offered to provide Dr. Roger Nasci (Centers for Disease Control, Fort Collins, CO) 15 or more pools of mosquito larvae from Nicholas Co., WV each week to test for presence of the LaCrosse encephalitis virus (several phone conversations and e-mail exchanges in May and June). Was informed that CDC was overwhelmed with West Nile virus testing this summer and would not be able to take advantage of our offer.

1-d. Provided Robert Gross (U.S. Department of Energy, NETL, Morgantown, WV) with information on tire-breeding mosquitoes (phone conversation and e-mails in July). Mr. Gross was involved in a public hearing to outline a proposal to co-fire waste tires in a fossil fuel power plant in the vicinity of Grant Town, WV. He wanted to play the “public health card;” i.e., make the case that getting rid of waste tires by burning for energy would be not only rid the state of unsightly tire piles, but also reduce potential mosquito breeding sites.

1-e. Provided field-training experience to Doug Crislip (WVHHS) who will be placed in charge of West Virginia’s West Nile virus surveillance program. (24 Aug; various Nicholas Co. locations).

1-f. Contacted by George Carico (Marshall University's Environmental Center, CEGAS) in September about location of waste tire piles. CEGAS was interested in the location and number of tires in dumps from standpoint of disposal.

1-g. Contacted Dr. Kirti Dave (Senior Project manager, Medical Analysis Systems, Inc.) about the feasibility of using the newly developed VecTest for West Nile and St. Louis encephalitis viruses. Unfortunately, all tests are intended for adult mosquitoes with no knowledge of how such tests might be applied to larval stages (e-mails of October).

1-h. Contacted Dr. Roger Nasci (CDC, Fort Collins, CO) about feasibility of utilizing VecTest on larval mosquitoes (e-mails of Oct). He noted that West Nile and St. Louis viruses were detected in adult mosquitoes, but only after an "extraordinary effort."

1-1 Contacted Dr. Michael Sardelis (Uniformed Services University of the Health Sciences, Division of Tropical Public Health) in October regarding feasibility of examining mosquito larval stages for presence of West Nile. Dr. Sardelis' work is with another introduced mosquito species, *Aedes japonicus*, in Maryland.

1-j. Contacted by Clark Judy (Glenville State University, WV) in October about waste tire disposal. Apparently, Mr. Judy is involved in research similar to that being conducted by Mr. Carico at CEGAS.

1-k. Consulted with Dr. Victor Fet (MU Department of Biological Sciences) in October about equipment needs and expendable supplies to test mosquitoes for the LaCrosse encephalitis virus. Dr. Fet informed me that we can do such work on campus, and offered to provide the requisite training for a student.

1-l. Consulted with Dr. Barry Beaty (Department of Microbiology; Veterinary Medicine and Biomedical Sciences; Colorado State University) via e-mail exchanges and phone conversations, about the feasibility of testing mosquito larvae in the field for the presence of LaCrosse encephalitis virus. Was informed that proposed work was quite feasible, especially given the expertise of Dr. Fet. Dr. Beaty also emphasized there was a considerable need for data relating to the prevalence of virus in mosquito populations, especially in West Virginia, where prevalence of LaCrosse is relatively high in the human population.

2. Research presentations

2-a. The WVHHS, Division of Disease Surveillance and Disease Control, has established a Zoonosis Task Force under Dr. Danae Bixler's organization plan. Two presentations were made at the 19 June meeting regarding LaCrosse encephalitis in West Virginia. Dr. James McJunkin, M.D., a pediatrician with Charleston Area Medical Center and West Virginia University Health Sciences Center, gave a paper on the clinical aspects of the disease, while I presented data relative to the mosquito vector throughout the state; specifically Nicholas Co.

2-b. Annual meeting of the West Virginia Association of Solid Waste Authorities, 25 to 27 October, Snowshoe, WV. I was one of three invited speakers (WVDEP Secretary Michael Callaghan and Rep. A. James Manchin were the other two) addressing the Association. My presentation dealt with; "Public Health Risk Assessment of Waste Tire Piles in West Virginia: The Mosquito Factor."

2-c. Committed (i.e., already scheduled, with abstracts submitted) speaking engagements.

2-c-1. Mr. Afif Hanna, project graduate research assistant, will be presenting a paper covering certain aspects of the Nick J. Rahall, II Appalachian Transportation Institute research at the West Virginia Entomological Society winter meeting, 4 Jan 02, North Bend State Park, Cairo, WV.

2-c-2. Mr. Hanna will be presenting data on the same subject matter at a biology lecture symposium, 24 Mar 02, Davis and Elkins College, Elkins, WV.

2-c-3. Ms. Brooke Kennedy, project undergraduate research assistant, will be presenting a paper at the Association of Southeastern Biologists (ASB) meetings, 11-13 Apr 02, Appalachian State University, Boone, NC.

2-c-4. Mr. Hanna will also be presenting at those ASB meetings.

2-c-5. Mr. Justin Godby, who did additional research on mosquitoes (i.e. senior thesis outlined in 3-a, following), will also present at the ASB meetings.

3. Ancillary research.

3-a. Marshall University requires 4th year students to undertake a "Capstone Project" which is comparable to a senior thesis. This work can take a variety of forms, but our most promising science students generally undertake some type of research problem under the direction of a faculty member. Four undergraduate students – Justin Godby, Brooke Kennedy, Faith Payne and Sara Lee – fulfilled their "Capstone" requirements by doing different projects related to the Rahall ATI initiative. These projects, constituting a significant undergraduate teaching and research component, were performed under the direction of the Project Director.

Appendix C: References cited in Section C.

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Appendix D: Tables 9 –18

Table 9

Summary Sheet – Sites collected and species present
Date: Mar 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1	Category 2	Category 3

[Summaries listed on each March collection date]

Summary of Nicholas County sites identified/visited (examined) for the month of March ‘01.

	Total	**No. of sites**		**No. of examined sites**	
Category	No. sites Identified/Visited	Not Examined	Examined	Pos. for larvae	Neg. for larvae
===	====	===	===	=====	=====
1	44	42	2	0	2
2	16	6	10	0	10
3	27	25	2	0	2
Totals	87	73	14	0	14

Species identified by site category; prevalence* of each species by site category.

	Species Designators						
Category	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
===	====	====	====	====	====	====	====
1							
2	[Note: No mosquito larvae collected in March ‘01]						
3							
Totals							

*No. examined sites positive for larvae / No. of sites examined.

Table 10

Summary Sheet – Sites collected and species present
Date: April 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1 Category 2 Category 3

[Summaries listed on each April collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	**Total** No. sites Identified/Visited	**No. of sites** Not Examined	Examined	**No. of examined sites** Pos. for larvae	Neg. for larvae
===	====	===	===	=====	=====
1	20	11	9	8	1
2	26	0	26	24	2
3	7	5	2	1	1
Totals	53	16	37	33	4

Species identified by site category; prevalence* of each species by site category.

Category	Species Designators [ocat]	[octr]	[cres]
===	====	====	====
1	2/9	7/9	0/9
2	2/26	23/26	4/26
3	0/2	1/2	0/2
Totals	4/37	31/37	4/37

*No. examined sites positive for larvae / No. of sites examined.

Table 11

Summary Sheet – Sites collected and species present
Date: May 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1 Category 2 Category 3

[Summaries listed on each May collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Examined	Examined	No. of examined sites Pos. for larvae	Neg. for larvae
1	62	35	27	25	2
2	55	9	46	45	1
3	33	29	4	0	4
Totals	150	73	77	70	7

Species identified by site category; prevalence* of each species by site category.

Category	Species Designators [ocat]	[octr]	[anpu]	[cres]	[cter]
1	1/27	20/27	1/27	18/27	3/27
2	1/46	37/46	0/46	28/46	8/46
3	0/4	0/4	0/4	0/4	0/4
Totals	2/77	57/77	1/77	46/77	11/77

*No. examined sites positive for larvae / No. of sites examined.

Table 12

Summary Sheet – Sites collected and species present
Date: June 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1	Category 2	Category 3

[Summaries listed on each June collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Exam.	Examined	No. of examined sites Pos. for larvae	Neg. for larvae
1	62	33	29	28	1
2	43	3	40	39	1
3	21	9	12	3	9
Totals	126	45	81	70	11

Species identified by site category; prevalence* of each species by site category.

Category	Species Designators [ocat]	[octr]	[anpu]	[cpip]	[cres]	[cter]	[trut]
1	1/29	21/29	1/29	1/29	21/29	9/29	0/29
2	4/40	34/40	5/40	0/40	17/40	12/40	1/40
3	0/12	1/12	0/12	0/12	3/12	0/12	0/12
Totals	5/81	56/81	6/81	1/81	41/81	21/81	1/81

*No. examined sites positive for larvae / No. of sites examined.

Table 13

Summary Sheet – Sites collected and species present
Date: July 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1 Category 2 Category 3

[Summaries listed on each July collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Examined	Examined	No. of examined sites Pos. for larvae	Neg. for larvae
1	61	12	49	44	5
2	39	0	39	38	1
3	12	5	7	2	5
Totals	112	17	95	84	11

Species identified by site category; prevalence* of each species by site category.

Category	Species Designators [aeal]	[ocat]	[octr]	[anba]	[anpu]	[cpip]	[cres]	[cter]	[osig]	[trut]
1	1/49	7/49	32/49	3/49	9/49	3/49	25/49	15/49	0/49	4/49
2	0/39	5/39	32/39	2/39	1/39	0/39	12/39	9/39	1/39	7/39
3	0/7	1/7	1/7	0/7	0/7	0/7	1/7	2/7	0/7	0/7
Totals	1/95	13/95	65/95	5/95	10/95	3/95	38/95	26/95	1/95	11/95

*No. examined sites positive for larvae / No. of sites examined.

Table 14

Summary Sheet – Sites collected and species present
Date: Aug. 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1 Category 2 Category 3

[Summaries listed on each August collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Examined	Examined	No. of examined sites Pos. for larvae	Neg. for larvae
1	62	13	49	46	3
2	59	8	51	49	2
3	17	14	3	2	1
Totals	138	35	103	97	6

Species identified by site category; prevalence* of each species by site category.

Categories	[aeal]	[ocat]	[octr]	[aeve]	[anba]	[anpu]	[cpip]	[cres]	[cter]	[osig]	[trut]
1	12/49	12/49	40/49	0/49	3/49	6/49	3/49	30/49	22/49	0/49	9/49
2	7/51	14/51	42/51	1/51	9/51	5/51	2/51	10/51	13/51	3/51	13/51
3	0/3	0/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Totals	19/103	26/103	84/103	1/103	12/103	11/103	5/103	40/103	35/103	3/103	22/103

*No. examined sites positive for larvae / No. of sites examined.

Table 15

Summary Sheet – Sites collected and species present
Date: Sept. 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1	Category 2	Category 3

[Summaries listed on each Sept. collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Examined	No. of sites Examined	No. of examined sites Pos. for larvae	No. of examined sites Neg. for larvae
1	58	11	47	41	6
2	30	0	30	29	1
3	6	1	5	3	2
Totals	94	12	82	73	9

Species identified by site category; prevalence* of each species by site category.

Cat.	[aeal]	[ocat]	[octr]	[anba]	[anpu]	[anqu]	[cpip]	[cres]	[cter]	[osig]	[trut]
1	13/47	14/47	21/47	0/47	10/47	0/47	4/47	16/47	19/47	0/47	10/47
2	9/30	6/30	21/30	5/30	2/30	1/30	0/30	7/30	11/30	2/30	10/30
3	1/5	1/5	2/5	1/5	0/5	0/5	0/5	1/5	2/5	0/5	1/5
Total	22/82	21/82	45/82	6/82	12/82	1/82	4/82	24/82	32/82	2/82	21/82

*No. examined sites positive for larvae / No. of sites examined.

Table 16

Summary Sheet – Sites collected and species present
Date: Oct. 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1 Category 2 Category 3

[Summaries listed on each Oct. collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Exam.	No. of sites Examined	No. of examined sites Pos. for larvae	No. of examined sites Neg. for larvae
1	38	4	34	27	7
2	38	6	32	22	10
3	5	1	4	2	2
Totals	81	11	70	51	19

Species identified by site category; prevalence* of each species by site category.

Category	[aeal]	[ocat]	[octr]	[anpu]	[cpip]	[cres]	[cter]	[osig]	[trut]
	Species Designators								
1	4/34	6/34	15/34	1/34	4/34	13/34	14/34	0/34	4/34
2	1/32	6/32	12/32	2/32	0/32	6/32	9/32	2/32	7/32
3	0/4	0/4	0/4	0/4	0/4	1/4	1/4	0/4	0/4
Totals	5/70	12/70	27/70	3/70	4/70	20/70	24/70	2/70	11/70

*No. examined sites positive for larvae / No. of sites examined.

Table 17

Summary Sheet – Sites collected and species present
Date: Nov. 2001

Give site number under each category below. Following the site number, note if site: was not examined (n.e.); was examined but no larvae found (neg.); or was examined and larvae were present. If larvae were present, give the larval species designator after the site number.

Category 1	Category 2	Category 3

[Summaries listed on each Nov. collection date]

Summary of Nicholas County sites identified/visited (examined) on above date(s).

Category	Total No. sites Identified/Visited	No. of sites Not Examined	Examined	No. of examined sites Pos. for larvae	Neg. for larvae
1	23	0	23	15	8
2	29	0	29	21	8
3	0	0	0	0	0
Subtotals	52	0	52	36	16

Species identified by site category; prevalence* of each species by site category.

Category	[aeal]	[ocat]	[octr]	[anpu]	[anqu]	[cpip]	[cres]	[cter]	[osig]	[trut]
	Species Designators									
1	0/23	3/23	5/23	2/23	0/23	2/23	2/23	2/23	1/23	3/23
2	1/29	3/29	7/29	1/29	1/29	0/29	10/29	5/29	1/29	3/29
3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Subtotals	1/52	6/52	12/52	3/52	1/52	2/52	12/52	7/52	2/52	6/52

*No. examined sites positive for larvae / No. of sites examined.

Table 18

Frequencies of mosquito species in Nicholas Co., W. Va. waste tire habitats by month and site category.

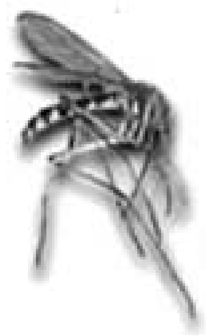
N = number of sites visited by category. Numbers in table represent frequencies of occurrence in percentages; i.e., 77.8% of the nine Category 1 sites in April were positive for *Ochlerotatus triseriatus* larvae.

	Mar			Apr			May		
Category	1	2	3	1	2	3	1	2	3
N_{visits} =	2	10	2	9	26	2	27	46	4
octr				77.8	88.5	50.0	74.1	80.4	
cres					15.4		66.7	60.9	
cter							11.1	17.4	
ocat				22.2	7.7		3.7	2.2	
trut									
aeal									
anpu							3.7		
anba									
cpip									
osig									
anqu									
aeve									

	Jun			Jul			Aug		
Category	1	2	3	1	2	3	1	2	3
N_{visits} =	29	40	12	49	39	7	49	51	3
octr	72.4	85.0	8.3	65.3	82.1	14.3	81.6	82.4	66.7
cres	72.4	42.5	25.0	51.0	30.8	14.3	61.2	19.6	
cter	31.0	30.0		30.6	23.1	28.6	44.9	25.2	
ocat	3.4	10.0		14.3	12.8	14.3	24.5	27.5	
trut			2.5	6.1	17.9		18.4	25.5	
aeal				2.0			24.5	13.7	
anpu	3.4	12.5		18.4	2.6		12.2	9.8	
anba				6.1	5.1		6.1	17.6	
cpip	3.4			6.1			6.1	3.9	
osig					2.6			5.9	
anqu									
aeve								2.0	

Table 18 Continued

	Sep			Oct			Nov		
Category	1	2	3	1	2	3	1	2	3
N_{visits} =	47	30	5	34	32	4	23	29	0
octr	44.7	70.0	40.0	44.1	37.5		21.7	24.1	
cres	34.0	23.3	20.0	28.2	18.8	25.0	8.7	34.5	
cter	40.4	36.7	40.0	41.2	28.1	25.0	8.7	17.2	
ocat	29.8	20.0	20.0	17.6	18.8		13.0	10.3	
trut	21.3	33.3	20.0	11.8	21.9		13.0	10.3	
aeal	27.7	30.0	20.0	11.8	3.1			3.4	
anpu	21.2	6.7		2.9	6.3		8.7	3.4	
anba		16.7	20.0						
cpip	8.5			11.8			8.7		
osig		6.7			6.3		4.3	3.4	
anqu		3.3						3.4	
aeve									



Numbers [n] in brackets = number of sites examined each month. Stippled bars represent the percent of [n] sites positive for larvae of each mosquito species (i.e., *Ochlerotatus triseriatus* larvae were found in 83.8% of the 37 sites examined in April).

Figure 5

Frequencies of each mosquito species in Nicholas Co., W. Va. waste tire habitats by month of collection.