Boulder County Mosquito Control District Integrated Mosquito Management Program 2021 Annual Report

Prepared for and in Cooperation with:

Boulder County Mosquito Control District

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Boulder County Mosquito Control District Integrated Mosquito Management Program

2021 Annual Report

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Program Objectives

Boulder County Mosquito Control District (BCMCD) and Boulder County Public Health Department (BCPH) contracted Vector Disease Control International, LLC (VDCI) to operate an Integrated Mosquito Management (IMM) program in 2021. The primary objective of BCMCD's IMM Program is to monitor and reduce mosquito populations through the use of environmentally sound control techniques in order to protect its residents from the threat of mosquito-borne diseases and suppress local populations of nuisance mosquitoes. VDCI prioritizes the detection and elimination of larval mosquitoes in aquatic habitats, in conjunction with the monitoring of adult mosquito populations through routine surveillance, in order to assess West Nile virus vector species abundance in the area.

Open communication is maintained by VDCI between the BCMCD, residents, HOAs, Property Management Companies, County and State Departments of Health & Environment, and surrounding municipalities in order to ensure that the highest level of mosquito control and epizootic response is achieved. This diligent and cooperative communication is important to the Boulder County Mosquito Control District mosquito management program and provides significant benefit to public health throughout the entire area.

VDCI's Commitment

Vector Disease Control International is a company built on the foundations of public health, ethics, professionalism, and technical expertise. VDCI is committed to providing our customers with scientifically based, environmentally sensitive and technologically advanced Integrated Mosquito Management (IMM) programs of the highest quality. All our employees are committed to excellence in vector control and public health and strive to improve the quality of human life in communities through public education and the control of mosquitoes and the diseases they can transmit. VDCI currently has programs across the state of Colorado, providing services for towns, cities, counties, homeowners associations, and encephalitis surveillance monitoring programs for county health departments.

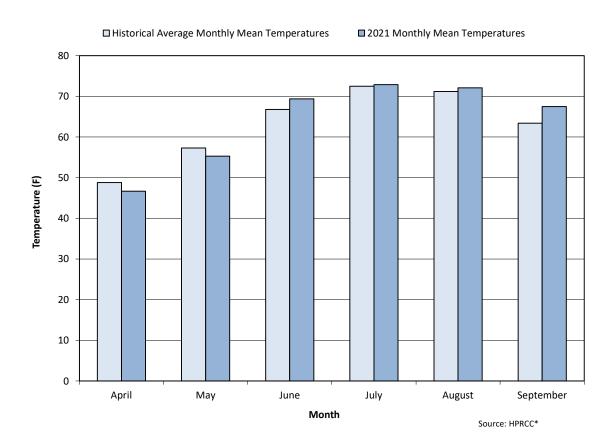
Vector Disease Control International, as the contractor for the Boulder County Mosquito Control District, will continue to use proven scientific Integrated Mosquito Management techniques to survey and control local mosquito populations using biorational larval controls and limited low-toxicity insecticide applications. All of the methods and materials used have been reviewed and registered by the US Environmental Protection Agency, the Centers for Disease Control, the Colorado Department of Agriculture and the American Mosquito Control Association.

2021 Season Perspective and Climate Data

At VDCI we have come to expect each Colorado summer to present a unique set of temperature, precipitation, irrigation, and human interactions that combine to create new and different challenges in both mosquito control and mosquito-borne disease proliferation. Boulder County is in a semi-arid environment with elevations in the BCMCD ranging from approximately 4,900 feet to 5,500 feet above sea level. The typical mosquito season for the BCMDC is from late April to September. Current and historical climate data from the National Oceanic Atmospheric Administration's (NOAA) High Plains Regional Climate Center's (HPRCC) Boulder, Colorado weather station was used to monitor regional temperature and precipitation patterns throughout the season.

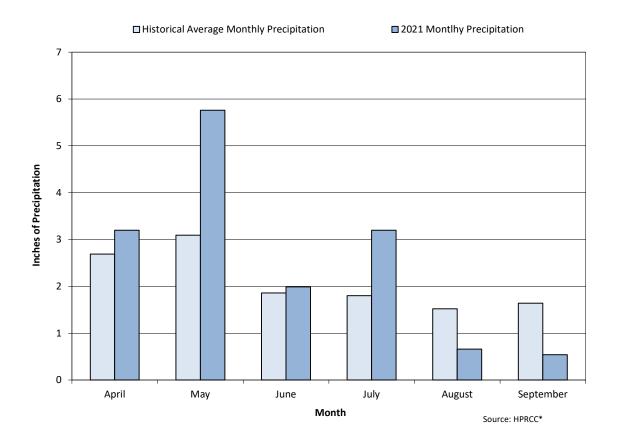
In 2021, every month of the mosquito season, except May, had temperatures above normal (Figure 1). The months of April and May were far below average at -2.1° and -2.0° while July and August were slightly above normal. However, June and September experienced the highest deviation from average, +2.6°, and +4.1° degrees. Overall, the 2021 season was approximately 21.3% warmer than an average year. The end of the season saw a record high temperature of (99°) on September 10th and 8 days still recording >90° in September. Even with these above average daytime temperatures, consistent nightly temperatures in the 50's triggered mosquitoes overwintering behavior and they stopped seeking blood meals (biting) as abundance plummeted in the last trap week.

Figure 1 2021 Monthly Mean Air Temperature and Historical Averages



The historical averages for monthly mean precipitation indicate that April, May and June are usually the wettest months of the year. The most significant variation during the 2021 mosquito season was the month of May, which received 86.4 percent more precipitation (5.76") than the average amount (3.09"), making it the wettest month of 2021 followed closely by July with 1.4" more than normal and April with 0.51". The total accumulated precipitation from April through September of 15.35 inches and was 21.8% higher than the historical average of 12.6 inches for the same time period (Figure 2). June was slightly above average, while August and September received approximately 43.4% and 32.9% of normal precipitation respectively, making those two months the driest of the mosquito season in 2021.

Figure 2 2021 Monthly Total Precipitation Data and Historical Averages*



Early and abundant spring precipitation in April and May followed by a really wet July likely influenced the historically higher than average nuisance and vector mosquito populations during 2021 mosquito season. A wet April and May presumably elevated the abundance of nuisance mosquitoes normally seen early in the season and unusually high humidity in June and July contributed to the longevity of adult mosquitoes, especially vector species, resulting in a historical abundance of all mosquito species and contributing to high West Nile virus incidence than what is normally seen throughout the front range Metro area.

2021 West Nile Virus Season – United States

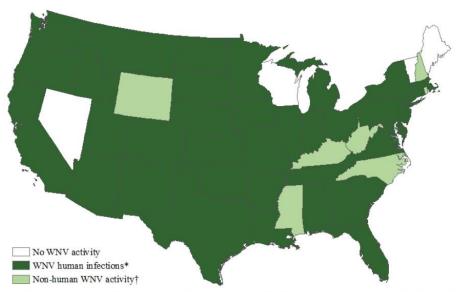
Since the introduction of West Nile virus to the United States in 1999, the virus has made a complete westward expansion to the West Coast. Starting in the Northeastern parts of the United States, the virus steadily spread through the South, the Midwest, the Rocky Mountain region and to the Western States. This extensive distribution is due to the ability of WNv to establish and persist in the wide variety of ecosystems present across the country. WNv has been detected in 65 different mosquito species in the U.S., though it appears that only a few *Culex* species drive epizootic and epidemic transmission (WNv Guidelines CDC 2013). Although West Nile virus has been endemic to the United States since 1999, researchers continue to seek an understanding for some of the factors which contribute to region specific spikes in vector abundance and human risk. We still do not understand why some humans develop West Nile fever while other infections develop into more serious West Nile encephalitis or West Nile meningitis cases. Additionally, physicians and researchers continue to seek answers to the variable recovery times and occurrence of deaths that result with some infections. WNV has expanded to the point that it can now

be found in all 48 contiguous states and has produced two additional, large nationwide epidemics in 2003 and 2012 (WNv Guidelines CDC 2013).

As of October 5th, 2021, a total of 43 states have reported West Nile virus infections in people, birds, or mosquitoes in 2021 (Figure 3). Overall, 725 cases of West Nile virus disease in humans have been reported to CDC. This is about 4x increase from the number of cases reported (174) in 2020 at this time last year and and compared to 627 cases in October 2019. Of these, 494 (68.1%) were classified as neuroinvasive disease (such as meningitis or encephalitis) and 231 (31.9%) were classified as non-neuroinvasive disease (Figure 4). A total of 35 deaths have resulted from these infections compared to 6 deaths last year at this time and 30 deaths in October 2019.

Figure 3 West Nile Virus Activity by State – United States, 2021 (as of October 5th, 2021)*

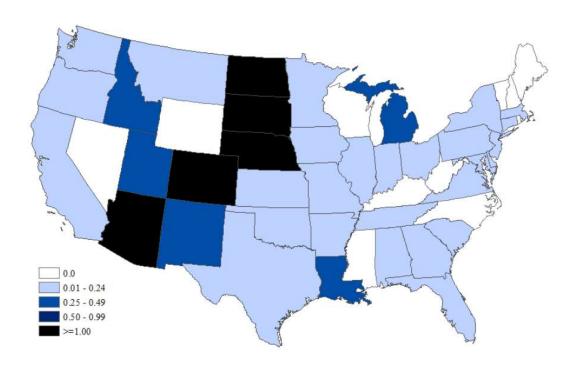
*CDC image https://www.cdc.gov/westnile/statsmaps/preliminarymapsdata2021/activitybystate2021.html



*WNV human disease cases or presumptive viremic blood donors. Presumptive viremic blood donors have a positive screening test which has not necessarily been confirmed.

 ${}^{\dagger}\!WNV\ veterinary\ disease\ cases,\ or\ infections\ in\ mosquitoes,\ birds,\ or\ sentinel\ animals.$

Figure 4 West Nile Virus Neuroinvasive Disease Incidence by State – United States, 2021 (as of September 22nd, 2021)* *CDC image https://www.cdc.gov/westnile/statsmaps/preliminarymapsdata2021/activitybystate2021.html



2021 West Nile Virus Season - Colorado

As of October 5th, 2021, the Colorado Department of Health and Environment has identified 139 cases of human West Nile virus (WNv) infections in Colorado (Figure 5) compared to 29 cases in 2020; a 4.8x increase, and six (6) deaths. The CDC reports 133 cases as of October 5th, 2021 with 22 (14.2%) asymptomatic blood donor, 74 (47.7%) neuroinvasive cases including symptoms of meningitis or encephalitis (including meningoencephalitis), and 59 (38.1%) non-neuroinvasive which includes cases where individuals are non-symptomatic or present with fever and other minor symptoms (Figure 6). There have been six (6) deaths (Figure 6) associated with West Nile virus infections in Colorado during the 2021 season to date. The discrepancy between CDPHE data and CDC data is likely due to lag time in the communication between these entities.

Figure 5 Weekly WNV Human Case Count 2021 (2014-2018 Average) *

*CDPHE image https://www.colorado.gov/pacific/cdphe/west-nile-virus-data

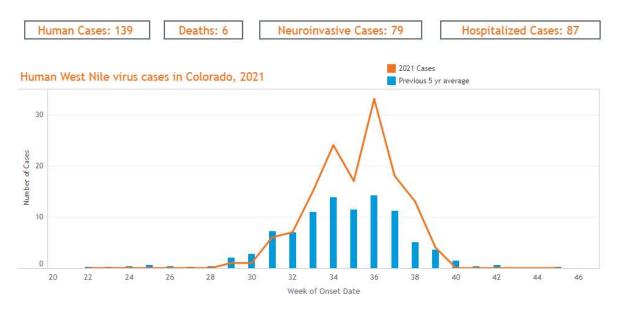


Figure 6 West Nile Virus Disease Cases and Presumptive Viremic Blood Donors by State – United States, **2021** (as of October 5th, 2021)*

 $\hbox{*CDC image $\underline{$https://www.cdc.gov/westnile/statsmaps/preliminarymapsdata2021/activitybystate2021.html}$}$

State	Neuroinvasive Disease Cases†	Non-neuroinvasive Disease Cases	Total cases	Deaths	Presumpti blood don	
Colorado	74	59	133	6	22	

2021 West Nile Virus Season - Boulder County

CDPHE data currently shows Larimer and Weld Counties both with the most West Nile virus human cases (15.1%) while Delta, ranks 2nd with 11.5% and Boulder and Denver Counties rank 3rd with 10.8% followed by Adams County with 10.1% of human cases. (**Figure 7**). For comparison, Boulder County had 10.3% of human cases cases out of only eight (8) counties reporting cases in 2020 compared to 16 counties reporting human cases this year.

Figure 7 Colorado WNV Human Cases per 1000 population by County, 2021*

Median Age

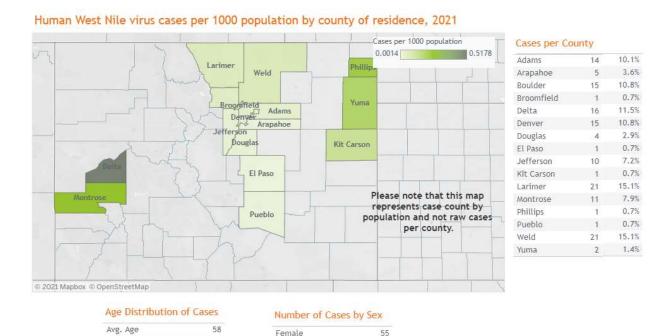
Max. Age

Min. Age

60

86 22 Male

*CDPHE image https://www.colorado.gov/pacific/cdphe/west-nile-virus-data



Adult mosquito surveillance data, submitted mosquito pools, and the resulting WNV infection rates were used by BCPH throughout the season to calculate Vector Index (VI) levels in order to help BCMCD officials determine local areas of concern for public awareness and safety. The VI is a tool used by health officials that takes into account the presence and density of *Culex* mosquitoes and their WNV infection rates, resulting in an early indicator for the risk of human WNV infection. Once the VI reaches levels above 0.75, state and local health departments typically recommend communities take additional action to control both larval and adult mosquitoes, increase public awareness, and encourage personal protection measures.

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The actual 2021 weekly Vector Index rates, as calculated by BCPH, for sentinel zones 1, 2 and 3 are illustrated below (**Table 1**). WNV activity in 2021 was very high compared to 2020, an increase in positive mosquito pools from 4.4% in 2020 to 28.5% in 2021.

Table 1 Vector Index, Boulder County Sentinel Zones 1 - 3, 2021

Boulder County Vector Index 2021 ¹						
	Sentinel Zone 1 ²	Sentinel Zone 2 ³	Sentinel Zone 3 ⁴			
Season Week	Vector Index	Vector Index	Vector Index			
Week 24 (June 7-13)						
Week 24 (June 13-19)						
Week 25 (June 20-26)						
Week 26 (June 27-July 3)	0.00	0.00	0.00			
Week 27 (July 4-10)	0.00	0.00	0.00			
Week 28 (July 11-17)	0.00	0.00	0.00			
Week 29 (July 18-24)	0.95	5.03	0.00			
Week 30 (July 25 -July 31)	0.76	1.86	0.49			
Week 31 (August 1-7)	1.53	2.56	0.37			
Week 32 (August 8-14)	0.39	1.35	2.03			
Week 33 (August 15-21)	0.00	0.59	0.59			
Week 34 (August 22-28)	1.54	1.09	0.53			
Week 35 (Aug 29 - Sept 4)	0.46	0.60	0.40			
Week 36 (Sept 5 - 11)	0.00	0.20	0.20			
1. Reported by BCPH as of September 17, 2021						
2. Boulder County; 3. Longmont, 4. Erie, Lafay ette, Louis ville, Superior						

Larval Mosquito Control

Larval mosquito control is the foundation of the Boulder County Mosquito Control District's Mosquito Control program and can be an extremely effective way to manage mosquitoes, thereby reducing the number of potential disease vectors and annoyances associated with biting adults. Years of research and practical experience have shown that the most effective way to control mosquito populations is through an aggressive Integrated Mosquito Management (IMM) approach. This approach aims at using a variety of concepts, tools, and products to reduce mosquito populations to a tolerable level.

Pre-season larval control work involved ground truthing GIS maps, remapping areas where new development and altered landscapes occurred. VDCI began larval site inspections the first full week in May. Hiring of seasonal field technicians began in April and continued into May. VDCI's Annual Field Technician Classroom Training Day looked different with continued Covid-19 pandemic restrictions on large gatherings. Instead, each Colorado VDCI office conducted small group class and field training during the week of May 17th. A less than normal contingent of returning technicians resulted in 13 newly trained field technicians for the 2021 season. Additional field training by VDCI management and veteran employees lasted through May and full-time field activities were in effect by early June.

In 2021, Vector Disease Control International performed 9,178 larval site inspections at 1,871 active breeding sites throughout the District. Of these individual inspections, 7,142 sites (77.8%) were wet upon inspection and 2,541 (27.7%) were producing mosquito larvae in the Boulder County Mosquito Control District. These inspections resulted in 2,516 (27.4%) applications in which VDCI applied 4,111.0 lbs. of VectoBac G (Bti), 7,194.3 lbs. of Vectomax FG (Bti+Bs), 8.0 lbs. of Vectolex FG/WSP (Bs), 1.9 lbs.of Vectolex WDG (Bs), 8.1 lbs. of Altosid XRG (S-Methoprene), and 89.2 gallons of BVA 2 larvicide oil (Table 2; Figures 8 and 9) to 1,026.5 acres of land within the Boulder County Mosquito Control District.



By comparison, in 2020, VDCI performed 10,340 larval site inspections at 1,851 active breeding sites throughout the District. Of these individual inspections, 7,826 sites (75.7%) were wet upon inspection and 2,811 (27.2%) were producing mosquito larvae in the Boulder County Mosquito Control District. These inspections resulted in 2625 (33.5%) applications in which VDCI applied 4,193.7 lbs. of VectoBac G (*Bti*), 1068.0 lbs. of Vectolex FG (*Bs*), 8.4 lbs.of Vectolex WDG (*Bs*), 1 Altosid briquet (S-Methoprene), 14.0 oz. of Altosid XRG (S-Methoprene), and 70.4 gallons of BVA 2 larvicide oil (Table 2 and Figures 9) to 811.6 acres of land within the Boulder County Mosquito Control District.

In 2019, VDCI performed 9,326 larval site inspections at 1,869 active breeding sites throughout the District. Of these individual inspections,

7,299 sites (78.3%) were wet upon inspection and 3,299 (35.4%) were producing mosquito

larvae in the Boulder County Mosquito Control District. These inspections resulted in 3,118 (42.7%) applications in which VDCI applied 5,201.8 lbs. of VectoBac G (*Bti*), 867.4 lbs. of Vectolex FG (*Bs*), 7.4 lbs. of Vectolex WDG (*Bs*), 4 Altosid briquets (S-Methoprene), 1.0 oz. Altosid XRG (S-Methoprene), and 79.4 gallons of BVA 2 larvicide oil (**Table 2 and Figure 9**) to 865.8 acres of land within the Boulder County Mosquito Control District.



Larval mosquito control can be achieved in several ways including biological, biochemical, chemical, and mechanical means. No single larvicide product will work effectively in every habitat where mosquito larvae are found, so a variety of products and methods should be employed. Additionally, although there are a variety of methods for reducing larval populations, some may have negative consequences that outweigh their benefits. Mechanical or physical habitat modification is a technique which VDCI uses on relatively small scale projects, as the area to be modified must be carefully reviewed.

VDCI's favored method of larval mosquito control is through the use of bacterial bio-rational products. The main product used by VDCI is a variety of bacteria (*Bacillus thuringiensis var. israeliensis*). *Bti*, as it is known, has become the cornerstone of mosquito control programs throughout the world. The benefits include its efficacy and lack of environmental impacts. When used in accordance with its label, successful control of mosquito larvae can be achieved without impact to non-target species such as other aquatic invertebrates, birds, mammals, fish, amphibians, reptiles, or humans. The label allows for the use of the product in the majority of the habitats throughout the service area. Another bacterial product closely related to *Bti* is *Bacillus sphaericus (Bs)*. *Bs* provides similar benefits to *Bti* while also providing residual control of certain species of mosquitoes. It is used specifically in difficult to treat areas where *Culex* larvae are the predominant species due to its limitations and high cost.

Other larval control products include the insect growth regulator S-methoprene (Altosid), and light mineral oils (BVA 2 larvicide oil). Methoprene is a synthetic version of a juvenile growth hormone in larval mosquitoes. The hormone prevents the normal development of larval mosquitoes into pupae and adults, eventually causing death. VDCI limits the use of chemical larvicides to areas with little biodiversity, such as road side ditches, or areas that chronically produce high mosquito populations. They are only used after a thorough assessment has been made of any habitat where their use is being considered. Mineral oil is the only product effective in controlling mosquito pupae and therefore is an essential tool when pupae are present.

VDCI made available predatory fathead minnows (*Pimephales promelas*) in limited habitats to serve as a biological control for mosquito larvae. Fathead minnows are a native fish species in Colorado that regularly feed off of surface-dwelling aquatic organisms, including mosquito larvae. VDCI will provide minnows to residents that have "closed system" habitats such as ornamental ponds or small farm ponds that are isolated from streams or other areas so the minnows cannot expand indiscriminately. VDCI received no requests for fathead minnows in 2021.

Table 2 2021 Summary of Larval Control Product Applications by Type

Larval Control Product Types	2017	2018	2019	2020	2021
Bacillus thuringiensis israelensis (Bti)					
Vectobac G (lbs) EPA Reg. #73049-10	4,056.8	2,129.5	5,201.8	4,193.7	4,111.0
Bacillus thuringiensis israelensis (Bti) +Bacillu	s sphaericus (Bs)				
Vectomax FG (lbs) EPA Reg. #73049-429	0.0	0.0	0.0	0.0	7,194.3
Bacillus sphaericus (Bs)					
Vectolex FG/WSP (lbs) EPA Reg. #73049-20	109.0	372.1	867.4	1,068.0	8.0
Vectolex WDG (lbs) EPA Reg. #73049-57	5.2	3.6	7.4	8.4	1.9
S-Methoprene					
Altosid Briquet (oz) EPA Reg. #2724-375	1.0	0.0	4.0	1.0	0.0
Altosid XRG (oz) EPA Reg. #2724-451	ı	1.1	1.0	14.0	8.1 lbs.
Mineral Oil					
BVA 2 Larvicide Oil (gal) EPA Reg. #70589-1	76.7	118.7	79.4	70.4	89.2

Figure 8 2021 Larval Site Inspections and Applications by Month

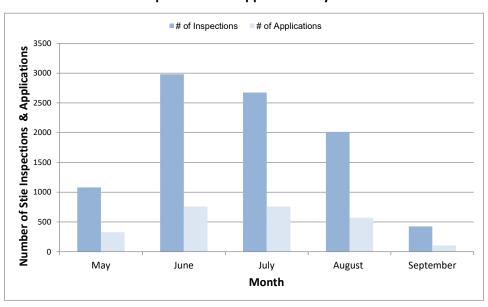
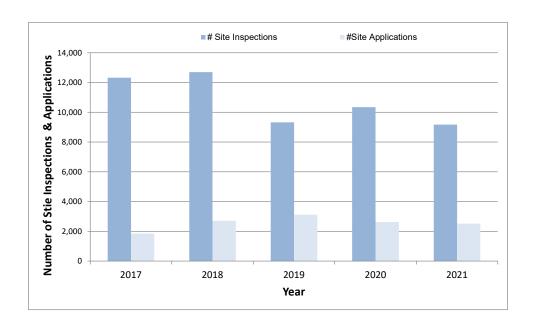


Figure 9 Comparison of Larval Site Inspections and Applications by Year



VDCI Adult Mosquito Surveillance and Laboratory

Information about mosquito abundance and species diversity is essential to any IMM program. Vector Disease Control International's most used adult mosquito surveillance tool is the CDC light trap which uses carbon-dioxide from dry ice as bait to attract female mosquitoes seeking a blood meal from a breathing animal. Once attracted by the CO_2 , the mosquitoes are lured by a small light to a fan that pulls them into a net for collection. Traps are set overnight at carefully selected sites with abundant harborage. They are collected the following morning and returned to VDCl's laboratory, where the contents of the trap nets are counted and speciated by trained technicians.

In 2021, Vector Disease Control International monitored a statewide network of hundreds of weekly trap sites, collecting 1,127,553 adult mosquitoes that were counted and identified to species by the VDCI Surveillance Laboratories compared to 516,629 in 2020, a 118.3% increase. A total of 233,922 adult mosquitoes were counted and identified in the entirety of Boulder County in 2021 compared to 166,058 in 2020, a 40.9% increase. (City of Boulder is not included for both years). While individual traps provide current seasonal information, trap data can be interpreted in the context of historical records for the same trap site if such data is available. Individual traps are also compared to other traps from around the region that were set on the same night and therefore exposed to similar weather



conditions. Technicians working in the Surveillance Laboratories at Vector Disease Control International are trained to provide accurate species-level identification of both larval and adult mosquitoes.

Additionally, the VDCI Surveillance Laboratory conducts an intensive larval identification program with larval mosquito samples collected by field technicians. This information is now invaluable in targeting mosquito control efforts as we gain a greater understanding of the habitat types preferred by Colorado mosquito species and the seasonality of these habitats as sites for mosquito development.

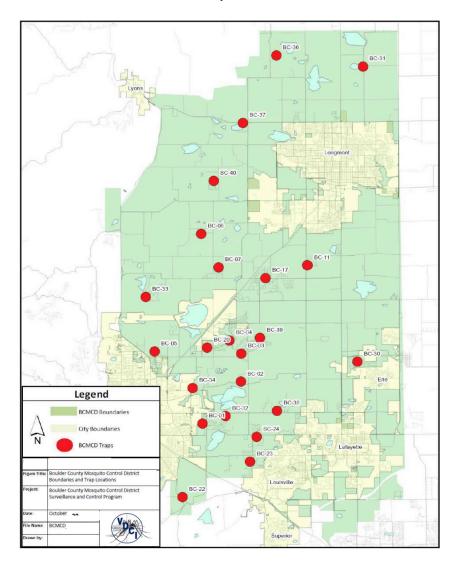
Specimens and data collected from these traps and larval identification are used in:

- Determining the effect of larval control efforts. Each mosquito species prefers specific types of habitats for larval development. If a trap includes large numbers, it could indicate the presence of an unknown larval habitat and, based on the species identification and known habitat preference for that species, direct field technicians as to possible sources of the mosquitoes collected.
- <u>Determining larval and adult mosquito species.</u> This helps to illustrate the threat of mosquitoborne disease amplification and transmission because different mosquito species can vector different diseases to people and animals.
- Determining where adult control efforts were necessary. While mosquito eradication is impossible, significant population reduction is achievable. In places where larval control is insufficient, such as neighborhoods where adult mosquitoes have migrated in from outside of the control area, it may be necessary to use adulticide methods, such as ULV truck fogging or barrier sprays of harborage areas. Trap counts that exceed an acceptable threshold for an area may trigger adult control measures.
- Surveillance for Mosquito-borne Disease. Historically, VDCI efforts were targeted primarily at controlling mosquito nuisance problems with limited disease surveillance. However, since the arrival of the West Nile virus in Colorado in August of 2002, the paradigm has shifted toward disease prevention and control. Accurate species identification of the mosquitoes in the traps is important when monitoring species population trends. It also is necessary for evaluating whether a population spike represents an actual increase in disease transmission potential or only an increased nuisance level.

BOULDER COUNTY SURVEILLANCE LIGHT TRAP DATA

In 2021, an average of 22 surveillance light traps monitored adult mosquito populations within the Boulder County Mosquito Control District on a weekly basis (Figure 10). Early season adult surveillance began with select sites (BC-02, 03, 04, 07, 30, 33, 47 & 49 traps) the week of May 25th. VDCI began full surveillance (22 traps) the week of June 1st and concluded on September 13th corresponding with low adult mosquito activity.

Figure 10 2021 BCMCD Boundaries and Trap Locations



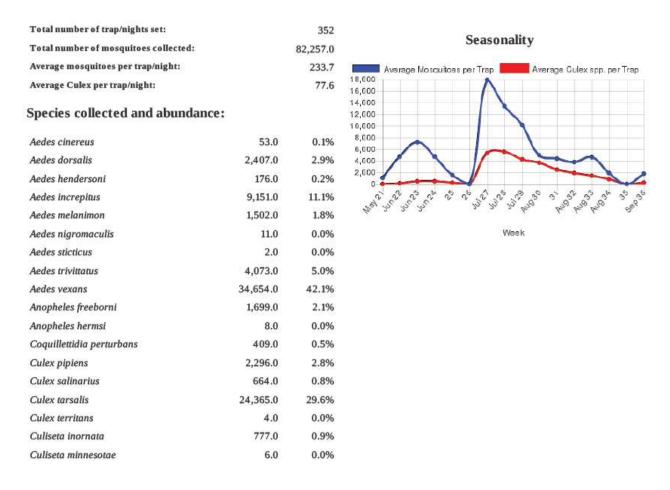
There were 352 CDC light surveillance trap nights set within Boulder County Mosquito Control District during the 2021 season. These traps collected a total of 82,257 mosquitoes. There was an average of 233.7 mosquitoes caught per trap per night and an average 77.6 *Culex spp.* mosquitoes per trap per night. The composition of mosquitoes collected was 52,029 (63.3%) *Aedes/Ochlerotatus spp.*, 1,707 (2.1%) *Anopheles spp.*, 409 (0.5%) *Coquillettidia spp.*, 27,329 (33.2%) *Culex spp.*, and 783 (1.0%) *Culiseta spp.* (Figure 11). Please refer to Appendix A for BCMCD Individual Light Trap Summaries.

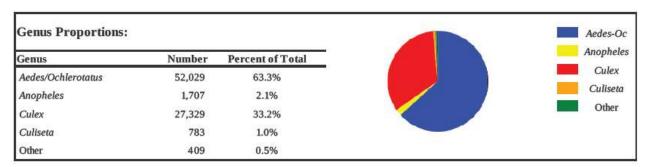
A total of 18 species were represented in 2021 in BCMCD. No exotic/introduced species (such as Asian Tiger Mosquitoes) were collected this season.

By comparison, in 2020 there were 352 CDC light surveillance trap nights set within Boulder County Mosquito Control District. These traps collected a total of 89,472 mosquitoes. There was an average of 254 mosquitoes caught per trap per night and an average 40 *Culex spp.* mosquitoes per trap per night. The composition of mosquitoes collected was 80.6% (72,087) *Aedes/Ochlerotatus spp.*, 2.0% (1,824) *Anopheles spp.*, 0.9% (821) *Coquillettidia spp.*, 15.9% (14,189) *Culex spp.*, and 0.6% (551) *Culiseta spp.*

In 2019 there were 330 CDC light surveillance trap nights set within Boulder County Mosquito Control District. These traps collected a total of 79,897 mosquitoes. There was an average of 242 mosquitoes caught per trap per night and an average 61 *Culex spp.* mosquitoes per trap per night. The composition of mosquitoes collected was 70.4% (56,285) *Aedes/Ochlerotatus spp.*, 1.1% (888) *Anopheles spp.*, 1.7% (1,343) *Coquillettidia spp.*, 25.0% (19,961) *Culex spp.*, and 1.8% (1,420) *Culiseta spp.*

Figure 11 2021 Boulder County Mosquito Control District Light Trap Composite Data





WEST NILE VIRUS MOSQUITO SAMPLE TESTING RESULTS - BOULDER COUNTY

VDCI and BCMCD used the adult mosquito data collected to help determine local areas of concern for public awareness and safety as well as to monitor the local vector mosquito populations. Many local health departments have moved towards mosquito-based surveillance indicators to assess the weekly risk of West Nile transmission and guide response decisions for adult mosquito control applications. The vector index and infection rate is derived by testing the mosquitoes VDCI collects for the presence of West Nile virus. This value is closely monitored by the CDPHE and local health departments to evaluate the risk posed by the vector mosquito population.

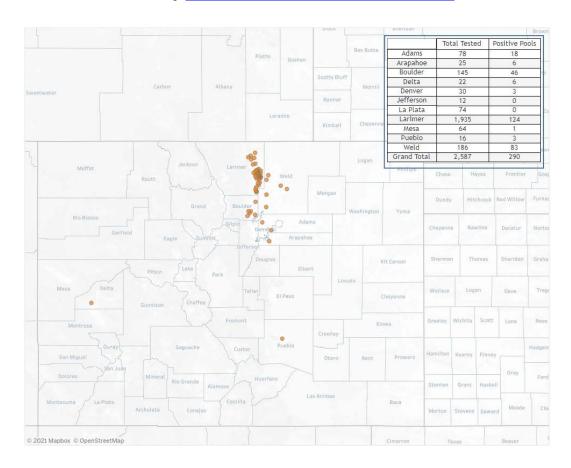
As defined in the CDC guidelines for West Nile virus surveillance, prevention and control, the vector index (VI) is an estimate of the number of West Nile virus infected mosquitoes in an area. This number can serve as a human health risk value. An operational value of 0.75, which was derived from the comparison of historical data for human infections, as well as relative abundance and infection in mosquitoes, serves as an indicator of high risk for West Nile virus transmission to humans in the corresponding area. As the value of the vector index increases there is a corresponding risk of human disease and this value can be used to offset epidemics.

Due to budget cutbacks associated with West Nile virus surveillance in recent years, the CDPHE does not have the ability to test mosquitoes from every trap set across the state. As a result, there is select testing done within three sentinel zones in Boulder County. *Culex species* mosquito samples are sent to CDPHE for WNV testing on a weekly basis as part of the state's Sentinel Encephalitis Surveillance program (Figure 12), which VDCI is contracted separately through BCPH to perform.

As of September 28th, 2021, CDPHE reported 290 positive mosquito pools from Colorado Sentinel Zones compared with 66 last year, approximately 4x increase. Larimer County had the most West Nile virus positive mosquito pools (124) while Weld County ranked 2nd with 83 positive pools followed by Boulder and Adams Counties with 46 and 18 respectively. The first Colorado West Nile virus positive mosquito sample pool in 2021 was the week of June 27th (week 26) in Weld County followed by City of Boulder and Larimer County the week of July 11th (week 28), while Boulder County sample pools tested positive the week of July 18th (week 29) 3-4 weeks earlier than last year (Figure 12).

Figure 12 Number of Colorado Positive WNV Specimens 2021*

*CDPHE image https://www.colorado.gov/pacific/cdphe/west-nile-virus-data



BOULDER COUNTY PUBLIC HEALTH ADULT MOSQUITO SENTINEL ZONES

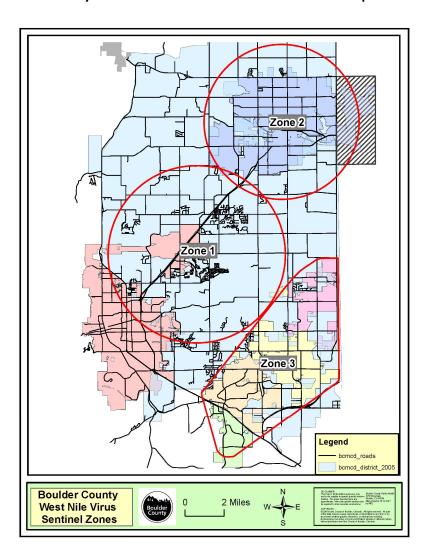
The Sentinel Encephalitis Surveillance Program was funded by the Colorado Department of Public Health and Environment and Boulder County Public Health in 2021. VDCI maintained the sentinel system with five surveillance traps at permanent locations in each of three Boulder County Sentinel Zones: Boulder County (BCZ1); City of Longmont (BCZ2); Town of Erie, City of Lafayette, City of Louisville, Town of Superior (BCZ3) (Figure 12). The sentinel CDC light traps were set once a week from June 14th (week 25) to September 13th, 2021 (week 37). WNv sentinel pool testing began June 28th (week 26) and ended September 8th, 2021 (week 36).

As of September 17th, 2021 (week 36), CDPHE tested a total of 144 mosquito pools from Boulder County Sentinel Zones. Of the tested mosquito pools, 41 pools tested positive for West Nile virus out of all three sentinel zones in 2021 (**Table 3**). The first Boulder County West Nile virus positive mosquito sample pools (3) in 2021 were on July 19th, 2021 (week 29) in BCZ1 and BCZ2 compared to last year on August 3rd (week 32) in BCZ3. (**Table 1**; **Figures 11 and 12**).

Table 3 Total Submitted and Positive Mosquito Pools, BCPH Sentinel Zones, 2014-2021

Positive WNV Mosquito Pools								
	2014	2015	2016	2017	2018	2019	2020	2021
# Submitted	131	132	145	188	189	206	114	144
# Positive	16	10	21	15	11	8	5	41
% Positive	12.2	7.6	14.5	8.0	5.8	3.9	4.4	28.8

Figure 13 Boulder County Public Health Sentinel Surveillance Zone Map



ADULT MOSQUITO CONTROL

The goal of Vector Disease Control International is to provide our customers with the best options for safe, effective, modern mosquito management. The primary emphasis of the BCMCD Integrated Mosquito Management Program is to control mosquitoes in the larval stage, using safe biological control products. When mosquito counts surpass nuisance and disease threshold numbers of adult mosquitoes, VDCI utilizes least toxic and EPA and CDC approved adulticides to reduce mosquito populations in the BCMCD.

In 2021, BCPH and VDCI continued to use a week to week evaluation of the adult mosquito populations and utilized approximately 250 as a threshold for nuisance mosquitoes and 50 for vector-disease mosquitoes in the Boulder County Mosquito Control District. BCPH's 2018, 2019 and 2020 week to week evaluation suggested that a threshold of 50 for vector disease mosquitoes be implemented for the 2021 season. Additionally, a continuing weekly evaluation of several factors was utilized to determine if a neighborhood or spray zone were to have ULV adult control operations conducted June through September. These factors included: the current weekly trap species diversity and abundance (*Aedes vs. Culex spp.*), previous weekly trap species diversity and abundance (*Aedes vs. Culex spp.*), was the trap diversity and abundance (*Aedes vs. Culex spp.*) declining or increasing, did the trap numbers decline the following week naturally or due to ULV adult control, the volume of resident annoyance calls from a neighborhood or spray zone area, the County human population density around the trap, and WNv activity in the area.

BCPH plans to further analyze current and historic adult surveillance and County WNv epidemiology data to aid in the evaluation of recommended threshold numbers for other Boulder County municipalities. Threshold recommendations might be for total adults, a separate *Aedes spp.* threshold and/or separate *Culex spp.* threshold for the 2022 season.

AUGUST 2021 INSECTICIDE RESISTANCE TRIALS: BOULDER COUNTY MCD

Resistance to insecticides has appeared in the major insect vectors from every genus. As of 1992, the list of insecticide resistant mosquito species included 56 anopheline species and 39 culicine mosquito species. Resistance has developed to every chemical class of insecticide, including microbial drugs and insect growth regulators.

Insecticide resistance has the potential to be a major obstacle to the control of mosquitoes and other insect pests and can have serious long terms impacts on a mosquito control program. Knowledge of the mechanisms of resistance and the susceptibly of local mosquito populations are critical to every program's success.

In 2017, the National Association of County and City Health Officials (NACCHO) mosquito control and surveillance <u>assessment</u> found that the 1,083 responding vector control organizations needed the most improvement in the following <u>core capabilities</u>: Pesticide resistance testing, making treatment decisions using surveillance data and routine standardized vector surveillance.

Armed with the NACCHO findings, the Boulder County Public Health discussed conducting a resistance trial with VDCI. VDCI's Research and Innovation team conducted a trial on August 23, 2021, with wild caught adult mosquitoes in all three sentinel zones. Bottle bioassay trial results found no cause for concern in any of the sentinel zones. VDCI is confident that local mosquito populations are susceptible to permethrin-based adult control products. The full report can be found in **Appendix C.**

2021 ULV ADULT CONTROL OPERATIONS

During the 2021 season a total of 475.8 Ultra Low Volume (ULV) miles of roads and access paths within BCMCD were sprayed using the adulticide Aqua Perm-X UL 30-30, Aqua-Kontrol® 30-30 or PermaSease UC (Active Ingredient – Permethrin) (Figure 14 and 15). In addition, a total of 14 gallons of Talstar Pro or Wisdom TC (Active Ingredient – Bifenthrin) were applied as a daytime adult barrier application. A detailed summary of adulticide applications, by neighborhood, can be found in Appendix D.

By comparison, in the 2020 season a total of 343.3 Ultra Low Volume (ULV) miles of roads and access paths within BCMCD were sprayed using the adulticide Aqua-Kontrol* 30-30 or PermaSease UC (Active Ingredient – Permethrin) (Figure 15). In addition, a total of 8 gallons of Talstar Pro or Wisdom TC (Active Ingredient – Bifenthrin) were applied as a daytime adult barrier application.

In 2019 a total of 282.4 Ultra Low Volume (ULV) miles of roads and access paths within BCMCD were sprayed using the adulticide Aqua-Kontrol* 30-30 (Active Ingredient – Permethrin) (Figure 15). In addition, a total of 4 gallons of Talstar Pro (Active Ingredient – Bifenthrin) was applied as a daytime adult barrier application.

VDCI uses state of the art technology, calibrated application timing, and least-toxic products to minimize non-target impacts. Adult mosquito control applications are accomplished using Ultra Low Volume (ULV) spray equipment and performed after dusk when the majority of mosquito species are most active. This type of equipment produces droplets averaging 10-25 microns in diameter and allows for a minimal amount of product to be put into the environment. These treatments take place in the evening when mosquitoes are flying in the greatest numbers and non-target insect activity



(for example, day-flying pollinators like bees) is greatly reduced. Using this application technique, the overall goal of minimal environmental impact and effective adult control is achieved in the targeted area.

Figure 14 ULV Adulticide Miles by Month

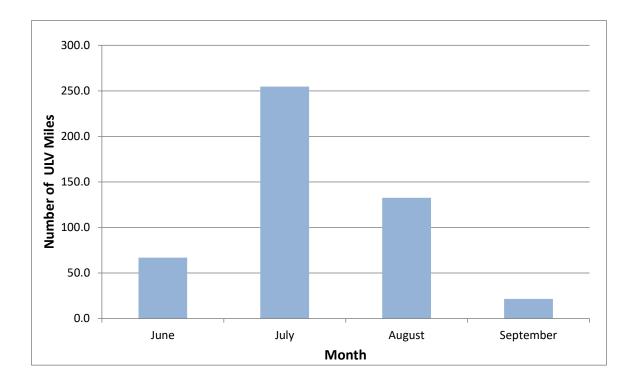
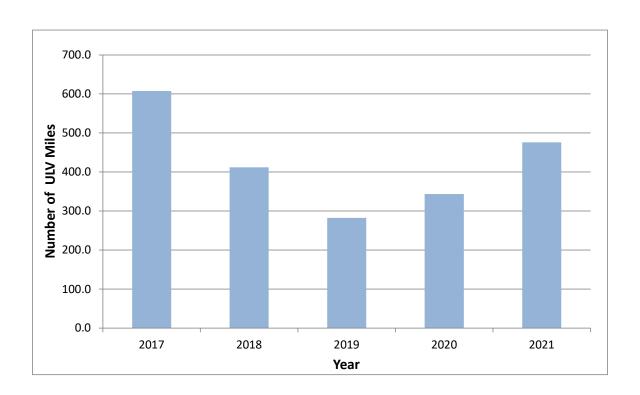


Figure 15 Comparison of ULV Adulticide Miles by Year



CALL NOTIFICATION & SHUTOFF SYSTEM

Both VDCI and the BCMCD acknowledge that adult mosquito control can be a sensitive matter to many residents; therefore, a Spray Shutoff and/or Notification request option was available to the public. Residents were able to call VDCI's MosquitoLineTM or submit a website request to be notified before adult control applications were performed and/or request that the ULV sprayer be shutoff in front of their address.

At the beginning of the 2021 season, VDCI mailed (95) and emailed (158) letters to all Boulder County shutoff and notification households on established ULV adult control routes to verify a current list for the 2021 season. During the season, the majority of new and renewed returned mail and/or website submissions were received mainly during May and June. The 2021 season concluded with 198 households of which 14 were shutoff only, 71 were shutoff and notification and 113 were notification. Residents on the shutoff and notification list were notified 48-24 hours in advance when their community was scheduled to be fogged. VDCI used an automated service to contact residents phone or text message and listed weekly ULV adult control operations on VDCI's website, www.vdci.net/colorado-schedules, which utilized Google Calendar and Maps. In 2021, VDCI completed 1,341 BCMCD automated notification calls.

By comparison, in 2020 VDCI mailed out 244 letters to all Boulder County shutoff and notification households to establish a current list for the 2020 season. The 2020 season shutoff and notification list concluded with 253 households of which 12 were shutoff only, 98 were shutoff and notification and 143 were notification only. In 2020, VDCI completed 846 BCMCD notification calls.

Public Relations and Education

VDCI is dedicated to providing strong Public Outreach and Education Programs to residents in all of our communities. Citizen complaints, inquiry, information and satisfaction surveys can aid in evaluating the effectiveness of a program. VDCI constantly looks for ways to better serve the communities we work with and encourages both the citizen and local media involvement in order to increase the effectiveness of our programs. We have clearly demonstrated that commitment and belief by proactively serving Boulder County Mosquito Control District (and all of our contracted communities) with numerous innovative programs, activities and services.

Customer service is always a high priority for VDCI. We take pride in training each and every technician so that they have the knowledge to provide residents with the correct answers to their questions. Each field technician spends part of their day responding to resident concerns in their work area. This in-field customer service personalizes the mosquito control program, provides VDCI with local information on mosquito activity and presents a valuable opportunity to educate our residents about mosquito biology and control.

MosquitoLine™

VDCI maintains a toll-free telephone line specific to Boulder County, (877) 276-4306 and a local line (303) 466-1892 to accept calls from the public concerning:

Information requests about mosquito biology and source reduction of mosquito habitats

- Mark Information on program components, operations and monitoring
- Seasonal West Nile virus activity
- * Personal protection options for mosquito annoyances and West Nile virus risk
- * Reports about mosquitoes and possible larval mosquito habitats
- * Requests to perform larvicide applications and/or opt-out of any adulticide spraying
- * Request notification when adulticide spraying is planned in their neighborhood
- ** Request health and safety information about mosquito control operations and pesticide products used

VDCI has provided Mosquito Hotlines to the residents in communities which we are contracted to also reduce workload by municipal personnel. This enables direct communication and response by mosquito control employees to resident's concerns about West Nile virus and larval site activity and treatment. VDCI maintains a log of calls received and will summarize call activity in monthly and annual reports.

In 2021 Vector Disease Control International received approximately 140 phone calls from residents of BCMCD. Most of these calls (98) were for adult mosquito complaints. Of the rest, 13 calls were requests for habitat assessment, 29 calls were requests for general information or other reasons and 198 requests for ULV adult control shut off and/or call notification (website and or letter submission) were received (Table 4; Figure 16 and 17).

By comparison, in 2020 Vector Disease Control International received approximately 176 phone calls from residents of BCMCD. Most of these calls (106) were for adult mosquito complaints. Of the rest, 27 calls were requests for habitat assessment, 43 calls were requests for general information or other reasons and 269 requests for ULV adult control shut off and/or call notification (website submission) (Figure 17). One habitat call resulted in a new larval site being added to the program.

In 2019 VDCI received 115 phone calls from residents of BCMCD. Most of these calls (58) were for adult mosquito complaints. Of the rest, 33 calls were requests for habitat assessment, 24 calls were requests for general information or other reasons and 97 website submission requests for shut off and/or call notification prior to ULV adult control (Figure 17). Several habitat calls resulted in new or expanded or reinstated previously denied larval sites being added to the program.

Table 4 2021 Mosquito Control Calls by Category

Call Category	2021			
Call Category	Number of Calls	Percentage		
Adult Complaint	98	29.0%		
Habitat Assessment	13	3.8%		
General Info/Other	29	8.6%		
ULV Shutoff/Notification*	85	25.1%		
ULV Notification*	113	33.4%		
Total	338	100.0%		
*VDCI website submissions				

Figure 16 2021 Mosquito Control Calls by Month

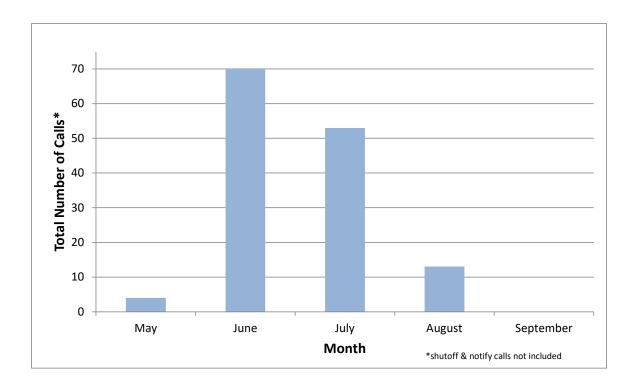
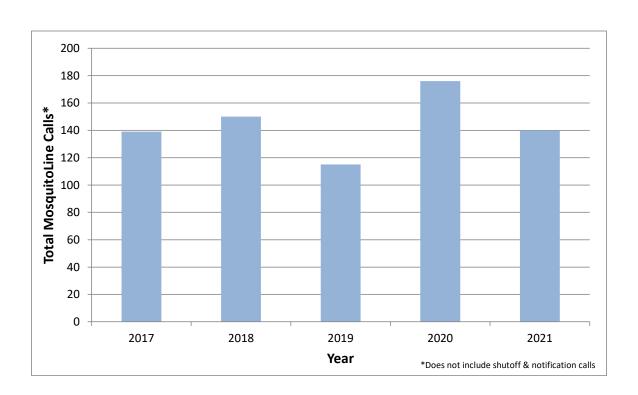


Figure 17 Comparison of Mosquito Control Calls by Year



Appendix A: Light Trap Su	Boulder County Immaries	Mosquito	Control Distric	ct Individual

Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap
Location: Cottonwood Kennels

GPS: 40.03469988689677, -105.18324997276066

Total number of trap/nights set:16.0Total number of mosquitoes collected:8,177.0Average mosquitoes per trap/night:511.1Average Culex per trap/night:65.1

Species collected and abundance:

Aedes dorsalis	60.0	0.7%
Aedes hendersoni	60.0	0.7%
Aedes increpitus	2,328.0	28.5%
Aedes melanimon	66.0	0.8%
Aedes trivittatus	229.0	2.8%
Aedes vexans	4,255.0	52.0%
Anopheles freeborni	58.0	0.7%
Coquillettidia perturbans	63.0	0.8%
Culex pipiens	34.0	0.4%
Culex salinarius	4.0	0.0%
Culex tarsalis	1,003.0	12.3%
Culiseta inornata	17.0	0.2%

Total Mosquitoes Culex spp. 2,000 1,000 1,000 Rule Spp. Culex spp. Culex spp. Culex spp.

Week

Seasonality

Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	6,998.0	85.6%
Anopheles	58.0	0.7%
Culex	1,041.0	12.7%
Culiseta	17.0	0.2%
Other	63.0	0.8%

Mayor

Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap

Location: Gunbarrel SE - Pali Way

GPS: 40.05284991156167, -105.18390007317066

Total number of trap/nights set: 17.0 Total number of mosquitoes collected: 6,584.0 Average mosquitoes per trap/night: 387.3 Average Culex per trap/night: 75.5

Species collected and abundance:

Aedes dorsalis	13.0	0.2%
Aedes hendersoni	3.0	0.0%
Aedes increpitus	2,562.0	38.9%
Aedes melanimon	58.0	0.9%
Aedes trivittatus	69.0	1.0%
Aedes vexans	2,388.0	36.3%
Anopheles freeborni	161.0	2.4%
Coquillettidia perturbans	26.0	0.4%
Culex pipiens	187.0	2.8%
Culex salinarius	109.0	1.7%
Culex tarsalis	987.0	15.0%
Culiseta inornata	21.0	0.3%

Total Mosquitoes Culex spp. 2,000 1,800 1,600 1,400 1,200-1,000-800 600 400 200 mu55

34127

Seasonality

Week

Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	5,093.0	77.4%
Anopheles	161.0	2.4%
Culex	1,283.0	19.5%
Culiseta	21.0	0.3%
Other	26.0	0.4%

Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap

Location: Gunbarrel NW - Red Fox Hills

GPS: 40.06164995704901, -105.19394997507334

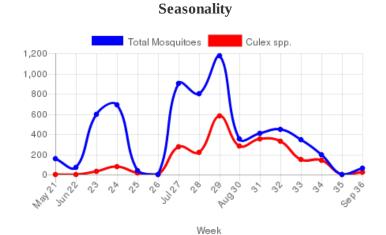
Total number of trap/nights set: 17.0

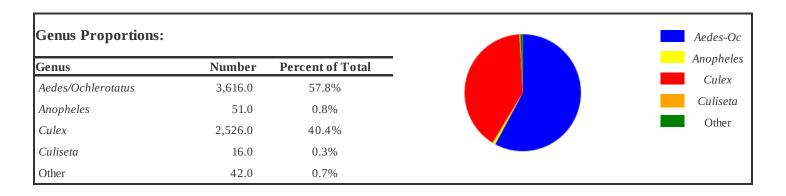
Total number of mosquitoes collected: 6,251.0

Average mosquitoes per trap/night: 367.7

Average Culex per trap/night: 148.6

Aedes cinereus	4.0	0.1%
Aedes dorsalis	183.0	2.9%
Aedes increpitus	642.0	10.3%
Aedes melanimon	82.0	1.3%
Aedes trivittatus	11.0	0.2%
Aedes vexans	2,694.0	43.1%
Anopheles freeborni	51.0	0.8%
Coquillettidia perturbans	42.0	0.7%
Culex pipiens	977.0	15.6%
Culex salinarius	193.0	3.1%
Culex tarsalis	1,356.0	21.7%
Culiseta inornata	10.0	0.2%
Culiseta minnesotae	6.0	0.1%





Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap

Location: Orange Orchard

GPS: 40.05409998143732, -105.2547999098897

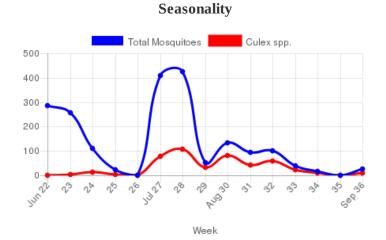
Total number of trap/nights set: 16.0

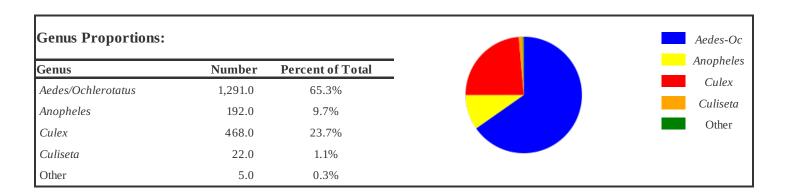
Total number of mosquitoes collected: 1,978.0

Average mosquitoes per trap/night: 123.6

Average Culex per trap/night: 29.3

Aedes dorsalis	3.0	0.2%
Aedes increpitus	197.0	10.0%
Aedes melanimon	9.0	0.5%
Aedes trivittatus	16.0	0.8%
Aedes vexans	1,066.0	53.9%
Anopheles freeborni	192.0	9.7%
Coquillettidia perturbans	5.0	0.3%
Culex pipiens	40.0	2.0%
Culex salinarius	22.0	1.1%
Culex tarsalis	406.0	20.5%
Culiseta inornata	22.0	1.1%





Season: 05/01/2021 - 09/30/2021

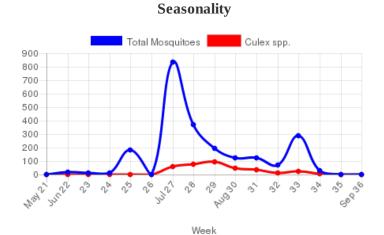
Trap Type: CDC Light Trap

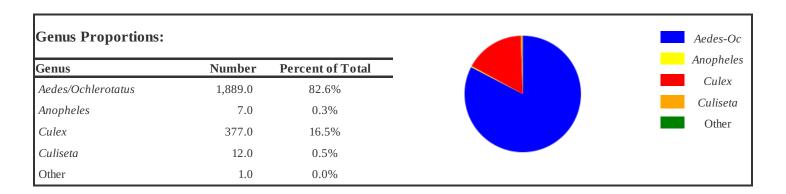
Location: Brigadoon Glen/Left Hand Creek

GPS: 40.10855006351473, -105.20234998315573

Total number of trap/nights set:16.0Total number of mosquitoes collected:2,286.0Average mosquitoes per trap/night:142.9Average Culex per trap/night:23.6

Aedes dorsalis	20.0	0.9%
Aedes hendersoni	3.0	0.1%
Aedes increpitus	10.0	0.4%
Aedes melanimon	81.0	3.5%
Aedes trivittatus	1,092.0	47.8%
Aedes vexans	683.0	29.9%
Anopheles freeborni	7.0	0.3%
Coquillettidia perturbans	1.0	0.0%
Culex pipiens	4.0	0.2%
Culex tarsalis	373.0	16.3%
Culiseta inornata	12.0	0.5%





Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap
Location: Boulder Hills

GPS: 40.13065002302139, -105.21675009280443

Total number of trap/nights set: 16.0

Total number of mosquitoes collected: 2,715.0

Average mosquitoes per trap/night: 169.7

Average Culex per trap/night: 84.4

Species collected and abundance:

Aedes dorsalis	86.0	3.2%
Aedes hendersoni	3.0	0.1%
Aedes increpitus	268.0	9.9%
Aedes melanimon	29.0	1.1%
Aedes trivittatus	130.0	4.8%
Aedes vexans	719.0	26.5%
Anopheles freeborni	82.0	3.0%
Culex pipiens	45.0	1.7%
Culex salinarius	10.0	0.4%
Culex tarsalis	1,296.0	47.7%
Culiseta inornata	47.0	1.7%

Week

Seasonality

Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	1,235.0	45.5%
Anopheles	82.0	3.0%
Culex	1,351.0	49.8%
Culiseta	47.0	1.7%
Other	0.0	0.0%

Season: 05/01/2021 - 09/30/2021

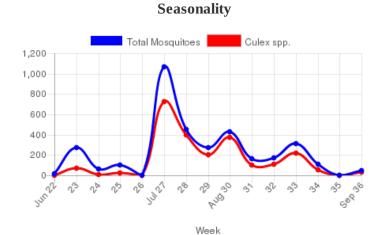
Trap Type: CDC Light Trap

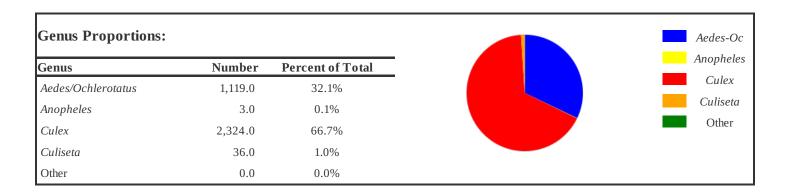
Location: Niwot East - Majestic Road

GPS: 40.1099998900239, -105.13030014932157

Total number of trap/nights set:16.0Total number of mosquitoes collected:3,482.0Average mosquitoes per trap/night:217.6Average Culex per trap/night:145.3

Aedes dorsalis	117.0	3.4%
Aedes increpitus	15.0	0.4%
Aedes melanimon	9.0	0.3%
Aedes nigromaculis	2.0	0.1%
Aedes trivittatus	9.0	0.3%
Aedes vexans	967.0	27.8%
Anopheles freeborni	3.0	0.1%
Culex pipiens	98.0	2.8%
Culex salinarius	48.0	1.4%
Culex tarsalis	2,178.0	62.6%
Culiseta inornata	36.0	1.0%





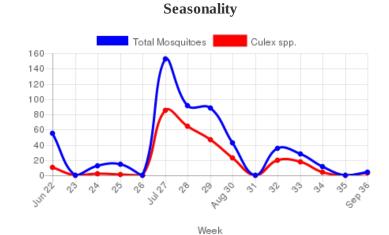
Season: 05/01/2021 - 09/30/2021

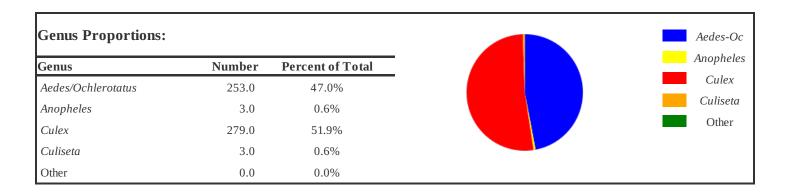
Trap Type: CDC Light Trap
Location: Niwot Central

GPS: 40.10180003989972, -105.16405005007982

Total number of trap/nights set:13.0Total number of mosquitoes collected:538.0Average mosquitoes per trap/night:41.4Average Culex per trap/night:21.5

Aedes dorsalis	3.0	0.6%
Aedes hendersoni	1.0	0.2%
Aedes increpitus	12.0	2.2%
Aedes trivittatus	3.0	0.6%
Aedes vexans	234.0	43.5%
Anopheles freeborni	3.0	0.6%
Culex pipiens	9.0	1.7%
Culex tarsalis	270.0	50.2%
Culiseta inornata	3.0	0.6%





Season: 05/01/2021 - 09/30/2021

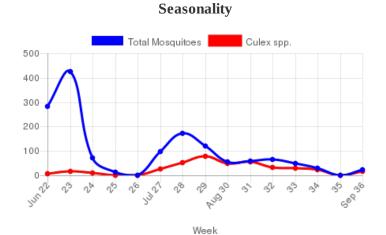
Trap Type: CDC Light Trap

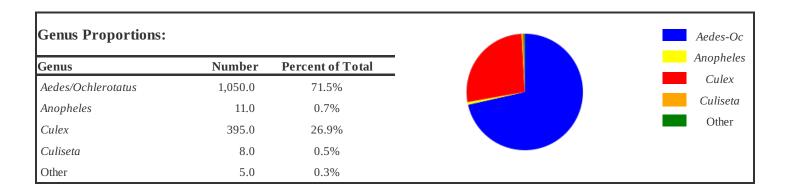
Location: Willows/Gunbarrel Commons Park

GPS: 40.05679996173248, -105.21199990063906

Total number of trap/nights set:15.0Total number of mosquitoes collected:1,469.0Average mosquitoes per trap/night:97.9Average Culex per trap/night:26.3

Aedes cinereus	3.0	0.2%
Aedes dorsalis	2.0	0.1%
Aedes hendersoni	1.0	0.1%
Aedes increpitus	536.0	36.5%
Aedes melanimon	4.0	0.3%
Aedes trivittatus	2.0	0.1%
Aedes vexans	502.0	34.2%
Anopheles freeborni	10.0	0.7%
Anopheles hermsi	1.0	0.1%
Coquillettidia perturbans	5.0	0.3%
Culex pipiens	162.0	11.0%
Culex salinarius	22.0	1.5%
Culex tarsalis	211.0	14.4%
Culiseta inornata	8.0	0.5%





Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap

Location: South Boulder Creek at Marshall Rd

GPS: 39.95944874334226, -105.23227907717228

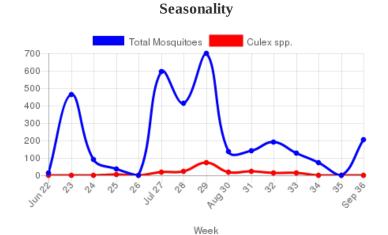
Total number of trap/nights set: 15.0

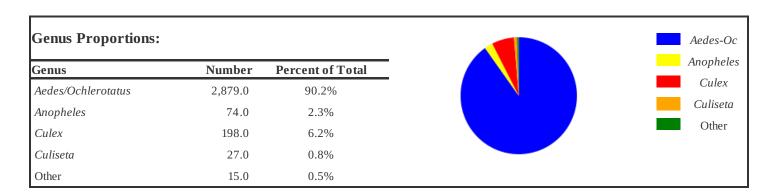
Total number of mosquitoes collected: 3,193.0

Average mosquitoes per trap/night: 212.9

Average Culex per trap/night: 13.2

Aedes cinereus	8.0	0.3%
Aedes dorsalis	4.0	0.1%
Aedes hendersoni	79.0	2.5%
Aedes increpitus	476.0	14.9%
Aedes melanimon	109.0	3.4%
Aedes trivittatus	138.0	4.3%
Aedes vexans	2,065.0	64.7%
Anopheles freeborni	74.0	2.3%
Coquillettidia perturbans	15.0	0.5%
Culex pipiens	11.0	0.3%
Culex salinarius	4.0	0.1%
Culex tarsalis	183.0	5.7%
Culiseta inornata	27.0	0.8%





Season: 05/01/2021 - 09/30/2021

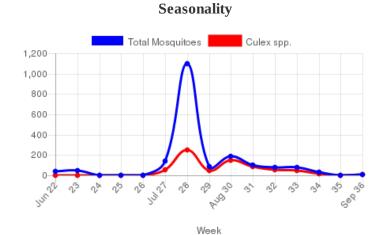
Trap Type: CDC Light Trap

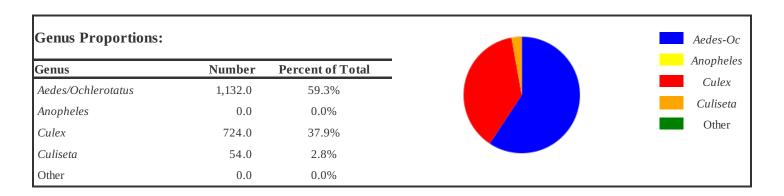
Location: Louis ville - Spanish Hills

GPS: 39.98264987357784, -105.17714995890856

Total number of trap/nights set:15.0Total number of mosquitoes collected:1,910.0Average mosquitoes per trap/night:127.3Average Culex per trap/night:48.3

Aedes dorsalis	123.0	6.4%
Aedes hendersoni	1.0	0.1%
Aedes increpitus	18.0	0.9%
Aedes melanimon	105.0	5.5%
Aedes nigromaculis	4.0	0.2%
Aedes trivittatus	2.0	0.1%
Aedes vexans	879.0	46.0%
Culex pipiens	51.0	2.7%
Culex salinarius	6.0	0.3%
Culex tarsalis	667.0	34.9%
Culiseta inornata	54.0	2.8%





Season: 05/01/2021 - 09/30/2021

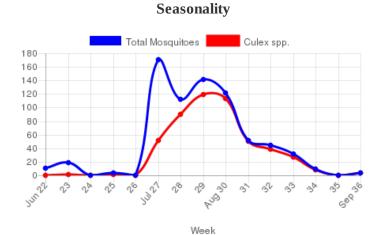
Trap Type: CDC Light Trap

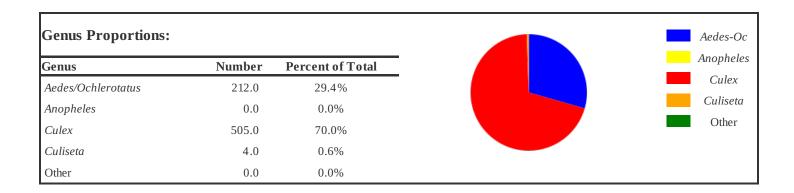
Location: Louisville - Wewoka Drive

GPS: 39.998750058526376, -105.17175000160933

Total number of trap/nights set:15.0Total number of mosquitoes collected:721.0Average mosquitoes per trap/night:48.1Average Culex per trap/night:33.7

Aedes dorsalis	6.0	0.8%
Aedes hendersoni	1.0	0.1%
Aedes increpitus	3.0	0.4%
Aedes melanimon	5.0	0.7%
Aedes vexans	197.0	27.3%
Culex pipiens	14.0	1.9%
Culex salinarius	1.0	0.1%
Culex tarsalis	490.0	68.0%
Culiseta inornata	4.0	0.6%





Season: 05/01/2021 - 09/30/2021

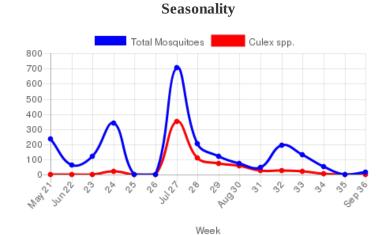
Trap Type: CDC Light Trap

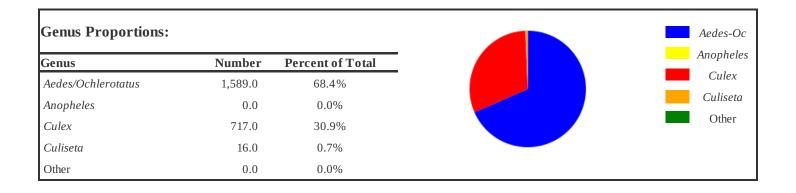
Location: Brownsville - Random Court

GPS: 40.04734994769696, -105.08964993059634

Total number of trap/nights set:16.0Total number of mosquitoes collected:2,322.0Average mosquitoes per trap/night:145.1Average Culex per trap/night:44.8

Aedes dorsalis	414.0	17.8%
Aedes increpitus	3.0	0.1%
Aedes melanimon	32.0	1.4%
Aedes nigromaculis	1.0	0.0%
Aedes trivittatus	12.0	0.5%
Aedes vexans	1,127.0	48.5%
Culex pipiens	11.0	0.5%
Culex salinarius	10.0	0.4%
Culex tarsalis	696.0	30.0%
Culiseta inornata	16.0	0.7%





Season: 05/01/2021 - 09/30/2021

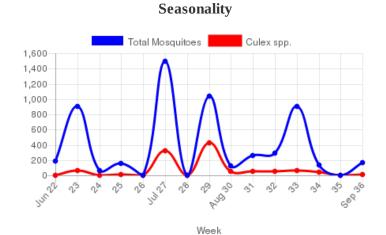
Trap Type: CDC Light Trap

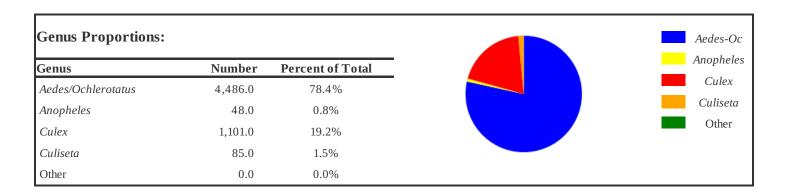
Location: Divide Reservoir

GPS: 40.23899997117141, -105.08389994502066

Total number of trap/nights set:15.0Total number of mosquitoes collected:5,720.0Average mosquitoes per trap/night:381.3Average Culex per trap/night:73.4

Aedes dorsalis	326.0	5.7%
Aedes increpitus	2.0	0.0%
Aedes melanimon	216.0	3.8%
Aedes nigromaculis	3.0	0.1%
Aedes trivittatus	334.0	5.8%
Aedes vexans	3,605.0	63.0%
Anopheles freeborni	48.0	0.8%
Culex pipiens	19.0	0.3%
Culex salinarius	18.0	0.3%
Culex tarsalis	1,064.0	18.6%
Culiseta inornata	85.0	1.5%





Season: 05/01/2021 - 09/30/2021

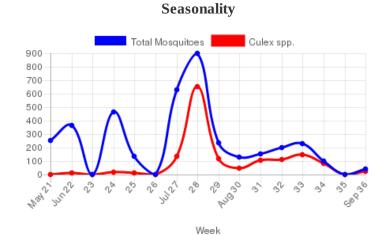
Trap Type: CDC Light Trap
Location: Lake Valley Estates

GPS: 40.0896500025398, -105.2624998614192

Total number of trap/nights set:16.0Total number of mosquitoes collected:3,855.0Average mosquitoes per trap/night:240.9Average Culex per trap/night:92.2

Species collected and abundance:

Aedes cinereus	38.0	1.0%
Aedes dorsalis	6.0	0.2%
Aedes hendersoni	1.0	0.0%
Aedes increpitus	556.0	14.4%
Aedes melanimon	47.0	1.2%
Aedes sticticus	2.0	0.1%
Aedes trivittatus	38.0	1.0%
Aedes vexans	1,425.0	37.0%
Anopheles freeborni	175.0	4.5%
Coquillettidia perturbans	1.0	0.0%
Culex pipiens	109.0	2.8%
Culex salinarius	21.0	0.5%
Culex tarsalis	1,345.0	34.9%
Culiseta inornata	91.0	2.4%



Genus Proportions: Aedes-Oc Anopheles Genus Number **Percent of Total** Culex Aedes/Ochlerotatus 2,113.0 54.8% Culiseta Anopheles 175.0 4.5% Other Culex 1,475.0 38.3% Culiseta 91.0 2.4% Other 0.0% 1.0

Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap
Location: Cline Trout Farm

GPS: 40.032999959590526, -105.22269990295172

Total number of trap/nights set: 15.0

Total number of mosquitoes collected: 1,962.0

Average mosquitoes per trap/night: 130.8

Average Culex per trap/night: 34.0

Species collected and abundance:

Aedes dorsalis	3.0	0.2%
Aedes hendersoni	1.0	0.1%
Aedes increpitus	272.0	13.9%
Aedes melanimon	11.0	0.6%
Aedes trivittatus	158.0	8.1%
Aedes vexans	773.0	39.4%
Anopheles freeborni	47.0	2.4%
Coquillettidia perturbans	153.0	7.8%
Culex pipiens	83.0	4.2%
Culex salinarius	16.0	0.8%
Culex tarsalis	411.0	20.9%
Culiseta inornata	34.0	1.7%

Week

Seasonality

Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	1,218.0	62.1%
Anopheles	47.0	2.4%
Culex	510.0	26.0%
Culiseta	34.0	1.7%
Other	153.0	7.8%

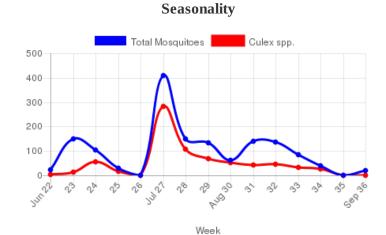
Season: 05/01/2021 - 09/30/2021

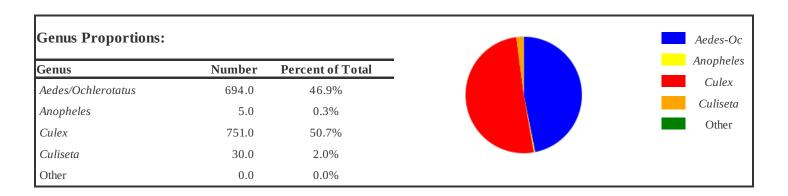
Trap Type: CDC Light Trap
Location: Yellowstone Road

GPS: 40.2467999152123, -105.15225000679492

Total number of trap/nights set:15.0Total number of mosquitoes collected:1,480.0Average mosquitoes per trap/night:98.7Average Culex per trap/night:50.1

Aedes dorsalis	31.0	2.1%
Aedes hendersoni	2.0	0.1%
Aedes increpitus	5.0	0.3%
Aedes melanimon	53.0	3.6%
Aedes trivittatus	21.0	1.4%
Aedes vexans	582.0	39.3%
Anopheles freeborni	5.0	0.3%
Culex pipiens	24.0	1.6%
Culex salinarius	3.0	0.2%
Culex tarsalis	724.0	48.9%
Culiseta inornata	30.0	2.0%





Season: 05/01/2021 - 09/30/2021

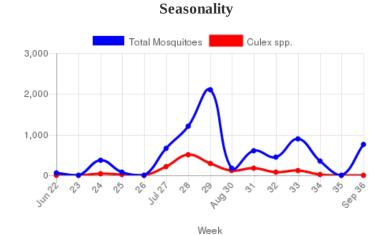
Trap Type: CDC Light Trap

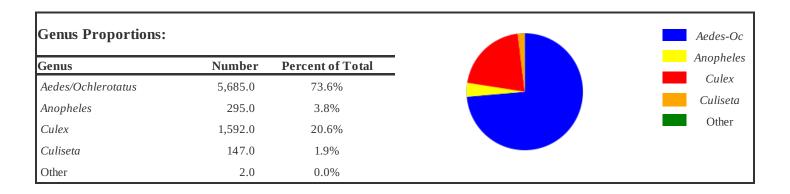
Location: Burch Reservior

GPS: 40.2025501103469, -105.18224984407425

Total number of trap/nights set:16.0Total number of mosquitoes collected:7,721.0Average mosquitoes per trap/night:482.6Average Culex per trap/night:99.5

Aedes dorsalis	9.0	0.1%
Aedes hendersoni	16.0	0.2%
Aedes increpitus	85.0	1.1%
Aedes melanimon	35.0	0.5%
Aedes trivittatus	1,571.0	20.3%
Aedes vexans	3,969.0	51.4%
Anopheles freeborni	295.0	3.8%
Coquillettidia perturbans	2.0	0.0%
Culex pipiens	59.0	0.8%
Culex salinarius	50.0	0.6%
Culex tarsalis	1,480.0	19.2%
Culex territans	3.0	0.0%
Culiseta inornata	147.0	1.9%





Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap

Location: Heatherwood

GPS: 40.062150077142704, -105.16924984753132

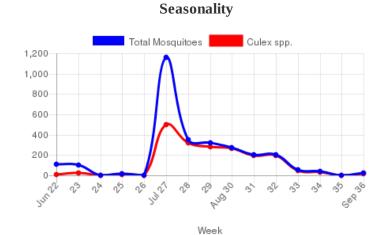
Total number of trap/nights set: 16.0

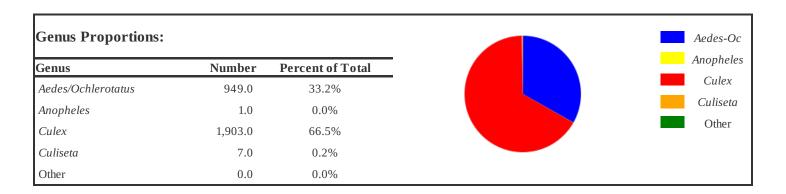
Total number of mosquitoes collected: 2,860.0

Average mosquitoes per trap/night: 178.8

Average Culex per trap/night: 118.9

Aedes dorsalis	316.0	11.0%
Aedes increpitus	51.0	1.8%
Aedes melanimon	42.0	1.5%
Aedes trivittatus	2.0	0.1%
Aedes vexans	538.0	18.8%
Anopheles freeborni	1.0	0.0%
Culex pipiens	128.0	4.5%
Culex salinarius	29.0	1.0%
Culex tarsalis	1,746.0	61.0%
Culiseta inornata	7.0	0.2%





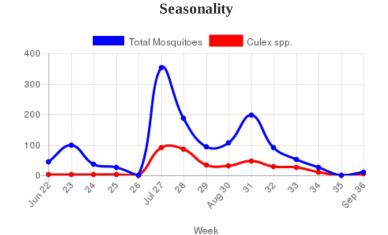
Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap **Location:** Chance Acres

GPS: 40.15964996228525, -105.20589988678694

Total number of trap/nights set:15.0Total number of mosquitoes collected:1,324.0Average mosquitoes per trap/night:88.3Average Culex per trap/night:24.9

Aedes dorsalis	27.0	2.0%
Aedes hendersoni	2.0	0.2%
Aedes increpitus	12.0	0.9%
Aedes melanimon	5.0	0.4%
Aedes nigromaculis	1.0	0.1%
Aedes trivittatus	179.0	13.5%
Aedes vexans	696.0	52.6%
Anopheles freeborni	8.0	0.6%
Culex pipiens	14.0	1.1%
Culex salinarius	3.0	0.2%
Culex tarsalis	357.0	27.0%
Culiseta inornata	20.0	1.5%



Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	922.0	69.6%
Anopheles	8.0	0.6%
Culex	374.0	28.2%
Culiseta	20.0	1.5%
Other	0.0	0.0%

Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap

Location: Baseline Heights - Chinook Way

GPS: 40.00951213723779, -105.19707709550858

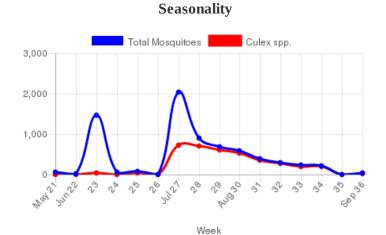
Total number of trap/nights set: 17.0

Total number of mosquitoes collected: 7,147.0

Average mosquitoes per trap/night: 420.4

Average Culex per trap/night: 222.0

Aedes dorsalis	577.0	8.1%
Aedes hendersoni	1.0	0.0%
Aedes increpitus	295.0	4.1%
Aedes melanimon	240.0	3.4%
Aedes trivittatus	15.0	0.2%
Aedes vexans	2,193.0	30.7%
Anopheles freeborni	3.0	0.0%
Coquillettidia perturbans	10.0	0.1%
Culex pipiens	109.0	1.5%
Culex salinarius	39.0	0.5%
Culex tarsalis	3,626.0	50.7%
Culiseta inornata	39.0	0.5%



Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	3,321.0	46.5%
Anopheles	3.0	0.0%
Culex	3,774.0	52.8%
Culiseta	39.0	0.5%
Other	10.0	0.1%

Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap **Location:** Gunbarrel Estates

GPS: 40.07825767706422, -105.18403518944979

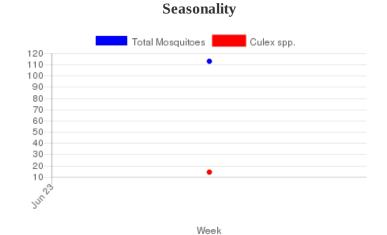
Total number of trap/nights set: 1.0

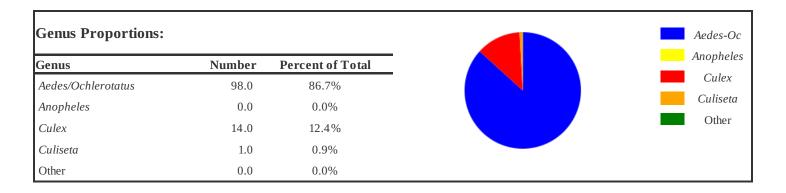
Total number of mosquitoes collected: 113.0

Average mosquitoes per trap/night: 113.0

Average Culex per trap/night: 14.0

Aedes hendersoni	1.0	0.9%
Aedes increpitus	37.0	32.7%
Aedes melanimon	2.0	1.8%
Aedes vexans	58.0	51.3%
Culex tarsalis	14.0	12.4%
Culiseta inornata	1.0	0.9%





Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap **Location:** Burke Lake

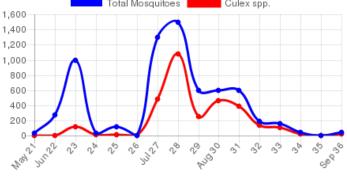
GPS: 40.016343976114676, -105.14925733208656

Total number of trap/nights set:17.0Total number of mosquitoes collected:6,509.0Average mosquitoes per trap/night:382.9Average Culex per trap/night:183.3

Species collected and abundance:

Aedes dorsalis	62.0	1.0%
Aedes increpitus	225.0	3.5%
Aedes melanimon	249.0	3.8%
Aedes trivittatus	2.0	0.0%
Aedes vexans	2,347.0	36.1%
Anopheles freeborni	452.0	6.9%
Anopheles hermsi	7.0	0.1%
Coquillettidia perturbans	6.0	0.1%
Culex pipiens	72.0	1.1%
Culex salinarius	49.0	0.8%
Culex tarsalis	2,995.0	46.0%
Culiseta inornata	43.0	0.7%

Seasonality Total Mosquitoes Culex spp.



Week

Genus Proportions:		
Genus	Number	Percent of Total
Aedes/Ochlerotatus	2,885.0	44.3%
Anopheles	459.0	7.1%
Culex	3,116.0	47.9%
Culiseta	43.0	0.7%
Other	6.0	0.1%

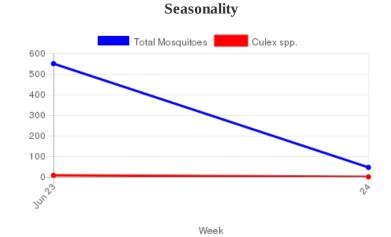
Season: 05/01/2021 - 09/30/2021

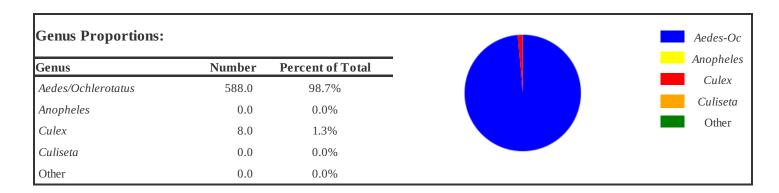
Trap Type: CDC Light Trap
Location: Hillcrest Reservoir

GPS: 40.015987828360906, -105.19760381430387

Total number of trap/nights set:2.0Total number of mosquitoes collected:596.0Average mosquitoes per trap/night:298.0Average Culex per trap/night:4.0

Aedes dorsalis	1.0	0.2%
Aedes increpitus	500.0	83.9%
Aedes melanimon	2.0	0.3%
Aedes vexans	85.0	14.3%
Culex tarsalis	8.0	1.3%





Season: 05/01/2021 - 09/30/2021

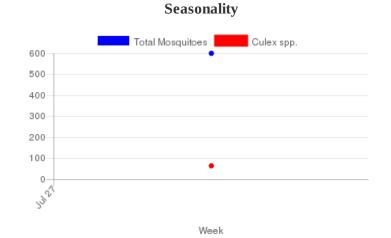
Trap Type: CDC Light Trap

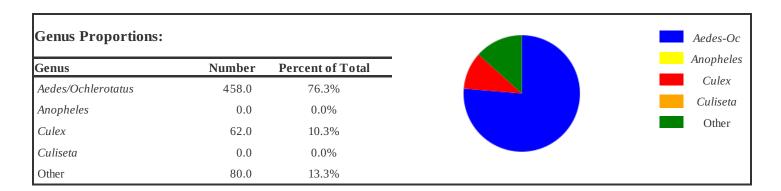
Location: Woodbourne Hollow Road

GPS: 40.04403281609449, -105.21681513637304

Total number of trap/nights set:1.0Total number of mosquitoes collected:600.0Average mosquitoes per trap/night:600.0Average Culex per trap/night:62.0

Aedes dorsalis	4.0	0.7%
Aedes increpitus	40.0	6.7%
Aedes trivittatus	8.0	1.3%
Aedes vexans	406.0	67.7%
Coquillettidia perturbans	80.0	13.3%
Culex tarsalis	62.0	10.3%





Season: 05/01/2021 - 09/30/2021

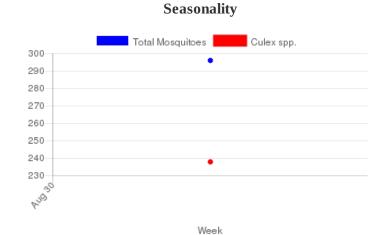
Trap Type: CDC Light Trap

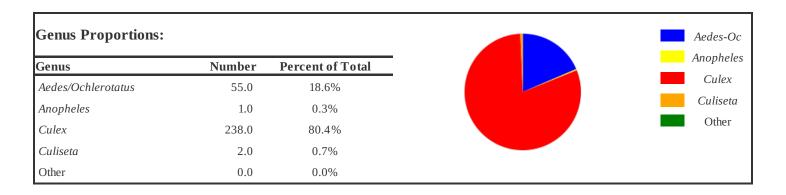
Location: Willow Creek Drive

GPS: 40.01816987819651, -105.16438398510218

Total number of trap/nights set:1.0Total number of mosquitoes collected:296.0Average mosquitoes per trap/night:296.0Average Culex per trap/night:238.0

Aedes dorsalis	10.0	3.4%
Aedes melanimon	11.0	3.7%
Aedes vexans	34.0	11.5%
Anopheles freeborni	1.0	0.3%
Culex pipiens	20.0	6.8%
Culex salinarius	2.0	0.7%
Culex tarsalis	216.0	73.0%
Culiseta inornata	2.0	0.7%





Season: 05/01/2021 - 09/30/2021

Trap Type: CDC Light Trap
Location: Stonehenge

GPS: 40.01522622570161, -105.10454088449478

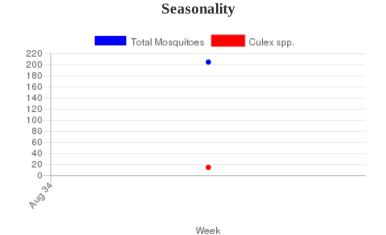
Total number of trap/nights set: 1.0

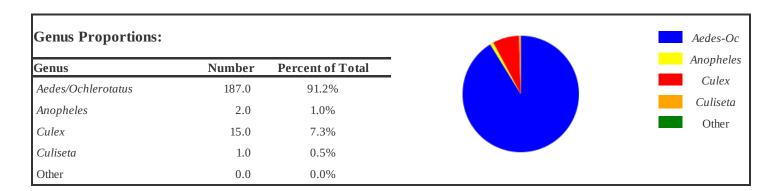
Total number of mosquitoes collected: 205.0

Average mosquitoes per trap/night: 205.0

Average Culex per trap/night: 15.0

Aedes dorsalis	1.0	0.5%
Aedes trivittatus	32.0	15.6%
Aedes vexans	154.0	75.1%
Anopheles freeborni	2.0	1.0%
Culex pipiens	2.0	1.0%
Culex tarsalis	13.0	6.3%
Culiseta inornata	1.0	0.5%





F4 Sombrero Marsh

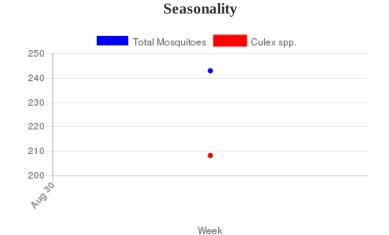
Season: 05/01/2021 - 09/30/2021

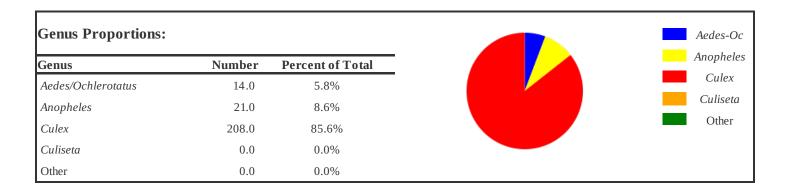
Trap Type: CDC Light Trap **Location:** Sombrero Marsh

GPS: 40.01072294147664, -105.2092308551073

Total number of trap/nights set:1.0Total number of mosquitoes collected:243.0Average mosquitoes per trap/night:243.0Average Culex per trap/night:208.0

Aedes increpitus	1.0	0.4%
Aedes vexans	13.0	5.3%
Anopheles freeborni	21.0	8.6%
Culex pipiens	14.0	5.8%
Culex salinarius	5.0	2.1%
Culex tarsalis	188.0	77.4%
Culex territans	1.0	0.4%





Appendix B: Adult Sample Pool Test Results for West Nile Virus Positive Location



Start Date: 06/01/2021 **End Date:** 09/30/2021

Boulder County Mosquito Control District

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area BC-06 T	est Results							
BC-08	06/27/2021	CDC Light Trap	06/30/2021	BC01002	Culex tarsalis	34	Negative	RT-PCR
BC-08	07/05/2021	CDC Light Trap	07/07/2021	BC01009	Culex tarsalis	65	Negative	RT-PCR
BC-08	07/11/2021	CDC Light Trap	07/14/2021	BC01027	Culex tarsalis	65	Negative	RT-PCR
BC-08	07/18/2021	CDC Light Trap	07/20/2021	BC01042	Culex tarsalis	65	Negative	RT-PCR
BC-08	07/25/2021	CDC Light Trap	07/27/2021	BC01057	Culex tarsalis	65	WNV+	RT-PCR
BC-08	08/01/2021	CDC Light Trap	08/04/2021	BC01072	Culex tarsalis	65	Negative	RT-PCR
BC-08	08/08/2021	CDC Light Trap	08/10/2021	BC01086	Culex tarsalis	33	WNV+	RT-PCR
BC-08	08/08/2021	CDC Light Trap	08/10/2021	BC01087	Culex tarsalis	65	Negative	RT-PCR
BC-08	08/15/2021	CDC Light Trap	08/17/2021	BC01102	Culex tarsalis	41	Negative	RT-PCR
BC-08	08/22/2021	CDC Light Trap	08/24/2021	BC01119	Culex tarsalis	65	WNV+	RT-PCR
BC-08	08/29/2021	CDC Light Trap	08/31/2021	BC01134	Culex tarsalis	39		RT-PCR
BC-08	09/07/2021	CDC Light Trap	09/08/2021	BC01142	Culex tarsalis	5		RT-PCR
Treatment Area BC-08 T	est Results							
BC-11	06/27/2021	CDC Light Trap	06/30/2021	BC01002	Culex tarsalis	20	Negative	RT-PCR
BC-11	07/05/2021	CDC Light Trap	07/07/2021	BC01010	Culex tarsalis	65	Negative	RT-PCR
BC-11	07/05/2021	CDC Light Trap	07/07/2021	BC01011	Culex tarsalis	65	Negative	RT-PCR
BC-11	07/11/2021	CDC Light Trap	07/14/2021	BC01028	Culex tarsalis	65	Negative	RT-PCR
BC-11	07/18/2021	CDC Light Trap	07/20/2021	BC01043	Culex tarsalis	65	Negative	RT-PCR
BC-11	07/25/2021	CDC Light Trap	07/27/2021	BC01058	Culex tarsalis	65	Negative	RT-PCR
BC-11	08/01/2021	CDC Light Trap	08/04/2021	BC01073	Culex tarsalis	65	WNV+	RT-PCR
BC-11	08/08/2021	CDC Light Trap	08/10/2021	BC01088	Culex tarsalis	65	Negative	RT-PCR
BC-11	08/15/2021	CDC Light Trap	08/17/2021	BC01102	Culex tarsalis	24	Negative	RT-PCR
BC-11	08/15/2021	CDC Light Trap	08/17/2021	BC01103	Culex tarsalis	65	Negative	RT-PCR

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
BC-11	08/22/2021	CDC Light Trap	08/24/2021	BC01120	Culex tarsalis	65	WNV+	RT-PCR
BC-11	08/22/2021	CDC Light Trap	08/24/2021	BC01121	Culex tarsalis	65	WNV+	RT-PCR
BC-11	08/29/2021	CDC Light Trap	08/31/2021	BC01135	Culex tarsalis	51		RT-PCR
BC-11	09/07/2021	CDC Light Trap	09/08/2021	BC01142	Culex tarsalis	14		RT-PCR
Treatment Area BC-10 T	est Results							
BC-03	06/27/2021	CDC Light Trap	06/30/2021	BC01001	Culex tarsalis	9	Negative	RT-PCR
BC-03	07/05/2021	CDC Light Trap	07/07/2021	BC01008	Culex tarsalis	65	Negative	RT-PCR
BC-03	07/11/2021	CDC Light Trap	07/14/2021	BC01025	Culex tarsalis	65	Negative	RT-PCR
BC-03	07/11/2021	CDC Light Trap	07/13/2021	BC01026	Culex tarsalis	6	Negative	RT-PCR
BC-03	07/18/2021	CDC Light Trap	07/20/2021	BC01040	Culex tarsalis	65	Negative	RT-PCR
BC-03	07/25/2021	CDC Light Trap	07/27/2021	BC01055	Culex tarsalis	65	Negative	RT-PCR
BC-03	07/25/2021	CDC Light Trap	07/27/2021	BC01056	Culex tarsalis	34	Negative	RT-PCR
BC-03	08/01/2021	CDC Light Trap	08/04/2021	BC01070	Culex tarsalis	65	WNV+	RT-PCR
BC-03	08/01/2021	CDC Light Trap	08/04/2021	BC01071	Culex tarsalis	7	Negative	RT-PCR
BC-03	08/08/2021	CDC Light Trap	08/10/2021	BC01085	Culex tarsalis	41	Negative	RT-PCR
BC-03	08/15/2021	CDC Light Trap	08/17/2021	BC01100	Culex tarsalis	65	Negative	RT-PCR
BC-03	08/22/2021	CDC Light Trap	08/24/2021	BC01117	Culex tarsalis	25	WNV+	RT-PCR
BC-03	08/29/2021	CDC Light Trap	08/31/2021	BC01134	Culex tarsalis	7		RT-PCR
BC-03	09/07/2021	CDC Light Trap	09/08/2021	BC01142	Culex tarsalis	5		RT-PCR
Treatment Area BC-11 T	est Results							
BC-05	06/27/2021	CDC Light Trap	06/30/2021	BC01001	Culex tarsalis	5	Negative	RT-PCR
BC-05	07/05/2021	CDC Light Trap	07/07/2021	BC01013	Culex tarsalis	30	Negative	RT-PCR
BC-05	07/11/2021	CDC Light Trap	07/14/2021	BC01026	Culex tarsalis	45	Negative	RT-PCR
BC-05	07/18/2021	CDC Light Trap	07/20/2021	BC01041	Culex tarsalis	65	Negative	RT-PCR
BC-05	07/25/2021	CDC Light Trap	07/27/2021	BC01056	Culex tarsalis	31	Negative	RT-PCR
BC-05	08/01/2021	CDC Light Trap	08/04/2021	BC01071	Culex tarsalis	58	Negative	RT-PCR
BC-05	08/08/2021	CDC Light Trap	08/10/2021	BC01086	Culex tarsalis	32	WNV+	RT-PCR
BC-05	08/15/2021	CDC Light Trap	08/17/2021	BC01101	Culex tarsalis	51	Negative	RT-PCR
BC-05	08/22/2021	CDC Light Trap	08/24/2021	BC01117	Culex tarsalis	17	WNV+	RT-PCR
BC-05	08/29/2021	CDC Light Trap	08/31/2021	BC01134	Culex tarsalis	5		RT-PCR
BC-05	09/07/2021	CDC Light Trap	09/08/2021	BC01142	Culex tarsalis	2		RT-PCR

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area BC-13 T	est Results							
BC-47	06/27/2021	CDC Light Trap	06/30/2021	BC01001	Culex tarsalis	48	Negative	RT-PCR
BC-47	07/05/2021	CDC Light Trap	07/07/2021	BC01012	Culex tarsalis	65	Negative	RT-PCR
BC-47	07/05/2021	CDC Light Trap	07/07/2021	BC01013	Culex tarsalis	35	Negative	RT-PCR
BC-47	07/11/2021	CDC Light Trap	07/14/2021	BC01026	Culex tarsalis	14	Negative	RT-PCR
BC-47	07/11/2021	CDC Light Trap	07/14/2021	BC01029	Culex tarsalis	65	Negative	RT-PCR
BC-47	07/18/2021	CDC Light Trap	07/20/2021	BC01044	Culex tarsalis	65	WNV+	RT-PCR
BC-47	07/25/2021	CDC Light Trap	07/27/2021	BC01059	Culex tarsalis	65	Negative	RT-PCR
BC-47	08/01/2021	CDC Light Trap	08/04/2021	BC01074	Culex tarsalis	65	Negative	RT-PCR
BC-47	08/08/2021	CDC Light Trap	08/10/2021	BC01085	Culex tarsalis	24	Negative	RT-PCR
BC-47	08/08/2021	CDC Light Trap	08/10/2021	BC01089	Culex tarsalis	65	Negative	RT-PCR
BC-47	08/15/2021	CDC Light Trap	08/17/2021	BC01101	Culex tarsalis	14	Negative	RT-PCR
BC-47	08/15/2021	CDC Light Trap	08/17/2021	BC01104	Culex tarsalis	65	Negative	RT-PCR
BC-47	08/15/2021	CDC Light Trap	08/17/2021	BC01105	Culex tarsalis	65	Negative	RT-PCR
BC-47	08/22/2021	CDC Light Trap	08/24/2021	BC01117	Culex tarsalis	23	WNV+	RT-PCR
BC-47	08/22/2021	CDC Light Trap	08/24/2021	BC01118	Culex tarsalis	65	WNV+	RT-PCR
BC-47	08/29/2021	CDC Light Trap	08/31/2021	BC01134	Culex tarsalis	14		RT-PCR
BC-47	08/29/2021	CDC Light Trap	08/31/2021	BC01135	Culex tarsalis	11		RT-PCR
BC-47	08/29/2021	CDC Light Trap	08/31/2021	BC01136	Culex tarsalis	65		RT-PCR
BC-47	09/07/2021	CDC Light Trap	09/08/2021	BC01142	Culex tarsalis	18		RT-PCR

Total Pools Tested: 70 Total Mosquitoes Tested: 3082 Total Negative: 45 Total Positive: 13



Start Date: 06/01/2021 **End Date:** 09/30/2021

Longmont

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area LM - Lon	gmont Test Results							
LM-03	06/27/2021	CDC Light Trap	06/30/2021	BC02003	Culex tarsalis	52	Negative	RT-PCR
LM-03	07/05/2021	CDC Light Trap	07/07/2021	BC02014	Culex tarsalis	65	Negative	RT-PCR
LM-03	07/05/2021	CDC Light Trap	07/07/2021	BC02019	Culex tarsalis	4	Negative	RT-PCR
LM-03	07/11/2021	CDC Light Trap	07/14/2021	BC02030	Culex tarsalis	65	Negative	RT-PCR
LM-03	07/19/2021	CDC Light Trap	07/20/2021	BC02045	Culex tarsalis	65	Negative	RT-PCR
LM-03	07/25/2021	CDC Light Trap	07/27/2021	BC02060	Culex tarsalis	65	Negative	RT-PCR
LM-03	08/01/2021	CDC Light Trap	08/04/2021	BC02075	Culex tarsalis	65	Negative	RT-PCR
LM-03	08/01/2021	CDC Light Trap	08/04/2021	BC02076	Culex tarsalis	65	WNV+	RT-PCR
LM-03	08/01/2021	CDC Light Trap	08/04/2021	BC02077	Culex tarsalis	4	WNV+	RT-PCR
LM-03	08/08/2021	CDC Light Trap	08/10/2021	BC02090	Culex tarsalis	65	WNV+	RT-PCR
LM-03	08/15/2021	CDC Light Trap	08/17/2021	BC02106	Culex tarsalis	65	Negative	RT-PCR
LM-03	08/15/2021	CDC Light Trap	08/17/2021	BC02107	Culex tarsalis	65	WNV+	RT-PCR
LM-03	08/15/2021	CDC Light Trap	08/17/2021	BC02108	Culex tarsalis	11	WNV+	RT-PCR
LM-03	08/22/2021	CDC Light Trap	08/24/2021	BC02122	Culex tarsalis	65	Negative	RT-PCR
LM-03	08/22/2021	CDC Light Trap	08/24/2021	BC02123	Culex tarsalis	65	WNV+	RT-PCR
LM-03	08/22/2021	CDC Light Trap	08/24/2021	BC02124	Culex tarsalis	13	WNV+	RT-PCR
LM-03	08/29/2021	CDC Light Trap	08/31/2021	BC02137	Culex tarsalis	65		RT-PCR
LM-03	08/29/2021	CDC Light Trap	08/31/2021	BC02138	Culex tarsalis	19		RT-PCR
LM-03	09/06/2021	CDC Light Trap	09/08/2021	BC02143	Culex tarsalis	12		RT-PCR
LM-17	06/27/2021	CDC Light Trap	06/30/2021	BC02004	Culex tarsalis	17	Negative	RT-PCR
LM-17	07/05/2021	CDC Light Trap	07/07/2021	BC02019	Culex tarsalis	61	Negative	RT-PCR
LM-17	07/11/2021	CDC Light Trap	07/14/2021	BC02031	Culex tarsalis	65	Negative	RT-PCR
LM-17	07/18/2021	CDC Light Trap	07/20/2021	BC02046	Culex tarsalis	65	Negative	RT-PCR

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
LM-17	07/25/2021	CDC Light Trap	07/27/2021	BC02061	Culex tarsalis	61	WNV+	RT-PCR
LM-17	08/01/2021	CDC Light Trap	08/04/2021	BC02077	Culex tarsalis	61	WNV+	RT-PCR
LM-17	08/08/2021	CDC Light Trap	08/10/2021	BC02091	Culex tarsalis	65	WNV+	RT-PCR
LM-17	08/15/2021	CDC Light Trap	08/17/2021	BC02108	Culex tarsalis	54	WNV+	RT-PCR
LM-17	08/22/2021	CDC Light Trap	08/24/2021	BC02124	Culex tarsalis	52	WNV+	RT-PCR
LM-17	08/29/2021	CDC Light Trap	08/31/2021	BC02138	Culex tarsalis	20		RT-PCR
LM-17	09/06/2021	CDC Light Trap	09/08/2021	BC02143	Culex tarsalis	8		RT-PCR
LM-28	06/27/2021	CDC Light Trap	06/30/2021	BC02005	Culex tarsalis	53	Negative	RT-PCR
LM-28	07/06/2021	CDC Light Trap	07/07/2021	BC02015	Culex tarsalis	65	Negative	RT-PCR
LM-28	07/06/2021	CDC Light Trap	07/07/2021	BC02016	Culex tarsalis	65	Negative	RT-PCR
LM-28	07/11/2021	CDC Light Trap	07/14/2021	BC02032	Culex tarsalis	65	Negative	RT-PCR
LM-28	07/18/2021	CDC Light Trap	07/20/2021	BC02047	Culex tarsalis	65	Negative	RT-PCR
LM-28	07/25/2021	CDC Light Trap	07/27/2021	BC02061	Culex tarsalis	4	WNV+	RT-PCR
LM-28	07/25/2021	CDC Light Trap	07/27/2021	BC02062	Culex tarsalis	65	Negative	RT-PCR
LM-28	08/01/2021	CDC Light Trap	08/04/2021	BC02078	Culex tarsalis	65	Negative	RT-PCR
LM-28	08/08/2021	CDC Light Trap	08/10/2021	BC02092	Culex tarsalis	65	Negative	RT-PCR
LM-28	08/08/2021	CDC Light Trap	08/10/2021	BC02094	Culex tarsalis	1	WNV+	RT-PCR
LM-28	08/15/2021	CDC Light Trap	08/17/2021	BC02109	Culex tarsalis	65	Negative	RT-PCR
LM-28	08/15/2021	CDC Light Trap	08/17/2021	BC02110	Culex tarsalis	15	Negative	RT-PCR
LM-28	08/22/2021	CDC Light Trap	08/24/2021	BC02125	Culex tarsalis	65	WNV+	RT-PCR
LM-28	08/22/2021	CDC Light Trap	08/24/2021	BC02126	Culex tarsalis	65	WNV+	RT-PCR
LM-28	08/22/2021	CDC Light Trap	08/24/2021	BC02128	Culex tarsalis	10	Negative	RT-PCR
LM-28	08/29/2021	CDC Light Trap	08/31/2021	BC02139	Culex tarsalis	39		RT-PCR
LM-28	09/06/2021	CDC Light Trap	09/08/2021	BC02143	Culex tarsalis	20		RT-PCR
LM-34	06/27/2021	CDC Light Trap	06/30/2021	BC02004	Culex tarsalis	7	Negative	RT-PCR
LM-34	07/06/2021	CDC Light Trap	07/07/2021	BC02017	Culex tarsalis	65	Negative	RT-PCR
LM-34	07/11/2021	CDC Light Trap	07/14/2021	BC02033	Culex tarsalis	65	Negative	RT-PCR
LM-34	07/19/2021	CDC Light Trap	07/20/2021	BC02048	Culex tarsalis	65	Negative	RT-PCR
LM-34	07/25/2021	CDC Light Trap	07/27/2021	BC02063	Culex tarsalis	65	WNV+	RT-PCR
LM-34	08/01/2021	CDC Light Trap	08/04/2021	BC02079	Culex tarsalis	65	WNV+	RT-PCR
LM-34	08/08/2021	CDC Light Trap	08/10/2021	BC02093	Culex tarsalis	65	Negative	RT-PCR

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
LM-34	08/15/2021	CDC Light Trap	08/17/2021	BC02110	Culex tarsalis	50	Negative	RT-PCR
LM-34	08/22/2021	CDC Light Trap	08/24/2021	BC02127	Culex tarsalis	65	Negative	RT-PCR
LM-34	08/29/2021	CDC Light Trap	08/31/2021	BC02138	Culex tarsalis	17		RT-PCR
LM-34	09/06/2021	CDC Light Trap	09/08/2021	BC02143	Culex tarsalis	6		RT-PCR
LM-42	06/27/2021	CDC Light Trap	06/30/2021	BC02004	Culex tarsalis	29	Negative	RT-PCR
LM-42	07/05/2021	CDC Light Trap	07/07/2021	BC02018	Culex tarsalis	65	Negative	RT-PCR
LM-42	07/11/2021	CDC Light Trap	07/14/2021	BC02034	Culex tarsalis	65	Negative	RT-PCR
LM-42	07/18/2021	CDC Light Trap	07/20/2021	BC02049	Culex tarsalis	65	Negative	RT-PCR
LM-42	07/25/2021	CDC Light Trap	07/27/2021	BC02064	Culex tarsalis	65	Negative	RT-PCR
LM-42	08/01/2021	CDC Light Trap	08/04/2021	BC02080	Culex tarsalis	65	Negative	RT-PCR
LM-42	08/08/2021	CDC Light Trap	08/10/2021	BC02094	Culex tarsalis	64	WNV+	RT-PCR
LM-42	08/15/2021	CDC Light Trap	08/17/2021	BC02111	Culex tarsalis	65	Negative	RT-PCR
LM-42	08/22/2021	CDC Light Trap	08/24/2021	BC02128	Culex tarsalis	55	Negative	RT-PCR
LM-42	08/29/2021	CDC Light Trap	08/31/2021	BC02139	Culex tarsalis	21		RT-PCR

Total Pools Tested: 68 Total Mosquitoes Tested: 3310 Total Negative: 39 Total Positive: 19



Start Date: 06/01/2021 **End Date:** 09/30/2021

City of Louisville

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area LO-01 T	est Results							
LO-01	06/27/2021	CDC Light Trap	06/30/2021	BC03007	Culex tarsalis	6	Negative	RT-PCR
LO-01	07/05/2021	CDC Light Trap	07/07/2021	BC03024	Culex tarsalis	24	Negative	RT-PCR
LO-01	07/11/2021	CDC Light Trap	07/14/2021	BC03037	Culex tarsalis	65	Negative	RT-PCR
LO-01	07/11/2021	CDC Light Trap	07/14/2021	BC03039	Culex tarsalis	19	Negative	RT-PCR
LO-01	07/18/2021	CDC Light Trap	07/20/2021	BC03052	Culex tarsalis	65	Negative	RT-PCR
LO-01	07/18/2021	CDC Light Trap	07/20/2021	BC03054	Culex tarsalis	14	Negative	RT-PCR
LO-01	07/25/2021	CDC Light Trap	07/27/2021	BC03067	Culex tarsalis	52	Negative	RT-PCR
LO-01	08/01/2021	CDC Light Trap	08/04/2021	BC03083	Culex tarsalis	43	Negative	RT-PCR
LO-01	08/08/2021	CDC Light Trap	08/10/2021	BC03099	Culex tarsalis	42	Negative	RT-PCR
LO-01	08/15/2021	CDC Light Trap	08/17/2021	BC03115	Culex tarsalis	33	Negative	RT-PCR
LO-01	08/22/2021	CDC Light Trap	08/24/2021	BC03132	Culex tarsalis	49	Negative	RT-PCR
LO-01	08/29/2021	CDC Light Trap	08/31/2021	BC03141	Culex tarsalis	12		RT-PCR
LO-01	09/07/2021	CDC Light Trap	09/08/2021	BC03144	Culex tarsalis	1		RT-PCR
LO-08	06/27/2021	CDC Light Trap	06/30/2021	BC03007	Culex tarsalis	22	Negative	RT-PCR
LO-08	07/05/2021	CDC Light Trap	07/07/2021	BC03023	Culex tarsalis	65	Negative	RT-PCR
LO-08	07/11/2021	CDC Light Trap	07/14/2021	BC03038	Culex tarsalis	65	Negative	RT-PCR
LO-08	07/11/2021	CDC Light Trap	07/14/2021	BC03039	Culex tarsalis	34	Negative	RT-PCR
LO-08	07/18/2021	CDC Light Trap	07/20/2021	BC03053	Culex tarsalis	65	Negative	RT-PCR
LO-08	07/18/2021	CDC Light Trap	07/20/2021	BC03054	Culex tarsalis	43	Negative	RT-PCR
LO-08	07/25/2021	CDC Light Trap	07/27/2021	BC03068	Culex tarsalis	65	Negative	RT-PCR
LO-08	07/25/2021	CDC Light Trap	07/27/2021	BC03069	Culex tarsalis	41	Negative	RT-PCR
LO-08	08/01/2021	CDC Light Trap	08/04/2021	BC03084	Culex tarsalis	65	WNV+	RT-PCR
LO-08	08/08/2021	CDC Light Trap	08/10/2021	BC03098	Culex tarsalis	58	WNV+	RT-PCR

Trap Number	Trap Date	Trap Type	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
LO-08	08/15/2021	CDC Light Trap	08/17/2021	BC03116	Culex tarsalis	50	WNV+	RT-PCR
LO-08	08/22/2021	CDC Light Trap	08/24/2021	BC03133	Culex tarsalis	65	Negative	RT-PCR
LO-08	08/29/2021	CDC Light Trap	08/31/2021	BC03141	Culex tarsalis	8		RT-PCR
LO-08	09/07/2021	CDC Light Trap	09/08/2021	BC03144	Culex tarsalis	3		RT-PCR

Total Pools Tested: 27 Total Mosquitoes Tested: 1074 Total Negative: 20 Total Positive: 3



Start Date: 06/01/2021 **End Date:** 09/30/2021

City of Lafayette

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area LA-01 T	est Results							
LA-11	06/27/2021	CDC Light Trap	06/30/2021	BC03006	Culex tarsalis	20	Negative	RT-PCR
LA-11	07/05/2021	CDC Light Trap	07/07/2021	BC03022	Culex tarsalis	65	Negative	RT-PCR
LA-11	07/11/2021	CDC Light Trap	07/14/2021	BC03036	Culex tarsalis	65	Negative	RT-PCR
LA-11	07/18/2021	CDC Light Trap	07/20/2021	BC03051	Culex tarsalis	65	Negative	RT-PCR
LA-11	07/25/2021	CDC Light Trap	07/27/2021	BC03066	Culex tarsalis	65	Negative	RT-PCR
LA-11	07/25/2021	CDC Light Trap	07/27/2021	BC03067	Culex tarsalis	13	Negative	RT-PCR
LA-11	08/01/2021	CDC Light Trap	08/04/2021	BC03082	Culex tarsalis	65	Negative	RT-PCR
LA-11	08/08/2021	CDC Light Trap	08/10/2021	BC03097	Culex tarsalis	65	WNV+	RT-PCR
LA-11	08/08/2021	CDC Light Trap	08/10/2021	BC03098	Culex tarsalis	7	WNV+	RT-PCR
LA-11	08/15/2021	CDC Light Trap	08/17/2021	BC03114	Culex tarsalis	65	WNV+	RT-PCR
LA-11	08/15/2021	CDC Light Trap	08/17/2021	BC03115	Culex tarsalis	32	Negative	RT-PCR
LA-11	08/15/2021	CDC Light Trap	08/17/2021	BC03116	Culex tarsalis	1	WNV+	RT-PCR
LA-11	08/22/2021	CDC Light Trap	08/24/2021	BC03130	Culex tarsalis	65	Negative	RT-PCR
LA-11	08/22/2021	CDC Light Trap	08/24/2021	BC03131	Culex tarsalis	65	WNV+	RT-PCR
LA-11	08/29/2021	CDC Light Trap	08/31/2021	BC03141	Culex tarsalis	34		RT-PCR
LA-11	09/07/2021	CDC Light Trap	09/08/2021	BC03144	Culex tarsalis	7		RT-PCR

Total Pools Tested: 16 Total Mosquitoes Tested: 699 Total Negative: 9 Total Positive: 5



Start Date: 06/01/2021 **End Date:** 09/30/2021

Town of Erie

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area ER-02 To	est Results							
ER-03	06/27/2021	CDC Light Trap	06/30/2021	BC03006	Culex tarsalis	27	Negative	RT-PCR
ER-03	07/05/2021	CDC Light Trap	07/07/2021	BC03020	Culex tarsalis	65	Negative	RT-PCR
ER-03	07/05/2021	CDC Light Trap	07/07/2021	BC03021	Culex tarsalis	65	Negative	RT-PCR
ER-03	07/11/2021	CDC Light Trap	07/14/2021	BC03035	Culex tarsalis	65	Negative	RT-PCR
ER-03	07/18/2021	CDC Light Trap	07/20/2021	BC03050	Culex tarsalis	65	Negative	RT-PCR
ER-03	07/25/2021	CDC Light Trap	07/27/2021	BC03065	Culex tarsalis	65	WNV+	RT-PCR
ER-03	08/01/2021	CDC Light Trap	08/04/2021	BC03081	Culex tarsalis	65	Negative	RT-PCR
ER-03	08/08/2021	CDC Light Trap	08/10/2021	BC03095	Culex tarsalis	65	WNV+	RT-PCR
ER-03	08/08/2021	CDC Light Trap	08/10/2021	BC03096	Culex tarsalis	65	WNV+	RT-PCR
ER-03	08/08/2021	CDC Light Trap	08/10/2021	BC03099	Culex tarsalis	10	Negative	RT-PCR
ER-03	08/15/2021	CDC Light Trap	08/17/2021	BC03112	Culex tarsalis	65	Negative	RT-PCR
ER-03	08/15/2021	CDC Light Trap	08/17/2021	BC03113	Culex tarsalis	65	Negative	RT-PCR
ER-03	08/22/2021	CDC Light Trap	08/24/2021	BC03129	Culex tarsalis	65	WNV+	RT-PCR
ER-03	08/29/2021	CDC Light Trap	08/31/2021	BC03140	Culex tarsalis	45		RT-PCR
ER-03	09/07/2021	CDC Light Trap	09/08/2021	BC03144	Culex tarsalis	21		RT-PCR

Total Pools Tested: 15 Total Mosquitoes Tested: 818 Total Negative: 9 Total Positive: 4



Start Date: 06/01/2021 **End Date:** 09/30/2021

Town of Superior

Trap Number	Trap Date	Тгар Туре	Date Tested	Pool No.	Mosquito Species	Pool Size	Results	Assay
Treatment Area SU-01 T	est Results							
SU-02	06/27/2021	CDC Light Trap	06/30/2021	BC03007	Culex tarsalis	4	Negative	RT-PCR
SU-02	07/05/2021	CDC Light Trap	07/07/2021	BC03024	Culex tarsalis	41	Negative	RT-PCR
SU-02	07/11/2021	CDC Light Trap	07/14/2021	BC03039	Culex tarsalis	12	Negative	RT-PCR
SU-02	07/18/2021	CDC Light Trap	07/20/2021	BC03054	Culex tarsalis	8	Negative	RT-PCR
SU-02	07/25/2021	CDC Light Trap	07/27/2021	BC03069	Culex tarsalis	24	Negative	RT-PCR
SU-02	08/01/2021	CDC Light Trap	08/04/2021	BC03083	Culex tarsalis	22	Negative	RT-PCR
SU-02	08/08/2021	CDC Light Trap	08/10/2021	BC03099	Culex tarsalis	13	Negative	RT-PCR
SU-02	08/15/2021	CDC Light Trap	08/17/2021	BC03116	Culex tarsalis	14	WNV+	RT-PCR
SU-02	08/22/2021	CDC Light Trap	08/24/2021	BC03132	Culex tarsalis	16	Negative	RT-PCR
SU-02	08/29/2021	CDC Light Trap	08/31/2021	BC03141	Culex tarsalis	1		RT-PCR

Total Pools Tested: 10 Total Mosquitoes Tested: 155 Total Negative: 8 Total Positive: 1

Appendix C: Boulder County Mosquito Control District Adulticide Application Data



Ground Adulticide Applications

Start Date: 06/01/2021 **End Date:** 09/30/2021

Boulder County Mosquito Control District

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
BC 119th St/Qu	iicksilver Road	l Applications						
July 2021	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	2.3	2.3	83.3	0.7
				Total	Aqua Peri	m-X UL 30-3	30 Applied:	0.7
June 2021	06/15/2021	_	PermaSease UC (53883-459-86291)	1:5	4.6	2.5	89.5	0.7
	06/22/2021		PermaSease UC (53883-459-86291)	1:5	1.9	1.8	66.2	0.5
		_			Total Pe	rmaSease L	IC Applied:	1.2
			BC 119th St/Quicksilv	er Road Totals:	8.8	6.6	238.9	1.9
BC 61st to 75tl	h & Valmont F	Rd Applications						
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	7.5	5.7	205.4	1.6
	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	7.4	5.5	201.4	1.6
		_		Total	Aqua Peri	m-X UL 30-3	30 Applied:	3.2
July 2021	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:3	7.4	5.6	202.5	1.6
				To	otal Aqua I	Kontrol 30-3	30 Applied:	1.6
			BC 61st to 75th & Val	mont Rd Totals:	22.3	16.8	609.4	4.8

BC 61st & Valmont Rd Applications

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
June 2021	06/09/2021	L	Aqua Kontrol 30-30 (73748-11)	1:4	2.2	1.7	63.3	0.5
	06/16/2021	l	Aqua Kontrol 30-30 (73748-11)	1:4	1.8	1.3	46.2	0.4
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	2.1	1.6	59.3	0.5
				To	tal Aqua	Kontrol 30-3	30 Applied:	1.3
			BC 61st & Vo	almont Rd Totals:	6.0	4.6	168.7	1.3
BC 6775 Arapa	ahoe Rd - floa	at Applications						
June 2021	06/15/2021		Aqua Kontrol 30-30 (73748-11)	1:4	0.6	0.6	20.0	0.2
				To	tal Aqua	Kontrol 30-3	30 Applied:	0.2
			BC 6775 Arapahoe	Rd - float Totals:	0.6	0.6	20.0	0.2
BC 75th & Val	mont Rd App	lications						
June 2021	06/16/2021		PermaSease UC (53883-459-86291)	1:5	3.8	2.3	82.9	0.7
					Total Pe	rmaSease U	C Applied:	0.7
June 2021	06/09/2021		Aqua Kontrol 30-30 (73748-11)	1:4	18.7	3.5	126.5	1.0
July 2021	07/07/2021	l	Aqua Kontrol 30-30 (73748-11)	1:4	3.8	2.3	83.3	0.7
				To	tal Aqua	Kontrol 30-3	30 Applied:	1.7
			BC 75th & Vo	almont Rd Totals:	26.4	8.1	292.7	2.3
BC Anhawa - f	loat Applicati	ons						
July 2021	07/14/2021	l	Aqua Kontrol 30-30 (73748-11)	1:4	3.9	2.8	100.7	8.0

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
				To	tal Aqua	Kontrol 30-3	0 Applied:	0.8
			BC Anh	nawa - float Totals:	3.9	2.8	100.7	0.8
BC Boulder Hill	ls Application	S						
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.5	3.9	142.9	1.1
	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.9	4.2	151.6	1.2
August 2021	08/04/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.9	4.0	145.8	1.2
	08/11/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.7	4.0	145.4	1.2
	08/25/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	4.6	4.0	143.6	1.1
				Total	Aqua Per	m-X UL 30-3	0 Applied:	5.8
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	4.8	3.9	143.3	1.1
	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:4	4.7	4.1	147.6	1.2
				To	tal Aqua	Kontrol 30-3	0 Applied:	2.3
			BC Bo	oulder Hills Totals:	33,2	28.1	1,020.3	8.1
BC Brigadoon	Glen/Rangevi	ew/Oriole Application	S					
July 2021	07/27/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	9.6	5.2	189.8	1.5
				Total	Aqua Per	m-X UL 30-3	0 Applied:	1.5
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	10.8	5.9	213.1	1.7
	07/13/2021		Aqua Kontrol 30-30 (73748-11)	1:4	10.8	6.1	222.9	1.7

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
	07/20/202	1	Aqua Kontrol 30-30 (73748-11)	1:3	10.6	5.9	215.6	1.7
				To	otal Aqua	Kontrol 30-3	30 Applied:	5.0
			BC Brigadoon Glen/Rangevi	ew/Oriole Totals:	41.8	23.1	841.4	6.5
BC Brownsville	/Canfield Ap	plications						
July 2021	07/22/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	9.6	6.2	224.0	1.8
	07/29/2022	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	9.6	6.2	225.1	1.8
August 2021	08/18/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:4	9.7	6.2	224.3	1.8
				Total	Aqua Peri	m-X UL 30-3	30 Applied:	5.4
June 2021	06/17/2022	1	PermaSease UC (53883-459-86291)	1:5	9.7	6.1	222.9	1.8
					Total Pe	rmaSease L	IC Applied:	1.8
July 2021	07/08/2022	1	Aqua Kontrol 30-30 (73748-11)	1:4	9.7	6.3	228.0	1.8
	07/15/2021	1	Aqua Kontrol 30-30 (73748-11)	1:4	9.7	6.2	224.0	1.8
				To	otal Aqua	Kontrol 30-3	30 Applied:	3.6
			BC Brownsville	/Canfield Totals:	58.0	37.1	1,348.2	10.8
BC Chance Acr	es Applicatio	ns						
July 2021	07/21/2022	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.0	2.1	76.4	0.6
				Total	Aqua Peri	m-X UL 30-3	30 Applied:	0.6
			BC Cha	nce Acres Totals:	: 3.0 2.1 76.4			0.6

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
BC Divide Rese	ervoir Applica	ations						
July 2021	07/21/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	2.5	1.4	52.4	0.4
	07/28/2021	l	Aqua Perm-X UL 30-30 (89459-76)	1:3	2.6	1.4	51.6	0.4
				Total .	Aqua Per	m-X UL 30-3	30 Applied:	0.8
June 2021	06/15/2021	_	PermaSease UC (53883-459-86291)	1:5	2.6	1.4	51.6	0.4
					Total Pe	ermaSease U	C Applied:	0.4
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	2.5	1.4	51.6	0.4
	07/14/2021	l	Aqua Kontrol 30-30 (73748-11)	1:4	2.5	1.4	52.0	0.4
		_		То	tal Aqua .	Kontrol 30-3	30 Applied:	0.8
		_	BC Divide Re	servoir Totals:	12.8	7.1	259.2	2.1
BC Fairview Es	tates/Indian I	Hills Applications						
July 2021	07/28/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.4	2.4	88.7	0.7
August 2021	08/04/2021	l	Aqua Perm-X UL 30-30 (89459-76)	1:3	8.4	5.3	192.0	1.5
				Total .	Aqua Per	m-X UL 30-3	30 Applied:	2.2
		_	BC Fairview Estates/India	ın Hills Totals:	11.8	7.7	280.7	2.2
BC Fairview Es	tates/Indian I	Hills/Spanish H <u>i</u> lls	/Paragon Estates Applications					
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	9.0	5.4	196.7	1.5
		_		Total	Aqua Per	m-X UL 30-3	30 Applied:	1.5
		_						

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
		_	BC Fairview Estates/Indian Hills/Span	ish Hills/Paragon Estates Totals:	9.0	5.4	196.7	1.5
BC Gunbarrel E	states - floa	t Applications						
June 2021	06/16/202	1	PermaSease UC (53883-459-86291)	1:5	7.7	4.5	162.5	1.3
		_			Total Pe	rmaSease U	C Applied:	1.3
			BC Gunbarrel Est	ates - float Totals:	7.7	4.5	162.5	1.3
BC Gunbarrel G	Green Applica	ations						
July 2021	07/21/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	7.4	5.2	188.3	1.5
	07/28/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	7.3	5.1	186.2	1.5
August 2021	08/04/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	7.3	5.1	186.5	1.5
	08/18/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	7.3	5.2	187.6	1.5
		_		Total	Aqua Peri	n-X UL 30-3	30 Applied:	5.9
June 2021	06/16/202	1	PermaSease UC (53883-459-86291)	1:5	7.4	5.2	188.7	1.5
		_			Total Pe	rmaSease U	C Applied:	1.5
June 2021	06/09/202	1	Aqua Kontrol 30-30 (73748-11)	1:4	23.0	5.3	190.8	1.5
July 2021	07/07/202	1	Aqua Kontrol 30-30 (73748-11)	1:4	7.9	5.1	185.1	1.4
	07/14/202	1	Aqua Kontrol 30-30 (73748-11)	1:3	7.5	5.1	185.8	1.5
				To	otal Aqua I	Kontrol 30-3	30 Applied:	4.4
		_	BC Gunba	rrel Green Totals:	75.0	41.2	1,499.0	11.9

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
BC Heatherwoo	d Application	ns						
July 2021	07/21/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	7.3	5.0	182.2	1.4
	07/28/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	7.1	4.9	177.8	1.4
August 2021	08/04/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	6.9	4.8	175.3	1.4
	08/11/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	6.9	4.8	173.1	1.3
	08/18/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	6.8	4.8	175.6	1.4
				Total	Aqua Peri	m-X UL 30-3	30 Applied:	6.9
June 2021	06/16/2021	1	PermaSease UC (53883-459-86291)	1:5	7.2	5.2	187.6	1.5
					Total Pe	rmaSease L	C Applied:	1.5
July 2021	07/07/2021	1	Aqua Kontrol 30-30 (73748-11)	1:4	7.2	4.8	172.7	1.4
	07/14/2021	1	Aqua Kontrol 30-30 (73748-11)	1:3	7.4	5.0	180.0	1.4
				To	tal Aqua l	Kontrol 30-3	30 Applied:	2.8
			BC Heati	herwood Totals:	56.7	39.2	1,424.2	11.2
BC Hillcrest He	ights Applica	ntions						
July 2021	07/21/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	6.3	3.0	110.5	0.9
	07/28/2021	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	5.2	3.4	124.0	1.0
August 2021	08/04/2022	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	4.8	2.9	105.1	8.0

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
	08/11/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.2	3.4	124.0	1.0
	08/18/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	4.6	2.9	106.9	0.9
				Total	Aqua Peri	n-X UL 30-3	30 Applied:	4.5
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	4.7	2.9	106.5	8.0
	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:3	5.0	3.2	115.3	0.9
		_		To	otal Aqua 1	Kontrol 30-3	30 Applied:	1.8
			BC Hillcrest	Heights Totals:	35.8	21.8	792.3	6.3
BC Hillcrest He	ights/Gaynor	Lake Applications	;					
August 2021	08/25/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	8.1	5.3	192.0	1.5
September 2021	09/01/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	1.7	1.1	41.1	0.3
	09/01/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	6.4	4.2	152.0	1.2
				Total	Aqua Peri	n-X UL 30-3	30 Applied:	3.1
			BC Hillcrest Heights/Gayn	or Lake Totals:	16.2	10.6	385.0	3.1
BC Hygiene/Hy	giene Height	s Applications						
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.9	3.7	134.5	1.1
	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.9	3.7	133.8	1.1
August 2021	08/04/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	6.0	3.7	134.5	1.1

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
	08/11/2021	-	Aqua Perm-X UL 30-30 (89459-76)	1:3	6.0	3.5	127.6	1.0
	08/25/2021	l	Aqua Perm-X UL 30-30 (89459-76)	1:4	5.6	3.4	123.3	1.0
September 2021	09/09/2021	l	Aqua Perm-X UL 30-30 (89459-76)	1:4	6.3	4.0	146.5	1.2
				Total	Aqua Peri	n-X UL 30-3	30 Applied:	6.3
June 2021	06/15/2021		PermaSease UC (53883-459-86291)	1:5	6.4	3.6	131.6	1.1
					Total Pe	rmaSease U	C Applied:	1.1
July 2021	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:4	5.9	3.8	136.7	1.1
				To	otal Aqua I	Kontrol 30-3	30 Applied:	1.1
			BC Hygiene/Hygien	e Heights Totals:	47.9	29.4	1,068.6	8.5
BC McCall Lak	e/Hygiene He	eights Applications						
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	7.1	4.9	177.1	1.4
				To	otal Aqua I	Kontrol 30-3	30 Applied:	1.4
			BC McCall Lake/Hygien	e Heights Totals:	7.1	4.9	177.1	1.4
BC North Rim/I	Lake Valley E	States Applications						
July 2021	07/21/2021	-	Aqua Perm-X UL 30-30 (89459-76)	1:3	9.0	5.6	205.1	1.6
	07/29/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	8.0	5.1	185.1	1.5
August 2021	08/11/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	7.6	4.6	168.7	1.3
	08/18/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	7.7	4.9	176.7	1.4

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
	08/25/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	7.3	4.5	164.4	1.3
September 2021	09/01/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	8.0	4.9	178.9	1.4
		_		Total	Aqua Peri	n-X UL 30-3	30 Applied:	8.5
June 2021	06/23/2021		PermaSease UC (53883-459-86291)	1:5	7.8	4.9	179.6	1.5
		_			Total Pe	rmaSease U	C Applied:	1.5
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	8.2	5.0	181.8	1.4
	07/14/2021	_	Aqua Kontrol 30-30 (73748-11)	1:4	9.6	5.0	183.3	1.4
				To	otal Aqua l	Kontrol 30-3	30 Applied:	2.8
		_	BC North Rim/Lake Valley	Estates Totals:	73.2	44.7	1,623.5	12.8
BC Park Lake A	Applications							
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	2.9	2.1	75.3	0.6
	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	2.9	2.1	75.6	0.6
August 2021	08/04/2021	-	Aqua Perm-X UL 30-30 (89459-76)	1:3	2.9	2.0	74.2	0.6
	08/11/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	2.9	2.0	73.8	0.6
	08/18/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	2.9	2.1	76.7	0.6
	08/25/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	2.9	2.0	72.4	0.6
		_		Total	Aqua Peri	n-X UL 30-3	30 Applied:	3.5
		_						

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
June 2021	06/16/2021		Aqua Kontrol 30-30 (73748-11)	1:4	2.9	2.0	73.5	0.6
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	3.0	2.1	77.1	0.6
	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:3	2.9	2.0	73.1	0.6
				To	otal Aqua	Kontrol 30-3	0 Applied:	1.8
		_	BC Pa	ırk Lake Totals:	26.2	18.5	671.6	5.3
BC Red Fox Hi	lls Application	าร						
June 2021	06/16/2021		Aqua Kontrol 30-30 (73748-11)	1:4	2.9	2.0	73.5	0.6
	06/16/2021		Aqua Kontrol 30-30 (73748-11)	1:4	7.3	3.6	131.3	1.0
				To	otal Aqua	Kontrol 30-3	0 Applied:	1.6
			BC Red F	ox Hills Totals:	10.2	5.6	204.7	1.6
BC Red Fox Hi	lls/Twin Lake	s Applications						
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.3	2.7	96.4	8.0
	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.4	2.6	93.1	8.0
August 2021	08/04/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.3	2.6	94.9	0.7
	08/11/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.2	2.5	90.5	0.7
	08/18/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.2	2.7	96.4	8.0
	08/25/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	4.1	2.6	94.2	0.7

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
September 2021	09/01/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	4.1	2.6	95.3	0.7
				Total	Aqua Peri	m-X UL 30-3	0 Applied:	5.2
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	4.3	2.7	97.4	0.8
	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:3	4.0	2.5	92.0	0.7
				To	otal Aqua I	Kontrol 30-3	0 Applied:	1.5
			BC Red Fox Hills/Tw	in Lakes Totals:	37.9	23.4	850.1	6.7
BC Ridglea Hill	s/Crestmoor .	Applications						
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.1	3.7	135.6	1.1
	07/28/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.2	3.7	134.2	1.1
August 2021	08/04/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.1	3.8	136.4	1.1
	08/11/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	5.1	3.7	135.3	1.1
	08/18/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	5.1	3.7	132.7	1.1
	08/25/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	4.9	3.6	130.5	1.0
September 2021	09/01/2021		Aqua Perm-X UL 30-30 (89459-76)	1:4	4.7	3.6	129.1	1.0
		_		Total	Aqua Peri	m-X UL 30-3	0 Applied:	7.3
June 2021	06/15/2021	_	Aqua Kontrol 30-30 (73748-11)	1:4	4.6	3.2	116.7	0.9
July 2021	07/07/2021	-	Aqua Kontrol 30-30 (73748-11)	1:4	4.6	3.5	128.4	1.0

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
	07/14/2021	1	Aqua Kontrol 30-30 (73748-11)	1:3	4.5	3.5	128.4	1.0
				To	otal Aqua I	Kontrol 30-3	30 Applied:	2.9
			BC Ridglea Hills/C	Crestmoor Totals:	49.1	36.0	1,307.1	10.3
BC South Mead	lows Applica	tions						
July 2021	07/21/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.2	2.1	76.0	0.6
	07/28/2022	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.2	2.1	74.5	0.6
August 2021	08/04/2023	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.2	2.1	75.3	0.6
	08/18/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.2	2.1	74.9	0.6
				Total	Aqua Peri	m-X UL 30-3	30 Applied:	2.3
June 2021	06/16/2021	1	PermaSease UC (53883-459-86291)	1:5	3.4	2.1	77.1	0.6
					Total Pe	rmaSease U	C Applied:	0.6
June 2021	06/09/2022	1	Aqua Kontrol 30-30 (73748-11)	1:4	5.7	2.2	79.3	0.6
July 2021	07/07/2022	1	Aqua Kontrol 30-30 (73748-11)	1:4	3.2	2.0	73.1	0.6
	07/14/2021	1 	Aqua Kontrol 30-30 (73748-11)	1:3	3.2	2.0	73.8	0.6
				To	otal Aqua 1	Kontrol 30-3	30 Applied:	1.8
			BC South	Meadows Totals:	28.2	16.6	603.9	4.8
BC Spanish Hill	s/Paragon Es	states Applications						
August 2021	08/11/2021	L	Aqua Perm-X UL 30-30 (89459-76)	1:3	3.7	2.6	96.0	0.8

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
		_		Tota	ıl Aqua Per	m-X UL 30-3	30 Applied:	0.8
			BC Spanish Hills/Parag	jon Estates Totals:	3.7	2.6	96.0	0.8
BC Stonehenge	- float Appl	cations						
September 2021	09/01/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:4	1.3	1.1	39.6	0.3
		_		Tota	ıl Aqua Per	m-X UL 30-3	30 Applied:	0.3
			BC Stoneho	enge - float Totals:	1.3	1.1	39.6	0.3
BC Willow Glen	/Fox Run A _l	oplications						
July 2021	07/21/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	1.8	1.3	45.8	0.4
	07/28/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	1.8	1.3	46.9	0.4
August 2021	08/04/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	1.8	1.3	46.5	0.4
	08/11/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:3	1.8	1.3	46.9	0.4
	08/18/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:4	1.9	1.3	48.4	0.4
	08/25/202	1	Aqua Perm-X UL 30-30 (89459-76)	1:4	1.8	1.3	47.6	0.4
				Tota	ıl Aqua Per	m-X UL 30-3	30 Applied:	2.2
June 2021	06/16/202	1	Aqua Kontrol 30-30 (73748-11)	1:4	1.8	1.2	44.7	0.3
July 2021	07/07/202	1	Aqua Kontrol 30-30 (73748-11)	1:4	1.8	1.3	48.4	0.4
	07/14/202	1	Aqua Kontrol 30-30 (73748-11)	1:3	1.8	1.3	46.9	0.4
					Total Aqua	Kontrol 30-3	30 Applied:	1.1

Month	Date M	J unicipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
			BC Willow Gle	en/Fox Run Totals:	16.4	11.6	422.1	3.3
BC Woodbourn	ne Hollow/Rustic	: Knolls - float Ap	plications					
June 2021	06/09/2021		Aqua Kontrol 30-30 (73748-11)	1:4	0.5	0.4	16.0	0.1
	06/09/2021		Aqua Kontrol 30-30 (73748-11)	1:4	2.3	0.4	15.3	0.1
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	1.2	0.8	30.5	0.3
				To	otal Aqua I	Kontrol 30-3	30 Applied:	0.5
			BC Woodbourne Hollow/Rustic K	nolls - float Totals:	4.0	1.7	61.8	0.5
BC Yellowston	e Road Applicati	ions						
July 2021	07/21/2021		Aqua Perm-X UL 30-30 (89459-76)	1:3	4.8	4.2	153.4	1.2
				Total	Aqua Peri	n-X UL 30-3	30 Applied:	1.2
July 2021	07/07/2021		Aqua Kontrol 30-30 (73748-11)	1:4	4.7	4.2	151.3	1.2
	07/14/2021		Aqua Kontrol 30-30 (73748-11)	1:4	4.7	4.2	151.6	1.2
				To	otal Aqua l	Kontrol 30-3	30 Applied:	2.4
			BC Yellow	stone Road Totals:	14.2	12.5	456.3	3.6
				Grand Totals:	748.3	475.8	17,298.8	136.8



Ground Adulticide Applications

Start Date: 06/01/2021 **End Date:** 09/30/2021

Boulder County Mosquito Control District

Month	Date	Municipality	Chemical	Mix Ratio	Trip Miles	Spray Miles	Spray Acres	Gallons Sprayed
BC Cline Trout F	arm - Barrier Appli	cations						
June 2021	06/17/2021		Wisdom TC (5481-520)	1:128	0.0	0.0	0.1	4.0
July 2021	07/13/2021		Wisdom TC (5481-520)	1:128	0.0	0.0	0.1	4.0
	07/29/2021		Wisdom TC (5481-520)	1:128	0.0	0.0	0.1	6.0
					Tota	ıl Wisdom T	C Applied:	14.0
		_	BC Cline Trout Farm	- Barrier Totals:	0.0	0.0	0.3	14.0
			·	Grand Totals:	0.0	0.0	0.3	14.0

Appendix D:	Boulder County M	1osquito (Control .	Insecticide
resistance Tri	als: August 2021			



Insecticide Resistance Trials: Boulder County MCD

August 2021

Business Office: 1320 Brookwood Dr. Suite H Little Rock, AR 72202

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Introduction to Insecticide Resistance

Insecticide resistance is defined as a genetic change in response to selection by toxicants that may impair control in the field (Sawicki, 1987). Resistance is believed to develop as genetic traits for detoxification, target-site insensitivity, or other means of survival increase amongst the insect population. Insecticide resistance in a vector population is typically detected and characterized by using some sort of bioassay to determine whether a particular insecticide is able to control a vector population.

Resistance to insecticides has appeared in the major insect vectors from every genus. As of 1992, the list of insecticide resistant mosquito species included 56 anopheline species and 39 culicine mosquito species. Resistance has developed to every chemical class of insecticide, including microbial drugs and insect growth regulators.

The development of resistance in insects to acutely toxic insecticides generally occurs by selection of rare individuals in a population that can survive the insecticide. It is pre-adaptive and not a mutational effect. Resistance develops largely as a result of natural selection of pre-adapted mutants that possess genetically controlled mechanisms of resistance. Common mechanisms of resistance in insects include:

- Altered Behavior
- Reduced Penetration
- Detoxification
- Target Site Insensitivity
- Excretion/Sequestration

Insecticide resistance has the potential to be a major obstacle to the control of mosquitoes and other insect pests and can have serious long terms impacts on a mosquito control program. Knowledge of the mechanisms of resistance and the susceptibly of local mosquito populations are critical to every program's success.

Mechanisms of Resistance

Insecticide resistance mechanisms have a biochemical basis. The two major forms of biochemical resistance seen in mosquito populations are target-site resistance, which occurs when the insecticide no longer binds to its target, and detoxification enzyme-based resistance, which occurs when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferases (GST) prevent the insecticide from reaching its site of action.



Alterations of amino acids responsible for insecticide binding at its site of action cause the insecticide to be less effective or even ineffective. The target of organophosphate (OP) (e.g., malathion, naled) insecticides is acetylcholinesterase in nerve synapses, and the target of synthetic pyrethroids are the sodium channels of the nerve sheath.

The enzymes responsible for detoxification of xenobiotics in living organisms are transcribed by members of large multigene families of esterases, oxidases, and GST. Perhaps the most common resistance mechanisms in insects are modified levels or activities of esterase detoxification enzymes that metabolize a wide range of insecticides. These esterases comprise six families of proteins. Individual members of the gene cluster may be modified in instances of insecticide resistance, for example, by changing a single amino acid that converts the specificity of an esterase to an insecticide hydrolase or by existing as multiple-gene copies that are amplified in resistant insects.

Pesticide Modes of Action

Resistance concerns in mosquito populations are further amplified due to the low number of chemicals available for adulticide applications. For adult mosquito control, we only have products available from 4 different classes of pesticide, representing 2 distinct modes of action.

Adulticide	Class of Pesticide	Targeted System	Mode of Action		
Deltamethrin	Pyrethroid	Nervous System	Sodium Channel Modulator		
Etofenprox	Ether Pyrethroid	Nervous System	Sodium Channel Modulator		
Malathion	Organophosphate	Nervous System	Cholinesterase Inhibitor		
Naled	Organophosphate	Nervous System	Cholinesterase Inhibitor		
Permethrin	Pyrethroid	Nervous System	Sodium Channel Modulator		
Pyrethrin	Pyrethrin	Nervous System	Sodium Channel Modulator		
Sumithrin	Pyrethroid	Nervous System	Sodium Channel Modulator		



Cholinesterase Inhibitors:

Organophosphate insecticides are known as cholinesterase inhibitors. They bind to the enzyme that is normally responsible for breaking down acetylcholinesterase (Ach) after it has carried its message across the synapse. When a mosquito has been exposed to a cholinesterase inhibitor, the cholinesterase is not available to help break down the ACh, and the neurotransmitter continues to cause the neuron to "fire," or send its electrical charge. This causes over stimulation of the nervous system and the insect dies.

Sodium Channel Modulators:

Pyrethrins are naturally occurring compounds derived from members of the chrysanthemum family. While they have a quick knock-down effect against insects, they are unstable in the environment, so may not last long enough to kill the pest. Pyrethroids are synthetic versions of pyrethrins, specifically designed to be more stable in the environment (although still lasting only days or weeks), and thus provide longer-lasting control. Etofenprox is not considered a traditional synthetic pyrethroid by the EPA because it is a non-ester or ether pyrethroid. Ether pyrethroids differ from traditional pyrethroids in the way that their carbon and oxygen molecules are bound together. However, etofenprox has a similar mode of action and, therefore, exhibits a similar concern for developing cross-resistance.

Pyrethrins and pyrethroids act on tiny channels through which sodium is pumped to cause excitation of neurons. They prevent the sodium channels from closing, resulting in continual nerve impulse transmission, tremors, and eventually, death.

Cross Resistance

When insects become resistant to one class of pesticides, they may evolve resistance more rapidly to new groups of insecticides having either similar modes of action or similar metabolic pathways for detoxification. For example, pyrethroids have had a short useful life against some pests because of a gene identified as *kdr*. This gene played a key role in the genetic evolution of DDT resistance and appears to provide some insects with protection against pyrethroids. The "knockdown resistance" (*kdr*) mechanism is a generic term that is associated with resistance to pyrethroids, which involves amino acid substitutions in the voltage-sensitive sodium channel, causing a reduction in the sensitivity of the insect nervous system to pyrethroids. Typically, resistance to organophosphates depends primarily on levels of esterase-based resistance while resistance to pyrethroids depends on *kdr* and esterase.



Testing for Resistance

The standard method used for measuring resistance in the United States is the bottle bioassay as described by the Centers for Disease Control (CDC). Bottle bioassays rely on time-mortality data, which measures the time it takes an insecticide to penetrate a vector, traverse its intervening tissues, get to the target site, and act on that site. Anything that prevents or delays the compound from achieving its objective — killing insects — contributes to resistance (McAllister and Scott, 2019).

The CDC bottle bioassay is used to determine if particular active ingredients are able to kill insect vectors, such as a mosquitoes, within a specific time. Bottles are coated with a predetermined dose of insecticide known to kill 100% of susceptible mosquitoes within a period. The expected time for a known amount of insecticide to achieve this objective is called the diagnostic time. The Centers for Disease Control Guidelines for Evaluating Insecticide Resistance in Mosquitoes suggest that the diagnostic dose for technical grade Permethrin is $43\mu g$ (see Table 1) and the diagnostic time across most *Culex* species is 30 minutes.

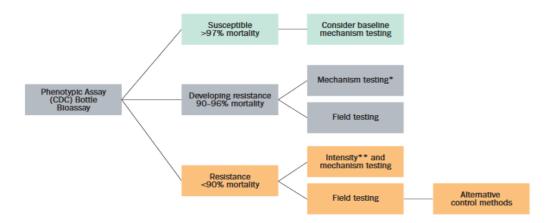
Table 1: Sample diagnostic time per species (McAllister and Scott, 2019).

Table 1: Sample diagnostic doses and diagnostic times for technical grade insecticides.												
Insecticide	Insecticide	Diagnostic time per species (minutes)										
	concentration (μg/bottle)	Ae. aegypti	Ae. albopictus	Cx. molestus	Cx. pipiens	Cx. quinquefasciatus	Cx. tarsalis					
Chlorpyrifos	20	45	45	45	90	45	60					
Deltamethrin	0.75	30	30		45	60						
Etofenprox	12.5	15	30	105	15	30	60					
Fenthion	800			30	75	45	45					
Malathion	400	15	30	30	45	45	45					
Naled	2.25	30	30	30	45	45	45					
Permethrin	43	10	10	30	30	30	30					
Prallethrin	0.05				60	60						
Pyrethrum	15	15	30		45	45	30					
Sumethrin	20	10	45	120	30	45	30					

Significant mortality (>97%) at times or doses less than the amounts listed indicate a highly susceptible population and indicate the pesticides are working as intended and one may consider baseline mechanism testing as time and resources allow (Figure 1). Mortality rates between 90 and 96% indicate that there may be some level of resistance to pesticides within the local vector population and mechanistic testing in addition to field testing are encouraged. Mortality rates of <90% within the diagnostic time indicate significant resistance and it is recommended that one consider alternative control strategies.



Figure 1: Suggested algorithm for further testing depending on level of resistance detected in the CDC bottle bioassay (McAllister and Scott, 2019).



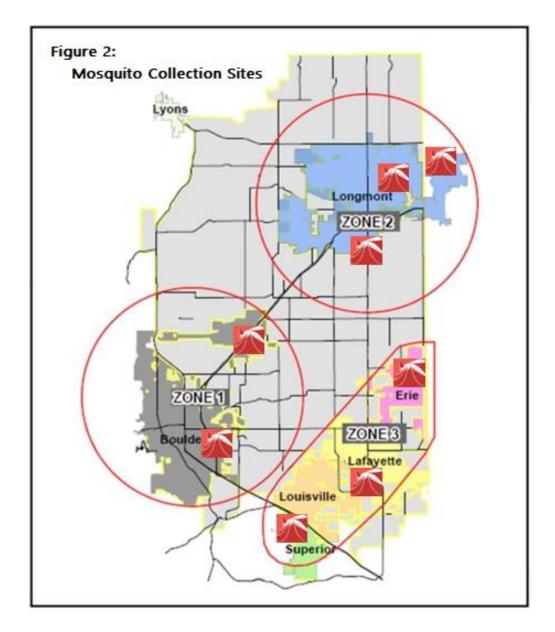
Project Methodology

- 1. Field collected mosquitos, of variable age and physiological status, were collected using CDC miniature light traps at multiple sites within each of 3 Sentinel Zone within Boulder County (see Figure 2).
- 2. Mosquitoes were transferred from collection nets to a holding cage and maintained on 10% sucrose solution for a minimum of 24-hours before testing.
- 3. 250-ml Wheaton bottles were coated with technical grade permethrin (43ug/bottle) allowed to dry per CDC guidelines (Brogdon *et al,* 1998).
- 4. Approximately 20 adult mosquitoes were aspirated and transferred to each treated 250-ml Wheaton bottle.
- 5. Mortality was recorded at the time of introduction and 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 minutes after exposure. Mosquitoes are considered dead if they can no longer stand.

Materials and Reagents Needed

- 15, 250-ml Wheaton bottles with PTFE lined lids
- Adjustable micropipettes with disposable tips
- Standard mouth aspirator and holding cages
- Clear 50 ml conical tube for measuring acetone
- Amber bottles/tube(s) for insecticide stock solutions
- Safety Data Sheets (SDS) for insecticides used in study





Zone 1: Gunbarrel, Sombrero Marsh

Zone 2: City of Longmont – St. Vrain Greenway, Garden Acres, Union Reservoir

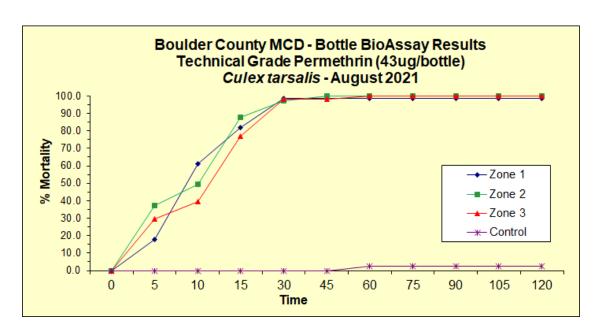
Zone 3: Superior, Lafayette and Town of Erie



Project Results

Our primary interest was to test for resistance to the public health pesticide permethrin which was originally registered for use by the US EPA in 1979 and is used as the primary control strategy for reducing active adult mosquito populations within northern Colorado. Most pyrethroid mosquito control products can be applied only by public health officials and trained personnel of mosquito control districts. Mosquito control professionals apply pyrethroids as an ultra-low volume (ULV) spray. ULV sprayers dispense very fine aerosol droplets that stay aloft and kill adult mosquitoes on contact. The product is often applied at rates less than 1/100th of a pound of active ingredient per acre.

The diagnostic dose of permethrin is 43ug per bottle and the diagnostic time (period in which we expect to see >97% mortality) is 30 minutes.



Time	Zone 1		Assay 1	Zone 2	2	Assay 2	Zone 3		Assay 3	Control			
	Dead	Alive	%Mortality	Dead	Alive	%Mortality	Dead	Alive	%Mortality		Dead	Alive	%Mortality
0	0	72	0.0	0	107	0.0	0	61	0.0		0	42	0.0
5	13	59	18.1	40	67	37.4	18	43	29.5		0	42	0.0
10	44	28	61.1	53	54	49.5	24	37	39.3		0	42	0.0
15	59	13	81.9	94	13	87.9	47	14	77.0		0	42	0.0
30	71	1	98.6	104	3	97.2	60	1	98.4		0	42	0.0
45	71	1	98.6	107	0	100.0	60	1	98.4		0	42	0.0
60	71	1	98.6	107	0	100.0	61	0	100.0		1	41	2.4
75	71	0	98.6	107	0	100.0	61	0	100.0		1	41	2.4
90	71	0	98.6	107	0	100.0	61	0	100.0		1	41	2.4
105	71	0	98.6	107	0	100.0	61	0	100.0		1	41	2.4
120	71	0	98.6	107	0	100.0	61	0	100.0		1	41	2.4



Conclusions:

Significant mortality (>97%) was noted within mosquito populations collected across all three sentinel zones of Boulder County, Colorado within the diagnostic time of 30 minutes. These results indicate no phenotypic resistance and a population highly susceptible to permethrin-based products. Its important to note that the wild caught mosquitoes utilized in these studies were collected from areas that were sprayed several days before collections took place meaning that there may be a higher likelihood of collecting resistant populations than if mosquitoes were collected from untreated areas.

The CDC bottle bioassay is designed to detect whether resistance is present in a population before you lose the use of a chemical and should be used to establish baseline data for comparison over time. Utilizing these tests, management decisions can be made in a timely manner to preserve susceptibility to specific chemical classes, and we can confidently choose pesticides that we know will work to protect public health within the communities we serve.



References:

Brogdon, WG and McAllister JC, 1998. Simplification of Adult mosquito bioassays through the use of time-mortality determinations in glass bottles. Journal of the American Mosquito Control Association. 14(2): 159-64.

McAllister, J. and Scott, M. (2019). CONUS Guidelines for Evaluating Insecticide Resistance in Mosquitoes Using the CDC Bottle Bioassay Kit.

Sawicki, RM. 1987. Definition, detection and documentation of insecticide resistance. In Combating Resistance to Xenobiotics: Biological and Chemical Approaches. Chrichester, UK. Ellis Horwood. pp 105-117.