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Diversity and ecology of host-seeking mosquitoes in irrigated agro-ecosystems of Clay County, Nebraska

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DIVERSITY AND ECOLOGY OF HOST-SEEKING MOSQUITOES IN IRRIGATED AGRO-ECOSYSTEMS OF CLAY COUNTY, NEBRASKA

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DIVERSITY AND ECOLOGY OF HOST-SEEKING MOSQUITOES IN IRRIGATED AGRO-ECOSYSTEMS IN CLAY COUNTY, NEBRASKA

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In the United States, Nebraska has the third highest incidence of human West Nile virus (WNV). Since WNV was first detected in the state in 2002, 3,422 confirmed cases and 57 deaths have been reported. Irrigated agro-ecosystems, which have been associated with elevated WNV incidences in other states, are prevalent in Nebraska. The objectives of this investigation were to 1) characterize mosquito abundance and diversity in irrigated agro-ecosystems, and 2) evaluate associations of two primary vectors of WNV, *Culex tarsalis* and *Culex pipiens*, with irrigation methods (sprinkler vs. surface) and crop type (corn vs. soybean). Investigations were conducted at South Central Agricultural Laboratory (SCAL) and privately owned operations in Clay County, Nebraska.

A total of 349,847 mosquitoes were collected during 2012 and 2013, representing 14 species and seven genera. The three most abundant species were *Aedes vexans* (53.7%), *Culex tarsalis* (37.6%) and *Culex pipiens* (2.4%). Other mosquitoes included *Anopheles punctipennis, Anopheles quadrimaculatus, Coquillettidia perturbans, Culiseta impatiens, Culiseta inornata, Ochlerotatus dorsalis, Ochlerotatus sollicitans, Ochlerotatus trivittatus, Psorophora ciliata , Psorophora columbiae,* and *Psorophora cyanescens*.

At SCAL, *Culex* abundance did not significantly differ between crop (P=0.11) or irrigation (P=0.98) types, but did significantly differ between years (P=<0.0001). A significant three-way interaction was detected for crop type, irrigation type, and year (F=9.76, P=0.0033) at privately owned fields. Significance was determined in the three-way interaction as a result between irrigation types and *Culex* mosquito abundance in corn fields in 2012 (mean= 52.45, P=0.0053); center pivot irrigated corn fields collected 52.5% fewer *Culex* mosquitoes than furrow surface irrigated corn fields. Field management practice differences, proximity of alternative larval developmental sites to study areas, and drought conditions during the study period potentially affected mosquito diversity and abundance. Continued surveillance during years with average or above average precipitation is recommended to adequately characterize adult mosquito populations in these systems.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Literature Review

Mosquito biology

Introduction

Mosquitoes belong to the Culicidae family in the order Diptera. Culicidae comprises two subfamilies: Anophelinae and Culicinae (Harbach 2007). Anophelinae includes three genera encompassing 467 species, while Culicinae is composed of 41 genera and over 3,000 species (Harbach 2007, Harbach and Howard 2007). Researchers are searching for isomorphic members of sibling species complexes, and if discovered, the number of recognized mosquito species may actually be three to five times what is currently recognized (Harbach 2007). Mosquitoes have been collected on all continents except Antarctica (Eldridge 2005), but approximately three-fourths of all species are found in the tropics and subtropics (Clements 1992).

Dipterans are holometabolous with complete metamorphosis occurring through egg, 4 larval instars, pupal, and adult stages (Eldridge 2005). Immature mosquitoes are anatomically different than adults, live in habitats dissimilar to adults, and require different sources of nutrition (Clements 1992).

Egg stage

All mosquito larvae are aquatic. Gravid females oviposit between 50 and several hundred eggs at a time, depending on species (Clements 1992). *Aedes* and *Ochlerotatus* females oviposit singly, typically directly above the waterline in moist substrates, and are

often resistant to desiccation (Eldridge 2005). As precipitation increases and water levels rise, eggs are submerged and a decrease in the concentration of atmospheric oxygen stimulates hatching (Horsfall 1956). Alternatively, most Culicines deposit eggs directly on the surface of water and form egg "rafts" that are suspended by surface tension (Eldridge 2005) while Anophelines oviposit individual eggs on the surface of water. Characteristic lateral floats allow eggs to float (Eldridge 2005). Larvae hatch within one to seven days, depending primarily on water temperature (Clements 1992). Anopheline and most Culicine eggs are not desiccation-resistant and are oviposited on stagnant bodies of water (Clements 1992).

Larval stage

Mosquito larvae are easily recognized by their legless bodies, well-developed head capsule, modified mouth parts, antennae, wide thorax, anal papillae, and if present, an elongate respiratory siphon (Clements 1992, Harbach 2007). Larval developmental sites vary considerably and can include leaf axils, pitcher plants, and even crab-holes (Harbach 2007). Typical sites, however, are temporary or permanent shallow bodies of freshwater with little water movement (Clements 1992, Harbach 2007). Though most prefer freshwater, some species are adapted to brackish or saline waters in salt marshes or inland saline pools (Clements 1992).

Nearly all mosquito larvae must obtain atmospheric oxygen to breathe. Culicine mosquitoes possess a siphon, from which they suspend at the water surface due to surface tension. This positions their bodies in a downward angle (Clements 1992). Anopheline

mosquito larvae lack a siphon, or have a significantly reduced one, and lie parallel to the water surface for respiratory purposes (Clements 1992, Eldridge 2005). Palmate hairs that line the larval bodies act as floats and aid Anopheline larvae in maintaining contact with the water surface (Eldridge 2005). In some genera, few physiological adaptations allow larvae to obtain atmospheric oxygen without having to go to the surface. *Mansonia, Coquillettidia,* and some species of *Mimomyia* have modified siphons that pierce aquatic plants' vasculature for obtaining oxygen (Eldridge 2005, Harbach 2007). Several *Aedeomyia* spp. are unique in that they use enlarged antennae for respiration (Harbach 2007). Other species have enlarged anal papillae that retain a great concentration of trachea and appear to utilize dissolved oxygen for respiration (Harbach 2007).

Most mosquito larvae feed on suspended particulate matter (i.e. detritus, bacteria, diatoms, algae and other micro-organisms) by filtering water with modified mouth parts often referred to as "mouth brushes" (Clements 1992). Others have a set of modified mandibles which are adapted for a predatory/cannibalistic life style (e.g. *Toxorhynchites spp.*) (Clements 1992, Harbach 2007). Largely dependent on food availability and water temperature, larvae can develop from first instar to pupae within several days to a few weeks (Clements 1992).

Pupal stage

During the relatively short pupal stage, all adult features form within the pupal casing but are concealed (Clements 1992). Pupae are motile with an abdomen that has been modified for propulsion to avoid potential predators (Clements 1992). An air bubble

in the cephalothorax region of the pupal body maintains buoyancy and assists pupae in maintaining contact with the water surface (Clements 1992). Respiration occurs through "respiratory trumpets" or "prothoracic horns" in which the mesothoracic spiracles lie (Clements 1992). Hydrophobic properties surrounding the respiratory trumpets break the surface layer, allowing the exchange of carbon dioxide and atmospheric oxygen (Clements 1992). The pupal stage lasts 1-14 days depending on water temperature.

Adult stage

Adult mosquitoes are characterized by elongate bodies, long delicate legs, one pair of flight wings, one pair of modified wings used for flight control and orientation (halteres), elongated mouthparts (proboscis), and scales present on wing veins and margins, and usually on the body (Clements 1992, Triplehorn and Johnson 2005). Males are distinguished from females by large antennae and by their non-blood feeding habits. Large antennae are used in detection of conspecific females (Clements 1992). The proboscis is utilized to feed on nectar as the initial energy source for hematophagous females and the sole energy source for males (Clements 1992). Most female mosquitoes are hematophagous (with the exception of *Toxorhychites* spp.) and are anautogenous, requiring blood meals to obtain protein for egg development (Clements 1992). Autogenous females are capable of developing eggs from larval reserves (Clements 1992). Most autogenous species, however, are facultatively autogenous (except *Wyeomyia smithii* (Coquillett)), in that after initial oviposition, subsequent egg development requires a blood meal (Clements 1992, Attardo et al. 2005).

Blood meals can be digested within hours or up to several days. Amino acids derived from blood proteins are incorporated to the mosquito's fat body, and synthesized to proteinaceous yolk (Clements 1992). Yolk is then transported to the ovaries and integrated into the oocytes (Clements 1992). The number of eggs produced depends on the amount of yolk produced per blood meal (Clements 1992, Attardo et al. 2005). Females do not continuously produce eggs; they produce one batch of eggs per blood meal (gonotrophic concordance) (Clements 1992, Attardo et al. 2005).

Adult mosquito host-seeking behavior

Mosquito host-seeking activity usually occurs during distinct time intervals in relation to sunset and sunrise, suggesting the behavior is controlled by a circadian rhythm (Clements 1999). When a blood meal is required, females disperse from refuge in search of a suitable host. After encountering wind-borne olfactory stimuli, females fly upwind in search of a host. Short-range cues then influence subsequent landing and probing (Clements 1999). Mosquito attraction to a vertebrate host requires a variety of chemical, visual, and physical cues. Host specificity is shown by many species and, therefore, responsiveness to host cues is widely varied between mosquito species (Clements 1999). Host-seeking behavior is modified by changes in olfactory receptor sensitivity as age, reproductive status, and diapause status changes (Bowen 1991).

Chemical cues

Kairomones, chemicals emitted from hosts, elicit a range of mosquito responses (Clements 1999). Kairomones may originate from respiration, epidermal secretions,

bacterial decomposition byproducts, flatus, and urinary and fecal contaminants (Clements 1999). Chemicals that elicit host-seeking behavior include, but are not limited to, carbon dioxide (CO_2), L-lactic acid, water vapor, and 1-octen-3-ol (octenol), (Bowen 1991, Clements 1999). Little is known about bird-derived kairomones that attract ornithophagous mosquitoes. It has been proposed that the uropygial (preen) gland found at the base of tail feathers may play a critical role in mosquito attraction (Clements 1999). Individual chemicals emitted by hosts may not elicit a strong host-seeking response, but may need to be presented in a mixture of chemicals to induce accurate behaviors (Bowen 1991, Clements 1999). Females have up to eight types of sensilla on the maxillary palpi and antennae that are used for mechanoreception, thermoreception, and olfaction (McIver 1978, Bowen 1991). Female mosquitoes are able to detect changes in CO_2 concentrations at 0.01% and are saturated by CO_2 concentrations above 4.0% (Bowen 1991).

Visual and physical cues

Mosquito response to visual cues is not understood as well as it is for other hematophagous dipterans (e.g. tsetse flies) (Clements 1999). Some researchers believe that mosquitoes use visual and chemical cues to determine speed and direction during host-seeking (Takken and Kline 1989, Bowen 1991). The use of visual cues during night host-seeking is still not well understood. Potentially, visual contrast and host movements augment other cues in short range orientation and host alighting (Sippel and Brown 1953, Clements 1999).

Other cues for host-seeking mosquitoes include temperature and moisture of the vertebrate host (Clements 1999). There is significant evidence showing that convection currents arising from warm-blooded hosts are an important cue for mosquitoes alighting on hosts (Bowen 1991, Clements 1999). Warm and moist convection currents are present in a "boundary layer" around the body of warm-blooded hosts (Clements 1999). These convection currents alone do not provide sufficient stimuli for descent and alighting of some mosquito species; however, convection currents carry many of the chemical cues that attract host-seeking mosquitoes (Clements 1999).

Mosquitoes and pathogens

Over the last century, mosquitoes earned the distinction as "most significant" arthropod of medical and veterinary importance (Durden and Mullen 2009). Vectors can be categorized as either a primary or secondary vectors (Clements 2012). Primary vectors are described as a prevalent species capable of sustaining transmission of a particular pathogen (Clements 2012). Secondary vectors are described as incidental, local, or bridge (Clements 2012). Incidental vectors are unable to maintain endemicity by itself, but can enhance transmission where it is sympatric with a main vector. Local vectors have a limited geographical range and possess sufficient vectorial capacity to preserve local endemicity without a main vector. Finally, bridge vectors can transmit an arthropod-borne virus (arbovirus) from an amplifying host to a "dead-end" or accidental host (e.g. *Culex spp.* transmit West Nile virus from bird host to humans). Dead-end hosts do not produce adequate parasitemia or viremia to perpetuate the pathogen transmission cycle

(Clements 2012). In order to assess presumed vectors of disease-causing pathogens, vector competence and vectorial capacity should be determined when possible (Clements 2012).

Due to a lack of vector competence, not all mosquitoes transmit pathogens that are ingested with the blood meal. Vector competence is dependent on the mosquito acquiring an infectious agent from a blood meal, replication of the infectious agent within the host, and transmission of the infectious agent through salivary secretions or fecal deposits during subsequent feedings (Reisen 2009, Clements 2012). Vectorial capacity is expressed as $C=ma^2(P^n)V/(-\ln P)$, where C = vectorial capacity as new infections per infection per day, m= bites per human per day, a= human biting habit, P= probability of daily survival, n= extrinsic incubation period in days, and V= vector competence (Reisen, 2009). This equation provides a checklist of key transmission components and can be utilized as an indirect method of measuring transmission rate by a vector population (Dye, 1992).

Often researchers will perform laboratory tests on potential vectors of an emerging disease. Much like Robert Koch's postulates, mosquitoes considered primary vector candidates should meet the following criteria: "(1) the mosquito and vertebrate hosts of the pathogen co-occur spatially and temporally; (2) the mosquitoes preferentially feed on an amplifying host species; (3) the pathogen has been isolated from wild populations of mosquitoes; (4) there is a high mosquito to amplifying host ratio; (5) in the laboratory, mosquitoes are readily susceptible to the pathogen and transmit it effectively; (6) the lifespan of wild females is longer than the extrinsic incubation period (time it

takes for pathogen to complete its development and able to be propagated in subsequent blood meals) of the pathogen (Clements 2012).

Potential vectors must be in the proper life stage for feeding on vertebrate hosts, at the right geographic location, and feed at the right time. The pathogen must go through its intrinsic incubation period in the host (period during which viruses develop significant titers in vertebrate host's blood and can infect mosquitoes via a blood meal) (Clements 2012). In the mosquito host, pathogens must overcome the mosquito's intrinsic barriers. One barrier is the midgut peritrophic membrane. Formation of peritrophic membrane is may require several hours to complete (Clements 1992). Functions associated with the peritrophic membrane in hematophagous insects include protection of the midgut epithelium from hematin crystals that are formed by partially digested blood and mechanically damage cells, and prevention of pathogen invasion from blood meals (Clements 1992).

Midgut epithelium infection and midgut escape barriers are major determinants of WNV vector competence (Turell et al. 2005, Blair 2009). If a virus, such as WNV, is able to avoid the peritrophic membrane, it may then bind to receptors on the midgut epithelial cells (Blair 2009). Once the virus has utilized the mosquito's cellular machinery for replication, the virus must then pass through the basal lamina for further dispersion within the mosquito (Blair 2009).

Upon dissemination from the midgut, WNV can infect and replicate in several tissues including the hemocytes, fat body, nervous system, and salivary glands (Blair

2009). In salivary gland tissues, WNV continues to amplify and is exuded, along with salivary proteins and other constituents, into the lumen for transmission during the next blood meal (Blair 2009). The extrinsic incubation period is directly influenced by initial virus dose, environmental temperatures, and mosquito and virus genetics (Reisen et al. 2006). In studies done by Styer et al. (2007) and Reisen et al. (2007) it was established that probing mosquitoes can inoculate mean doses of WNV of 10^{2}-$10^{4.2}$ Plaque Forming Units (PFU)/ml^{-1} directly into the blood stream of vertebrate hosts.

Zoonotic pathogens and mosquitoes

Transmission of zoonotic pathogens via a mosquito vector is dependent on intrinsic factors such as vector competence, feeding behavior, and mosquito life span, and extrinsic factors such as larval nutrition, temperature, precipitation, and host immunity (Kramer and Ebel 2003, Kilpatrick et al. 2006, Blair 2009). These factors are essential in determining when epidemics are likely to occur and the force of infection (Kramer and Ebel 2003, Kilpatrick et al. 2006, Blair 2009). A vital step in determining when an epidemic is likely to occur and how to mitigate infection is to establish host preferences of main vectors and how they shift spatially and temporally (Kilpatrick et al. 2006).

West Nile Virus

West Nile virus history

West Nile virus is a member of the virus family Flaviviridae and is a member of the Japanese encephalitis virus serogroup of the genus *Flavivirus* (Clements 2012). The

genus *Flavivirus*, consists of over 70 viruses, with many being arthropod-borne viruses (arboviruses) (Clements 2012). Medically important arboviruses, from the genus *Flavivirus*, include West Nile virus (WNV), St. Louis encephalitis virus (SLEV), Dengue virus (DENV), Yellow Fever virus (YFV), and Japanese encephalitis virus (JEV) (Clements 2012).

West Nile virus was first isolated from a febrile woman in the West Nile district of Uganda in 1937 (Smithburn et al. 1940). Throughout the 1940's and 1950's, WNV was isolated from humans in the Old World and was indicated as a causative agent of severe human neurologic infections (Spigland et al. 1958, Hayes 2001, Komar 2003). By the early 1960's, human epidemics were taking place throughout the Middle East and Africa and the first WNV induced equine encephalitis cases were noted in Egypt (Schmidt and El Mansoury 1963, Komar 2003). In 1974, the largest single season outbreak occurred in South Africa with approximately 10,000 cases of encephalitis (Jupp 2001, Komar 2003). From 1996 to 1999 there three major epidemics (Hayes 2001) occurring in large urban areas to include: Volgograd, Russia, Bucharest, Romania, and New York City, New York, USA (Han et al. 1999, Platanov et al. 2001, Hayes 2001).

West Nile virus in North America

Currently there are five lineages of WNV (Lineage 1-5) that are accepted with the majority of strains belonging to Lineage 1 (Clements 2012). The WNV strain that was initially isolated in North America was >99.8% homologous with an isolate extracted from a dead goose in Israel in 1998 (Hayes 2001). This implies that the origin of the

initial North American strain (NY99) originated from the Middle East/Mediterranean region (Hayes 2001).

West Nile virus's arrival in New York City in 1999 marked the first confirmed case of WNV in the Western Hemisphere (Hayes 2001). It is unclear how WNV was transported, but potentially, infected wild migratory bird populations served as the vehicle for dissemination throughout the Old World (Clements 2012). Alternatively, the introduction of an infected mosquito or a tick that hitch-hiked on a commercial airliner, an infected bird brought through the illegal pet trade, or migratory birds (Lanciotti et al. 1999, Rappole et al. 2000).

The first confirmed human case of WNV in the Western Hemisphere occurred in mid-August, 1999 (Lanciotti et al. 1999, Asnis et al. 2000, Rappole et al. 2000). In late August 1999, five patients were treated for a neurologic illness exhibiting symptoms of apparent encephalitis (Marra et al. 2004). Based upon symptoms and the majority of patients being elderly, infectious disease specialists of Flushing Medical Center, Queens, New York, suspected a mosquito-borne virus as the causative agent (Marra et al. 2004). From August-October 1999, 62 cases of human disease were confirmed in the northeastern United States with 70% in the borough of Queens (Rappole et al. 2000). Patients were initially misdiagnosed as having St. Louis encephalitis virus (SLEV) due to its close relation and cross-reactivity to SLEV immunoassays (Marra et al. 2004). Patients were tested for WNV once massive bird die-offs were reported in the area and correctly diagnosed (Anderson et al. 1999, Rappole et al. 2000, Marra et al. 2004).

West Nile virus spread across most of the continental United States and many provinces of Canada by 2003. Several dispersal agents may have been involved the proliferation, including infected migratory birds and local bird populations, dispersing mosquitoes, and possible human-mediated mosquito movement (Marra et al. 2004; Goldberg et al. 2010, Venkatesan and Rasgon 2010). Viremic migratory birds are considered the most likely main dispersal agent of WNV due to the propensity for mosquitoes to stay near larval developmental sites and being weak fliers (Marra et al. 2004).

United States West Nile virus statistics

According to the CDC between 1999 and 2012, 16,196 neuroinvasive disease cases have been reported resulting in 1,443 deaths (9%), 20,892 non-neuroinvasive disease cases resulting in 106 deaths (1%), totaling 37,088 cases and 1,549 deaths (4%) (CDC 2013a). Preliminary data for 2013 United States' cases include 1,205 neuroinvasive disease, 1,169 non-neuroinvasive disease, 420 presumptive viremic blood donors, and 114 total deaths (CDC 2014).

North American mosquito vectors

According to the CDC (2013b), from 1999-2012, 65 species of mosquitoes in the United States have been found to be infected with WNV. Of the 65 species identified, 34 have been collected in Nebraska (Darsie and Ward 2005, CDC 2013b). Though mosquito species test positive for WNV infection, most are unable to continue the transmission cycle (Turell et al. 2005). If a mosquito is able to transmit WNV in a laboratory setting it

doesn't necessarily mean that it will play a significant part in transmission under natural conditions. Laboratory conditions may alter the mosquito's natural feeding behavior and do not mirror host-feeding preference in the field (Turell et al. 2005). For example, Turell and colleagues (2005) demonstrated that *Psorophora ferox* (Humbolt) developed disseminated infections of WNV under laboratory conditions, however, when six were tested for transmission via blood-feeding, none transmitted the virus to naïve chickens.

Since WNV is maintained in a bird-mosquito-bird enzootic cycle, several ornithophilic *Culex* spp. have been implicated as primary and secondary vectors (Turell et al. 2005). Ornithophilic mosquitoes tend to be the most proficient vectors and include *Culex nigripalpus* Theobald, *Cx. pipiens pipiens* L., *Cx. tarsalis* Coquillett, *Cx. quinquefasciatus* Say, *Cx.restuans* Theobald, and *Cx. salinarius* Coquillett (Turell et al. 2005, Kilpatrick et al. 2005). Some ornithophilic mosquitoes such as *Cx. tarsalis* and *Cx. nigripalpus* undergo a host-shift during the summer and transition from bird to mammal feedings (Tempelis et al. 1967, Kilpatrick et al. 2006). In spring and early summer these mosquitoes serve as amplification vectors and later summer serve as bridge vectors to mammals including humans and horses (Turell et al. 2005).

Other mosquitoes that have been implicated as potential bridge vectors include *Ochlerotatus canadensis canadensis* (Theobald), *Oc. cantator* (Coquillett), *Oc. triseriatus* (Say), and *Aedes vexans* (Meigan) (Turell et al. 2005). *Aedes vexans* may potentially be an important bridge vector of WNV due to high population densities (Janousek and Kramer 1999, Turell et al. 2005) and its preference for feeding on

mammals (Turell et al. 2005, Molaei and Andreadis 2006); however, its limited preference for feeding on birds (<10% of blood meals), an assumed criterion for acquirement of the virus because birds are amplifying and reservoir hosts, has been suggested as a significant aspect that decreases its vectorial capacity (Andreadis et al. 2001, Molaei and Andreadis 2006).

Mosquitoes acquire WNV through two modes of transmission: horizontal and vertical. Horizontal transmission is the most common transmission method and occurs when a vector competent mosquito imbibes a blood meal from an amplifying vertebrate host that has an adequate viremic titer (approximately $10^{5.0}$-$10^{7.0}$ PFU/ml^{-1} species dependent) (Komar 2003, Hayes et al. 2005, Blair 2009, Clements 2012). Some North American birds have expressed viremic titers as high as $10^{12.6}$ PFU/ml^{-1} which enable marginal vectors to become infected (Komar et al. 2003, Blair 2009). Once a female mosquito acquires a systemic WNV infection, she has the potential to transmit virus to new vertebrate hosts upon subsequent feedings for the rest of her life.

Vertical transmission of WNV is the transference of the virus from one generation to the next. The mechanism of vertical transmission of flaviviruses is thought to involve virus entry into fully formed eggs during oviposition (Rosen 1988, Miller et al. 2000). This form of transmission is less common than horizontal transmission, but is thought to be a possible mechanism to supplement horizontal transmission during warmer months and for virus to persist in a location during winter months when mosquito activity is low (Miller et al. 2000, Goddard et al. 2003). West Nile virus vertical transmission was first

described in 1993 when *Aedes aegypti* L., *Aedes albopictus* (Skuse), and *Culex tritaeniorhynchus* (Wiedemann) were experimentally inoculated with WNV (Baqar et al. 1993). Natural vertical transmission has been discovered in male *Culex univittatus* complex mosquitoes in Africa (Miller et al. 2000) and in overwintering, unfed, female *Culex pipiens* in New York City (Nasci et al. 2001). Vertical transmission has been demonstrated in the laboratory for North American *Cx. pipiens* (Dohm et al. 2002b), *Cx. tarsalis*, and *Cx. quinquefasciatus* (Goddard et al. 2003).

North American avian reservoir hosts of West Nile virus

Since WNV's introduction into North America in 1999, human cases have been reported every year in the United States (Kilpatrick et al 2006, CDC 2013a). There have been more reported epidemics in North America, and of a greater magnitude than those reported in the Old World, with 2,000-10,000 cases reported in North America and <400 cases reported in Europe during epidemic years (Hubalek and Halouzka 1999, Kilpatrick et al. 2006).

In many Old World WNV strains, virulence to many native avian species has been low; however, in North America, WNV strains are highly virulent to many native avian species (Clements 2012). Of the 330 avian species that have been WNV-positive in the United States from 1999-2012, 272 are native (Clements 2012, CDC 2013c). Bird species naturally infected come from the orders Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, Galliformes, Strigiformes, Phoenicopteriformes,

Falconiformes, and Passeriformes (Steele et al. 2000, Swayne et al. 2000, Komar et al. 2003, Hayes et al. 2005, McLean 2006).

Komar et al. (2003) determined WNV viremia profiles of 25 bird species, representing 17 families and 10 orders. WNV viremias in Passeriformes and Charadriiformes were greater in magnitude and longer in duration than the other eight orders represented. Elevated WNV viremias in passerine birds, especially Corvididaes, are considered important factors in the transmission cycle to *Culex* mosquito vectors and are consistent with the role of this family of birds in the transmission cycles of other related flaviviruses (Komar et al. 2003, Clements 2012).

The prevalence of WNV in vector mosquitoes is positively correlated with human density and avian species community competence index, and negatively correlated with avian species diversity (Allan et al. 2009, Clements 2012). Potentially, the loss of bird diversity that accompanies urbanization contributes to intensifying WNV human infection rates due to a reduction in the "dilution effect" (Allan et al. 2009, Clements 2012).

Non-avian West Nile virus hosts

West Nile virus is unique among flaviviruses in North America due to a large potential range of hosts that can be infected (Marra et al. 2004). This virus has been reported to have infected 29 species of mammals throughout North America, including marsupials, rodents, carnivores, bats, equids, and humans (Marra et al. 2004, Clements 2012). Fox squirrels (*Sciurus niger*), eastern chipmunks (*Tamias striatus*), and eastern

cottontail rabbits (*Sylvilagus floridianus*) have been implicated as potential amplifying hosts for WNV with viremias sufficient to infect competent mosquito vectors (Platt et al. 2007, Platt et al. 2008, Tiawsirisup et al. 2005, Clements 2012).

The American alligator (*Alligator mississippiensis*) (Jacobson et al. 2005) and a Russian frog (*Rana ridibunda*) (Marra et al. 2004) produce viremic titers ($10^{5.0}$ PFU/ml^{-1}) significant enough to infect *Cx. pipiens* and *Cx. quinquefasciatus* (Sardelis et al. 2001). Additional research on reptiles and amphibians in WNV endemic areas should be carried out to determine their importance as possible WNV amplifying hosts.

Transmission of WNV by ingestion has been demonstrated. The ingestion of mosquitoes infected with WNV by the house sparrow (*Passer domesticus*) and by mice showed subsequent infection in both vertebrates (Komar et al. 2003, Marra et al. 2004). Two insectivorous North American bats, little brown bat (*Myotis lucifagus*) and northern long-eared bat (*M. septentrionalis*) are believed to have become viremic after ingesting infected mosquitoes (Pilipski et al. 2004, Clements 2012). Another form of non-mosquito-borne WNV transmission is through close contact, including oral-fecal routes or allopreening (Komar et al. 2003, Marra et al. 2004, Hayes et al. 2005).

Most mammals, including humans, are dead-end hosts for WNV. It is thought that arboviruses tend to be more virulent in dead-end hosts (Clements 2012). In Old World equine cases, infections were typically mild or asymptomatic infections, and only occasionally severe (Bunning et al. 2002). However, in North America during 1999 outbreak, high equine death rates were recorded due to equine encephalomyelitis

(Bunning et al. 2002). In a survey of horses near Riverhead, NY, 36 out of 83 horses sampled tested seropositive for WNV and eight of the horses died from infection (Bunning et al. 2002). Though horses can develop severe illness from WNV infection they are still considered dead-end hosts.

Several equine vaccines are available. Three main vaccine types have proven very efficacious and include a vaccine that uses inactivated WNV, a modified-live vaccine containing the WNV prM and E proteins expressed by a canarypox virus vector, and the third is a live-chimera vaccine containing WNV prM and E proteins yellow fever vector (YF17D) (Seino et al. 2007).

Like horses, humans are susceptible to infection with virulent strains of WNV and are dead-end hosts due to the development of low virus titers within the blood ($<10^{3.2}$ PFU/ml^{-1}) (Hayes et al. 2005). Those predisposed to severe WNV infection tend to be >50 years of age and many have pre-existing conditions such as diabetes, hypertension, and/or a weakened immune system (Nash et al. 2001; Weiss et al. 2001). Intrinsic incubation period in humans ranges from three to 15 days (Hubalek and Halouzka 1999; Nash et al. 2001) and approximately one out of 100 who are infected with WNV will develop neuroinvasive disease (Olejnik 1952; Nash et al. 2001). Those with neuroinvasive disease can show symptoms of fever, nausea, vomiting, headache, stiff neck, altered mental status, meningitis, encephalitis, rash on upper body, and in the most severe cases can ultimately result in flaccid paralysis, coma, and death (Nash et al. 2001; Weiss et al. 2001). Long-term sequelae reported from months to years post-infection are

characterized by persistent symptoms such as fatigue, headache, myalgias, gait and movement disorders, loss of limb functions and strength, and mental deficits (Murray et al. 2011). No human vaccine is available to date.

Non-mosquito-borne human WNV transmission may occur through blood transfusions (Pealer et al. 2003), organ transplantation (Iwamoto et al. 2003), transplacentally (Alpert et al. 2003), through breast milk (CDC 2002a), via laboratory accidental inoculations (CDC 2002b), and through aerosolization at a bird production facility (CDC 2003, Hayes and Gubler 2006).

Climatic events and West Nile virus epidemics

In North America, epidemics of WNV have been associated with mild winters followed by dry spring and summer months (Epstein and Defillipo 2001, Marra et al. 2004). In such years it is proposed that a "watering hole effect" takes place where birds may gather around limited water sources where mosquitoes may develop, and consequently increasing the rate of WNV transmission within susceptible bird and mosquito populations (Marra et al. 2004). Drought conditions may also kill off mosquito predators such as lacewings, ladybird beetles, dragonflies, and frogs, while also concentrating surface pools with organic material, which is optimal for larval development in several competent mosquito species (Marra et al. 2004).

Though drought conditions may lead to higher WNV transmission rates, prolonged drought and very high temperatures can negatively impact mosquito populations. When high temperatures average >30°C in July and August, in northern

latitudes, decreases in mosquito abundance are often observed due to larval and adult mortality (Reisen et al. 1992, Reisen 1995). Additionally, extended periods of drought can be detrimental to standing-water mosquito populations by ultimately reducing larval habitat availability.

Other climatic scenarios may also predict when WNV epidemics may take place. One such scenario is when a region experiences a wet spring followed by hot, dry summers (as experienced in 1999 in the New York City area and 2003 in Colorado) (Marra et al. 2004). These conditions were conducive to higher than normal mosquito numbers and maximized rates of WNV replication within mosquitoes due to warmer temperatures (Dohm et al. 2002a, Marra et al. 2004, Reisen et al. 2006) and led to shortened transmission and amplification cycles (Marra et al. 2004). Lastly, significant late summer rains can lead to increased amplification due to availability of ovipositioning sites (Marra et al. 2004).

Local land-use patterns can also affect vector population dynamics and vector-host interactions (Marra et al. 2004). Water management strategies can influence where and when suitable vector developmental sites arise by influencing the size and number of aquatic habitats (Marra et al. 2004). Often irrigation practices have been implicated as a main culprit in the transmission cycle of mosquito-borne diseases throughout the world (Herrel et al. 2004, Miramontes et al. 2006, Wimberly et al. 2008, Gates and Boston 2009, Sugumaran et al. 2009, Eisen et al. 2010, Jaleta et al. 2013).

Agricultural land-use practices and West Nile virus

Irrigation types

Center pivot irrigation is the most common form of irrigation in the state of Nebraska (Johnson et al. 2011, UNL 2014). It is characterized by a single sprinkler lateral (hollow pipe configuration), anchored at one end to a fixed pivot structure, where the well pump is located, while the other end moves around the pivot point (Pair et al. 1975). The sprinkler lateral is supported by towers or trusses, which are usually 24 m to 76 m apart, and typically move on wheels (Pair et al. 1975). Laterals have an alignment system, with mechanisms mounted on each support tower, and ensure that the system moves together and avoids damage (Pair et al. 1975). These arms can vary in length from 61 to 792 m (Pair et al. 1975). Typically, laterals are 402 m long and can cover approximately 133 acres (Nutt-Powell and Landers 1979). Center pivots can complete one cycle in as few as 12 hours, but most take three to four days (Nutt-Powell and Landers 1979).

Advantages of center pivot versus surface irrigation include: relatively low labor costs (mostly automated), high adaptability for use on certain soils and landscapes, increased utilization of acreage, increased yield, and the capability of watering high intake soils efficiently, thus, reducing runoff or deep percolation (IHD 1972). Disadvantages include relatively high initial costs, increased maintenance costs, increased energy costs, and require operational skills different from surface irrigation practices (IHD 1972).

Linear-move sprinkler irrigation is very similar in principle and equipment to center pivot irrigation. These assemblies travel in a straight line path versus a circular path and require flexible "drag hoses" that connect to underground water piping for a water source (CU 2014). A major benefit of linear-move irrigation is that it can cover all acreage of a square or rectangular field, whereas, a center pivot cannot usually irrigate the corner acres (CU 2014).

Surface irrigation is the second most common irrigation method in Nebraska (UNL 2014, Johnson et al. 2011) and refers to the addition of water to land by overland flow (Walker 1989). The principle behind surface irrigation is to start flow at one end of the field and allow for the water to move down a gradient, usually through gravitational forces (Walker 1989). This method of irrigation may make up as much as 95% of the common irrigation today (Walker 1989). There are three main types of surface irrigation utilized and include: level basin, border strip, and furrow (Burt 1995).

In Nebraska, furrow irrigation is the predominant type of surface irrigation practiced (UNL 2014). Furrow irrigation is characterized by small ditches or trenches (furrows), in between rows, which channel the water applied (Burt 1995). In Clay County, irrigation wells are used to pump either ground water or recycled water from reuse pits, through large diameter metal pipes, and out to the fields. As water moves down the field through gravity, water is slowly absorbed by the soil, infiltrating the root zone (Burt 1995). Advantages of surface irrigation over sprinkler irrigation and include a "lower initial investment cost and lower pumping costs per acre-inch of water pumped"

(UNL 2014). Disadvantages include increased labor costs to setup piping and forming the furrows, and lower application efficiency when compared to sprinkler and sub-surface drip irrigation (UNL 2014). Many Nebraskan farmers are converting from surface irrigation to center pivot irrigation and has resulted in a nearly one million acre decrease in furrow irrigated acreage over the last decade (UNL 2014).

Water runoff is a characteristic of typical furrow irrigation systems in the United States, but is needed for even distribution of water infiltration along the furrow and for flexible management (Burt 1995). Nearly half of the total time of irrigation will result in runoff, but can increase irrigation efficiency of sloping furrows if it is collected and reused (e.g. reuse pits) (Burt 1995). However, if runoff is not collected and reused, it may pool along field borders or in roadside ditches. This can contribute to mosquito larval habitat production and overall mosquito abundance in these areas that experience poor field management practices.

Due to current and expected limitation on water resources, throughout the United States, alternative methods than sprinkler and surface irrigation, to irrigate row crops are gaining much interest (Payero et al. 2008). Subsurface drip irrigation (SDI) is one of these alternative methods. Subsurface drip irrigation provides water directly to the crop root zone through tubing called a dripline (Payero et al. 2005). Dripline vary in diameter depending on length of field irrigated (Payero et al. 2005). Diameter of the dripline corresponds with the length of field to be irrigated meaning the longer the field, the larger the diameter of dripline. This ensures uniform water distribution (Payero et al. 2005).

Small holes, spaced evenly along the length of driplines, allow for water to escape and pressure in the tubes determines the rate at which water escapes (Payero et al. 2005).

Driplines are usually buried every other row and buried at a depth optimal for crop root zone watering. This allows for the soil to accept naturally occurring precipitation events and for soil surface to remain dry resulting in nearly no water loss to evaporation or runoff (Payero et al. 2005). If properly managed, SDI can deliver water with an efficiency of 95% (Payero et al. 2005). Advantages include increased water efficiency, reduced water costs, reduced energy costs, decreased manual labor once installed due to automation, allows for injections of chemical, and has potential for increased yield (crop dependent) (Payero et al. 2005). Disadvantages include limitations in rolling or hilly terrain due to pressure differentials in driplines, investment costs are nearly double center pivot per acre, limitations in length of dripline and, therefore, limited field size, and management and maintenance time is more than other irrigation methods (Payero et al. 2005).

Northern Great Plains and WNV

Northern Great Plains states of Colorado, Nebraska, North Dakota, South Dakota, and Wyoming has a combined 2.9% of the total United States population (USCB 2010) and have extremely high WNV case rates (CDC 2014). Colorado had the greatest number of human WNV infections, from 1999-2012, with 4,672 confirmed cases (CDC 2014). From data derived from the 2010 United States Census, and WNV cases reported per state, the top five states that reported WNV cases from 1999-2012 per 100,000 people are

as follows: 1. South Dakota (240.98), 2. North Dakota (206.07), 3. Nebraska (170.94), 4. Wyoming (120.82), and 5. Colorado (92.90) (CDC 2014).

An explanation as to why this sparsely populated area of the country experiences high case rates of WNV may be due to differences in human behavior between those in rural, agricultural settings versus those in urban/suburban settings. Those in rural areas may be at higher risk because they may spend more time outdoors, may have a decreased attention to public health control measures, and/or may have increased exposure to irrigated land. Underlying differences between human populations, rural versus urban, may need to be studied further to help in refining WNV risk models (Gates and Boston 2009).

States and counties with the greatest cumulative incidences are located mostly in the northern Great Plains states including South Dakota, North Dakota, Wyoming, Nebraska, and Colorado (Lindsey et al. 2008). Based on the cases reported to CDC's ArboNET, an internet database that tracks suspected and confirmed cases of arboviruses in the US, from 2002-2013 these five states still remain at the top of the list of incidences per 100,000 population (CDC 2014).

According to the 2008 Farm and Ranch Irrigation Survey, which expands on the basic irrigation data collected in the 2007 Census of Agriculture, United States' farmers and ranchers irrigated 54.9 million acres, an increase of 2.4 million acres from 2003 (USDA 2012). Acreage irrigated by sprinkler systems (center pivot or linear overhead)

was estimated at nearly 31 million acres and the area irrigated with gravity systems (surface flooding or furrow) was approximately 22 million acres (USDA 2012).

Nebraska has more than 107,000 registered irrigation wells and an additional 16,000 registered water wells (Johnson and Lukassen 2009, Johnson et al. 2011). As of 2008, 80.38% of irrigated acres in Nebraska used sprinkler systems, 19.96% used gravity systems, and 0.05% used drips systems or other forms of irrigation (Johnson et al. 2011, USDA 2012). Of the sprinkler systems used, 98% or ~55,000 are center pivot and irrigate nearly 6.7 million acres (Johnson et al. 2011, USDA 2012). The highest density of wells is located in the Central Platte Valley with densities often >16 wells per square mile (UNL 2014). Due to the extensive use of center pivot systems, and in particular low-pressure systems (under 30 psi) which equate to 40% of the center pivot systems, Nebraska is considered as a state on the leading edge of efficient water resource management (Johnson et al. 2011, UNL 2014).

As of 2007, Nebraska had 8.56 million acres and has earned the distinction of being the most irrigated state in the United States (Johnson et al. 2011, USDA 2012). Between 2002 and 2007, Nebraska added over 934,000 irrigated acres while most other states declined (Johnson et al. 2011, USDA 2012). Nebraska irrigates approximately 565,000 of the 8.56 million irrigated acres utilize surface water diverted from streams and rivers (UNL 2014). Most other irrigated acres utilize ground water from the Ogallala Aquifer (UNL 2014).

There have been many studies linking agricultural practices to an increase in WNV risk and disease incidences in North America. Studies utilizing spatial and temporal clustering of WNV cases, while also incorporating variables such as agricultural land-use, and environmental and demographic factors, address potential associations to WNV risk (Miramontes et al. 2006, DeGroote et al. 2008, Wimberly et al. 2008, Sugumaran et al. 2009, Eisen et al. 2010, Bowden et al. 2011). Spatial distribution and abundance of vector mosquitoes, such as *Cx. tarsalis*, is related to climatic conditions and suitable mosquito larval developmental sites (Eisen and Eisen 2007). Spatial patterns of abundance of *Cx. tarsalis* are varied and assessments of probable WNV exposure sites are complicated by insufficient knowledge of the fine-scale spatial distribution (Eisen and Eisen 2007). Studies should be refined from a state or county spatial unit for presentation of incidence of vector-borne diseases to finer ZIP code or census tract scales (typically 1,500-8,000 persons) (Eisen and Eisen 2007). Eisen et al. (2010) point out a need for research in determining the importance of different irrigation practices in production of vector mosquitoes, primarily *Cx. tarsalis*, on irrigated agricultural lands in the WNV endemic region of the northern Great Plains.

Mosquito sampling and surveillance

There are multiple ways in which mosquito populations can be sampled. Mosquito surveillance is an important component of a mosquito-borne disease surveillance program (Brown et al. 2008).

Larval sampling

Larvae may be sampled utilizing a larval dipper consisting of a wooden or aluminum pole with a cup attached to the end. Cups are typically 12.7 cm diameter and hold 350 ml. Sampling methods are not standardized and vary with landscape and preference. Methods include skimming the surface or scooping quickly at the water surface. Larvae collected can be quantified and identified to species or can be housed to allow for pupation and eventual eclosion (Silver 2008).

Adult mosquito sampling

The most common method for collecting adult, host-seeking females employs CDC miniature light traps. These traps are often baited with CO_2 and equipped with a small incandescent light bulb to increase capture rates (Clements 1992, Brown et al. 2008, Silver 2008). Mosquitoes are attracted to these traps through visual and chemical cues. The downdraft created by the trap fan, forces and keeps mosquitoes in the collection container. A resting box is used to collect blood-fed females. Resting boxes provide refuge for engorged females and allow a suitable habitat for digestion of the blood meal (Brust 1990). Relatively inactive females can then be collected from these boxes with an aspirator. Another trap type, used to collect gravid females, is the gravid trap. These traps consist of an updraft fan placed over a container that mimics natural oviposition sites (Brust 1990). Gravid traps are set up utilizing water with infusions of organic materials like Bermuda grass, hay, brewer's yeast, and steer manure (Brust 1990, Jackson et al. 2005). Byproducts from the breakdown of organic material in the infusions

are chemical cues used by mosquitoes to detect potential oviposition sites (Kramer and Mulla 1979, Jackson et al. 2005).

Though trapping is a key component in monitoring mosquito populations, standard techniques for interpreting the results of trapping are still deficient (Brown et al. 2008). Trap design, use of attractants, biotic and abiotic factors of trap location, and even trap placement height may introduce bias into mosquito abundance estimates and these biases must be accounted to have an efficient and practical surveillance program (Brown et al. 2008).

Entomological Risk Assessment

Kilpatrick et al. (2005) developed an equation to estimate the risk that a mosquito will infect a human with WNV. The equation:Risk=A x F_m x P x C_v, where A is mosquito abundance, F_m is the fraction of blood meals taken from mammals, P is WNV infection prevalence, and C_v is an index of vector competence (the fraction of WNV-infected mosquitoes that will transmit virus in a subsequent bite). The equation is utilized to estimate the relative number of WNV-infectious bites on mammals by each mosquito species (Kilpatrick et al. 2005).

The first step in evaluating entomological risk is by capturing adult female mosquitoes and determining abundance of primary and secondary vector species. Following methods of Bolling et al. (2009), seasonal patterns for abundance of the primary vectors of WNV, *Cx. tarsalis* Coquillett and *Cx. pipiens* L., was determined for Clay County, Nebraska during the summer months of 2012 and 2013. Further processing

of mosquitoes collected is needed to fully evaluate human risk of contracting WNV from specific mosquito species.

Objectives

West Nile virus is now endemic in North America and human cases continue to occur annually. Though many studies have examined factors associated with WNV at a large scale (e.g. county based statistics), studies examining factors at the field level have been limited. Agricultural practices, especially heavily irrigated lands, have been associated with increased risk and incidence rates of WNV, but investigation into specific irrigation types (center pivot and surface) and their contributions to vector mosquito species abundance are still needed.

To address these issues the objectives of this study are: 1) identify mosquito species and abundance in agricultural fields in Clay County, Nebraska; 2) determine how irrigation types influence WNV vector mosquitoes; 3) determine how crop types influence WNV vector mosquitoes.

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CHAPTER 2

Mosquito diversity and abundance in irrigated agro-ecosystems of Clay County, Nebraska, and entomological risk for human West Nile virus (Flaviviridae: *Flavivirus*) incidence

Abstract

In the United States, Nebraska has the third highest incidence of human West Nile virus (WNV). Since WNV was first detected in the state in 2002, 3,422 confirmed cases and 57 deaths have been reported. Irrigated agro-ecosystems, which have been associated with elevated WNV incidences in other states, are prevalent in Nebraska. The objectives of this investigation were to 1) characterize mosquito abundance and diversity in irrigated agro-ecosystems, and 2) evaluate associations of two primary vectors of WNV, *Culex tarsali*s and *Culex pipiens*, with irrigation methods (sprinkler vs. surface) and crop type (corn vs. soybean). Investigations were conducted at South Central Agricultural Laboratory (SCAL) and privately owned operations in Clay County, Nebraska.

A total of 349,847 mosquitoes were collected during 2012 and 2013, representing 14 species and seven genera. The three most abundant species were *Aedes vexans* (53.7%), *Culex tarsalis* (37.6%) and *Culex pipiens* (2.4%). Other mosquitoes included *Anopheles punctipennis, Anopheles quadrimaculatus, Coquillettidia perturbans, Culiseta impatiens, Culiseta inornata, Ochlerotatus dorsalis, Ochlerotatus sollicitans, Ochlerotatus trivittatus, Psorophora ciliata , Psorophora columbiae,* and *Psorophora cyanescens*.

At SCAL, *Culex* abundance did not significantly differ between crop (P=0.11) or irrigation (P=0.98) types, but did significantly differ between years (P=<0.0001). A significant three-way interaction was detected for crop type, irrigation type, and year (F=9.76, P=0.0033) at privately owned fields. Significance was determined in the three-way interaction as a result between irrigation types and *Culex* mosquito abundance in corn fields in 2012 (mean= 52.45, P=0.0053); center pivot irrigated corn fields collected 52.5% fewer *Culex* mosquitoes than furrow surface irrigated corn fields. Field management practice differences, proximity of alternative larval developmental sites to study areas, and drought conditions during the study period potentially affected mosquito diversity and abundance. Continued surveillance during years with average or above average precipitation is recommended to adequately characterize adult mosquito populations in these systems.

Introduction

West Nile virus (WNV) is a flavivirus initially isolated in 1937 from a woman in the West Nile District of Uganda (Smithburn et al. 1940). Since its initial isolation it has become the most widely dispersed arthropod-borne flavivirus in the world (Kauffman et al. 2011). West Nile virus is maintained in an enzootic cycle, primarily through transmission between viremic avian species and ornithophilic mosquitoes, mainly of the genus *Culex* (Kramer and Bernard 2001, Kilpatrick et al. 2006). The virus has been isolated from 65 mosquito species and 272 native avian species in the United States (CDC 2013) suggesting that WNV is an ecological generalist, when compared to other arboviruses, and may explain its sizable geographic range (Bowden et al. 2011).

In 1999, WNV was isolated in the Western Hemisphere for the first time (Han et al. 1999, Platanov et al. 2001, Hayes 2001). By 2003, WNV had spread across the continental United States and to other parts of North America (CDC 2014). Several dispersal agents may have been involved in spreading WNV, including infected migratory and local bird populations, dispersing mosquitoes, and possibly human-mediated mosquito movement (Marra et al. 2004; Goldberg et al. 2010, Venkatesan and Rasgon 2010, Clements 2012).

To date, the Centers for Disease Control and Prevention (CDC) has confirmed 16,196 cases of human neuroinvasive WNV, 20,892 confirmed cases of non-neuroinvasive WNV, and 1,549 deaths associated with WNV (CDC 2014). West Nile virus was first isolated in Nebraska in 2002 and 3,422 confirmed human cases and 57 deaths from WNV have been documented since (NE DHHS 2014). Data derived from the 2010 US Census and WNV incidences per state per 100,000 people, from 1999-2012, lists Nebraska at 170.94, making it third highest for WNV incidences (USCB 2010, CDC 2013). The northern Great Plains states, including Nebraska, have high incidence of WNV. A proposed explanation is a prominent vector species of mosquito of this region, *Cx. tarsalis* Coquillett, (Lindsey et al. 2008).

Regional differences in WNV human disease incidences have been elucidated utilizing human WNV case reports at a county levels and incorporating land cover type. Bowden et al. (2011) found a positive association between human WNV disease incidences and developed land cover (i.e. urban environments) in the Eastern regions, and

a positive association between human WNV disease incidences with crop or grassland cover in the Western regions of the United States.

Mosquito vectors are often associated with landscape because abundance and distribution of vectors are determined by environment (Brownstein et al. 2002, Bowden et al. 2011). *Culex tarsalis* is the main WNV vector west of the Mississippi River and its preferred larval developmental sites are in standing pools of water that receive abundant sunlight (Bowden et al. 2011). *Culex pipiens* is the main vector in the northeastern United States, but also occurs in areas west of the Mississippi River (north of 39°N latitude) (Darsie and Ward 2005, Bowden et al. 2011). *Culex quinquefasciatus* is the primary vector of WNV in the southeastern United States, but also occur throughout the warmer southern states west of the Mississippi River (south of 39°N latitude) (Bowden et al. 2011). *Culex pipiens* and *Cx. quinquefasciatus* typically thrive in urban and suburban environments where the larvae flourish in organically polluted waters, but can also be found in rural areas as well (Calhoun et al. 2007, Bowden et al. 2011).

Local land-use can affect vector population dynamics and vector-host interactions (Marra et al. 2004). Water management strategies can determine when and where suitable mosquito larval developmental sites arise by influencing the size and number of aquatic habitats (Marra et al. 2004). Irrigation practices have been implicated as principal factors in the transmission cycle of mosquito-borne diseases throughout the world (Miramontes et al. 2006, Lindsey et al. 2008, Wimberly et al. 2008, Gates and Boston 2009, Sugumaran et al. 2009, Eisen et al. 2010).

Several studies have related agricultural activity to mosquito abundance and increased WNV human disease incidences (Miramontes et al. 2006, DeGroote et al. 2008, Gates and Boston, 2009, Eisen et al. 2010, Bowden et al. 2011). These county-level studies show an increased relative risk of human WNV incidence as irrigated land composition and crop sales increased. Spatial patterns of abundance of *Cx. tarsalis* are highly heterogeneous and assessments of probable WNV exposure sites are complicated by inadequate knowledge of the fine-scale spatial distribution; studies should be refined from a state or county spatial unit, to a finer scale (Eisen and Eisen 2007). Furthermore, Eisen et al. (2010), point out a need for research in determining the importance of different irrigation practices in production of vector mosquitoes, primarily *Cx. tarsalis*, on irrigated agricultural lands in the WNV endemic region of the northern Great Plains.

The objectives of this field study were to: 1) identify mosquito species diversity of irrigated crop fields in Clay County, Nebraska, 2) determine abundance of mosquitoes with emphasis on WNV vector species (*Culex* species), 3) compare irrigation types (overhead vs. surface) and their influence on *Culex* species abundance, and 4) determine how predominant crop types (corn vs. soybean) influence *Culex* species abundance at a spatially refined level.

Materials and Methods

The study was conducted in Clay County, Nebraska (Figure 2.1) during the summers of 2012 and 2013.

Study Area 1

Study Area 1 was the South Central Agricultural Laboratory (SCAL), a University of Nebraska-Lincoln Extension facility (Figure 2.2). It is located in south-central Nebraska in Clay County, approximately 154.5 km west from Lincoln and 22.5 km east of Hastings (40.5753° N and 098.1378° W). The site is surrounded on the south and east by pasture land owned by the United States Department of Agriculture's Meat Animal Research Center (USMARC), to the southwest by waterfowl refuge land, to the west by a hog feeding facility and World War II era bunkers, and to the north by US Highway 6 and private irrigated crop fields. The facility is approximately 600 acres and comprised primarily of corn and soybean plots. Irrigation methods used include linear-move, center pivot, furrow surface, and subsurface drip irrigation. Several buildings are located near the center of the property. These buildings house employee offices, farming equipment, irrigation pumps, and often times roosting birds.

There are two gravel roads that bisect SCAL. Along both gravel roads are ditches that collect water from irrigation runoff and naturally occurring precipitation. Two large water-filled pits are located on the property, one in the northwest corner and one near the center, near the main office building. These are referred to as "reuse" pits. Reuse pits are used for surface irrigation purposes. Excess water drains back into the pits via ditches, which is then pumped from the pits, through large diameter metal pipes, and sprayed onto fields.

Study area 2

The second study area was composed of 12 privately owned fields located south, southwest, and northwest of Clay Center (Figure 2.3). Total acreage sampled encompassed approximately 1,150 acres.

In 2012, three fields produced soybeans (*Glycine max*) with center pivot irrigation, two fields produced soybeans with furrow surface irrigation, three produced corn (*Zea mays*) with center pivot irrigation, and four produced corn with furrow surface irrigation.

In 2013, crop type and irrigation practices in some fields were changed. Field composition included: one-half fields produced soybeans with center pivot irrigation (other half was corn), one field produced soybeans with furrow surface irrigation, three fields produced corn with furrow surface irrigation, and seven and a half fields produced corn with center pivot irrigation (other half was soybeans, listed above).

Many of the fields that utilized surface irrigation contained reuse pits. Several fields that had once been surface irrigated and had been converted to center pivot still contained remnant reuse pits.

Mosquito collections and identification

Due to the landscape of Clay County, naturally available resources, such as trees and bushes, were not always available to hang CO_2-baited CDC miniature light traps (John W. Hock, Gainesville, FL). To maintain consistency, shepherd's hooks (2.13 m) were utilized to hang light trap assemblies 1.65 m above ground. Light traps were powered by rechargeable 6-volt batteries. Dry ice (1.5 kg pelleted) was the source of CO_2

and was contained and hung in 3.78 L drink coolers (Igloo, Shelton, CT). Drink coolers were hung directly behind the light trap assembly, spout open, allowing for CO_2 to escape. Collection cups were attached to light traps by a mesh cloth (Figure 2.4).

Trap assemblies set up at SCAL were arranged in a 5x5 grid pattern with a spacing of approximately 322 m (Table 2.1, Figure 2.2). Two traps were placed around privately owned fields. They were placed at the southwest and northeast corners of each field (Tables 2.2 & 2.3). To avoid crop damage, and possible damage to the trap setup from irrigation and farm machinery, all CO_2-baited CDC light trap assemblies were placed along the periphery of the fields.

In 2012, trapping was conducted from June 7-September 7, with a sampling of 12 weeks during this period. Twenty-five trap assemblies were placed during a single night per week sampled at SCAL and 24 were placed during visits to privately owned fields. In 2013, trapping at SCAL was conducted from June 18-September 4, and six weeks were sampled during this period. The same quantities of traps were used for 2013 as 2012.

Light trap assemblies were placed between 1800 and 2030 h and were collected from 0700 to 0900 h the following morning. Trap set up was randomized each week to avoid placement of the same traps at the same time every week. Individual light traps were randomized as well to minimize bias.

Mosquitoes in collection cups were placed in ice chests to keep them cool and protected during transport to University of Nebraska-Lincoln and placed in freezers at -

15°C overnight. Mosquitoes were counted and identified utilizing a stereo dissecting-scope (Olympus, Center Valley, Pennsylvania) and sorted on a thermoelectric chill table at -13°C (Bioquip, Rancho Dominguez, CA). All mosquitoes were identified to species (Darsie and Ward 2005). West Nile virus vector species of interest, *Cx. tarsalis* Coquillett and *Cx. pipiens* L., were separated and labeled according to date, trap number, and species. Vector mosquitoes were stored in -70°C freezers until needed for further testing. Males were not utilized for this study.

Statistics

Data were analyzed using a general linear mixed model (GLIMMIX) with a negative binomial distribution via Statistical Analysis Software (SAS Institute, Cary, NC). This analysis allowed for investigation into relationships between irrigation types, crop type, year, and *Culex* mosquito populations. Relative rates were determined for the number of *Culex* mosquitoes collected and took into account year, crop type, and irrigation type.

Results

Study Area 1

In SCAL fields during 2012, 300 trap nights were conducted, with 14,593 mosquitoes collected (Table 2.4), equaling an average of 48.6 mosquitoes collected per trap night. In 2013, a total of 150 trap nights were conducted, with 110,101 collected (Table 2.4), equaling an average of 734 mosquitoes collected per trap night. A trap night is expressed as one trap operated one night (Janousek and Kramer 1999).

Thirteen mosquito species representing seven genera were collected in 2012 and 2013 (Table 2.4). The same 13 species were collected during both summers. In 2012, the most abundant species collected were *Culex pipiens* L. (39%), *Cx. tarsalis* Coquillett (31%), and *Aedes vexans* (Meigan) (16%) (Table 2.4). In 2013, the most abundant species were *Cx. tarsalis* (79%), *Ae. vexans* (14%), and *Ochlerotatus sollicitans* (Walker) (4%) (Table 2.4). When combining totals from both years, the most abundant species collected was *Cx. tarsalis* with 73% of the total. Other abundant mosquitoes were *Aedes vexans* (14.5%); *Cx. pipiens* (5%); and *Oc. sollicitans* (3.6%) (Table 2.4).

In 2012 and 2013, statistical analysis showed no significant differences in irrigation type or crop type with *Culex* abundance (F=0.00, P=0.9792; F=2.70, P=0.1077, respectively).. There was significant difference between years with *Culex* abundance (F=333.53, P=<0.0001). The Least Squares Means were 400.24 (SE=38.35) and 3332.33 (SE=327.97), for 2012 and 2013, respectively (Figure 2.5). The relative ratio thus was 8.32, meaning 8.32 times more *Culex* mosquitoes were collected per trap night in 2013 than 2012.

Study area 2

In the private fields during 2012, a total of 284 trap nights were conducted, with 136,776 mosquitoes collected (Table 2.5**)**, equaling an average of 481.6 mosquitoes collected per trap night. In 2013, a total of 144 trap nights were conducted, with 88,377 collected (Table 2.5), equaling an average of 613.7 mosquitoes collected per trap night.

Fourteen mosquito species representing seven genera were collected during 2012 and 2013 (Table 2.5). The same 14 species were collected during both summers. The species collected in privately owned fields that was not collected at SCAL was *Anopheles punctipennis* (Say).

In Study Area 2 during 2012, the most abundant species collected were *Ae. vexans* (90.5%), *Cx. tarsalis* (3.8%), and *Ps. columbiae* Dyar and Knab (1.2%) **(**Table 2.5**)**. In 2013, the most abundant species collected were *Ae. vexans* (52%), *Cx. tarsalis* (40%), and *Anopheles quadrimaculatus* Say (2.6%) (Table 2.5). When combining totals from both years, the most abundant mosquito collected was *Ae. vexans* with 75.4% of the total. Other abundant species were *Cx. tarsalis* (18%), *Oc. sollicitans* (1.6%), and *An. quadrimaculatus* (1.4%) (Table 2.5).

Statistical analysis showed a significant three-way interaction between crop type, irrigation type, and year with total *Culex* species abundance (F=9.76, P=0.0033). Since there was a three-way interaction, main effects could not be analyzed independently so simple effects were examined. Significance was determined utilizing estimates of the three-way interaction. Estimates compared the average number of *Culex* mosquitoes collected per trap night by center pivot irrigation versus furrow surface irrigation, with specific crop type per year (four estimates total). The estimate that showed a significant difference was the average number of *Culex* collected in center pivot irrigated corn fields versus furrow surface irrigated corn fields in 2012 (Relative Rate=0.5245, P=0.0053). The means for *Culex* collected per trap night in center pivot irrigated corn and furrow

surface irrigated corn were 174.67 (SE=28.957) and 333.00 (SE= 47.4137) (Figure 2.6), respectively. *Culex* mosquito abundance in center pivot irrigated corn fields was 52.45% less than *Culex* abundance in furrow surface irrigated corn fields in 2012. All other estimates were not significant.

Discussion

This study examined mosquito species diversity and associations between *Culex* vector species, irrigation types, and predominant crop type. In order to determine if WNV cases reported from Clay County could have been from local transmission, mosquito populations in the local area were sampled. Data from this field study may aid in determining entomological risk of WNV in irrigated agro-ecosystems of Clay County, Nebraska.

The floodwater mosquito species, *Aedes vexans* (Meigan), and two standing-water mosquito species, and primary WNV vectors, *Culex tarsalis* Coquillett and *Cx. pipiens* L., were the three most abundant species collected during this field study comprising 93.7% of the total mosquitoes collected. The other 6.3% was comprised of *Anopheles punctipennis* (Say), *An. quadrimaculatus* Say, *Coquillettidia perturbans* Dyar, *Culiseta impatiens* (Walker), *Cs. inornata* (Williston), *Ochlerotatus dorsalis* (Meigan), *Oc. sollicitans* Walker), *Oc. trivittatus* (Coquillett), *Psorophora ciliata* (Fabricius), *Ps. columbiae* (Dyar & Knab), and *Ps. cyanescens* (Coquillett).

The same 13 species were collected at SCAL during both summers, meaning species richness was equal. Relative abundance of each species, however, was not the

same over the two years (Table 2.4). In 2012, 86.95% of total abundance was comprised of *Ae. vexans*, *Cx. pipiens*, and *Cx. tarsalis* (Shannon diversity index of 1.52). In 2013, 92.87 % of total abundance was made up by *Ae. vexans* and *Cx. tarsalis* (Shannon diversity index of 0.77).

The same 14 species were collected in the privately owned fields during both summers, meaning species richness was equal. Relative abundance was more evenly distributed over the 14 species collected at privately owned fields in 2013 than in 2012, the opposite of what was observed at SCAL (Table 2.5). In 2012, 90.5% of total mosquitoes collected were *Ae. vexans* (Shannon diversity index of 0.49). In 2013, 94.1% of total mosquitoes collected were made up by *Ae. vexans* and *Cx. tarsalis* (Shannon diversity index of 1.04).

Anopheles punctipennis was not collected at SCAL either year, but was collected in 2012 and 2013 in privately owned fields. The presence of windbreaks, barriers used to reduce wind speed comprised primarily by trees and shrubs, and used in Nebraska agro-ecosystems (Brandle et al. 2004), may have supported the life cycle of *An. punctipennis* (Dorsey 1944, Fairley et al. 2000). Windbreaks were not utilized at SCAL, but five privately owned field had windbreaks and traps were set up in close proximity (<5 m).

Anopheles quadrimaculatus, *Coquillettidia perturbans*, *Culiseta impatiens*, and *Cs. inornata* are all standing-water mosquitoes that prefer permanent freshwater sites for larval development, feed primarily on large mammals, host-seek around dusk, and are attracted to artificial light (Carpenter and LaCasse 1955, Hill et al. 2013). Presence of

livestock around trapping locations (private farms and on USDA MARC), combined with the visual cue on the traps, may explain how and why these species were collected.

Floodwater mosquitoes of the genera *Aedes*, *Ochlerotatus*, and *Psorophora* did not have consistent seasonal abundance, most likely due to 2012 and 2013 being drought years. Large populations were experienced in both study areas 10-20 days after precipitation events, and declined until the next rainfall period (Figures 2.7 & 2.8). This is characteristic of their life cycle (Carpenter and LaCasse 1955, Meisch 1994, Foster and Walker, 2009). These genera are characterized as primarily mammalophilic and strong fliers (Carpenter and LaCasse 1955, Edman and Downe 1964, Tempelis 1975). The species collected can be found three to 15 miles away from larval emergence sites, and host-seek principally around dusk (Gjullin et al. 1950, Meisch 1994, PU 2014). Bionomics of species in these genera helps explain how they were collected and why they were usually collected in great abundance during certain times over the summers.

Anecdotally, a large amount of floodwater mosquitoes was collected on June 26, 2012, approximately 12 days after a significant precipitation event (Figure 2.7). Three traps in privately owned fields, all in close proximity (<5 m) to windbreaks, and one near a horse pasture, collected between ~13,000 to ~30,000.

Culex tarsalis and *Cx. pipiens* are standing-water mosquitoes that utilize temporary, shallow freshwater pools for larval habitat. Both have been documented to undergo a host-shift from birds to mammals as summer progresses (Tempelis et al. 1967, Kilpatrick et al. 2006). These species were collected during both summers, with

Cx.tarsalis being more abundant than *Cx. pipiens* (Tables 2.4 & 2.5). They have been implicated as primary and bridge vectors of WNV, with *Cx. pipiens* playing a more significant role in the northeastern United States, and *Cx. tarsalis* playing the most significant role in the western United States (Bowden et al. 2011). The role that *Culex* species play in WNV transmission cycle is why added emphasis was placed on these species throughout this study.

South Central Agricultural Laboratory's 5x5 grid trap set up was primarily designed to determine if certain locations around the property would have higher *Culex* abundance due to their proximity to suspected larval habitat (i.e. reuse pits). Traps near reuse pits (central area and NW corner), however, did not collect as many *Culex* per trap night as traps in the two southern rows (Figure 2.2)

Culex mosquitoes are considered short-range fliers with average dispersal flights of <100 yards per night from larval habitat for *Cx. tarsalis* (Reisen 1993) and one-quarter to one mile for *Cx. pipiens* (EPA 2014). The proximity of pasture lands to the south, waterfowl refuge land to the southwest, hog feeding facility to the west, and southerly summer winds, may have all contributed to significant levels of *Culex* at SCAL. Mosquitoes may have come from these surrounding areas, did not continue to host-seek once chemical and visual cues were detected, and were collected by the southern traps.

Culex pipiens abundance surged at the end of the 2012 trapping season at SCAL. It is unclear why the population increased dramatically, but *Cx. pipiens* is known as a late

season mosquito, with population peaks in late summer and early fall (Madder et al. 1980, Jackson et al. 2005).

Total *Culex* mosquito abundance for 2012 and 2013 showed similar seasonal abundance patterns with a peak around mid- to late June followed by a decline in late July to early August and rose in the latter part of the trapping season (Figures 2.9 & 2.10). The early season peak of *Culex* in mid- to late spring is consistent with previous studies and is a result of overwintering mated females exiting diapause (Mitchell 1981). The overwintering strategy for *Culex* mosquitoes may coincide with their preference to feed on fledgling and nesting birds during spring and early summer months (Tempelis 1975). The late season peak in *Culex* populations, from mid-August to early September (Figures 2.9 & 2.10), correlates with the proposed seasonal host-shift from birds to more readily available vertebrates such as mammals (Tempelis 1975). This late season population peak also correlates to the peak reporting time for human WNV cases in Nebraska (Figure 2.11).

The influence of furrow surface irrigation and center pivot irrigation to *Culex* abundance was investigated due to their prevalence within Nebraska and the northern Great Plains region. Associations have been made linking higher human WNV incidences to irrigation (Gates and Boston 2009, Eisen et al. 2010); however, specific irrigation types were not specifically sampled to show how they contribute to overall WNV risk in burdened areas. Water runoff from furrow surface irrigated fields was expected and therefore, surface irrigated fields were expected to have a significantly higher total of

mosquitoes, including *Culex*, than the more efficient center pivot irrigated fields. Since 2012 and 2013 were both drought years, this may have impacted mosquito larval developmental site production by increasing evaporation rates and decreasing overall standing surface pool abundance.

The influence of corn and soybeans to *Culex* abundance was investigated due to their prevalence within Clay County. Due to the relatively flat and wind-blown landscape of Clay County, crops may provide refuge for mosquitoes. After a female mosquito takes a blood meal she seeks refuge to digest her meal, which could take hours (Clements 1992). Crops, especially corn, were expected to provide adequate resting locations for mosquitoes in a landscape that is not heavily forested. Additionally, crops may also provide a source of nectar for males and females alike. Soybean flowers, broken corn kernels, damaged vegetative tissues, and even aphid honeydew could provide sugars necessary for mosquito development (Haeger 1955, Clements 1992).

One factor that may have influenced statistical results was that many land owners did not rotate crops (usually corn to soybean) from 2012 to 2013. An increase in corn fields and a decrease in soybean fields sampled were seen in 2013 versus 2012. The decision to plant corn two years in a row may have been reflective of higher corn prices as a result of the 2012 drought (USDA 2013).

Another factor that may have contributed to a lack of significance between irrigation and crops with *Culex* abundance, for SCAL and for privately owned fields, was

a lack of sampling in 2013 when compared to 2012. This may have reduced statistical power in leading to a type II error.

Substantially more *Culex* were collected in 2013 than 2012, even though 2012 had double the trap nights (Tables 2.4 & 2.5). Based upon meteorological data, there does not appear to be a significant difference between 2012 and 2013. One explanation why *Cx. tarsalis* populations were higher in 2013, may be elevated annual temperatures in 2012 and 2013, and reduced precipitation amounts (NCDC 2014), which may have been important factors in survival of overwintering female *Culex* mosquitoes (Foster and Walker 2009). Several climatic variables have been shown to influence WNV epidemiology; however, temperature and precipitation have emerged as the strongest predictors of viral transmission activity (Landesman et al. 2007, Ruiz et al. 2010, Johnson and Sukhdeo 2013). As temperatures increase, larval development time decreases, and viral replication within adult mosquitoes increases (Clements 2012).

A study by Miramontes et al. (2006) did not show a correlation with precipitation and human WNV incidence. Other studies associated mosquito outbreaks with drought conditions from the previous year (Chase et al. 2003, Wang et al. 2010, Johnson and Sukhdeo 2013). It is believed that increased rainfall can negatively impact *Culex* species due to a flushing of preferred organically polluted, eutrophic, larval habitat (Jacob et al. 2009, Johnson and Sukhdeo 2013). Drought conditions also reduce mosquito predators and competitors, allowing for mosquito populations to increase (Chase et al. 2003, Wang et al. 2010). Shaman et al. (2005) and Johnson and Sukhdeo (2013) noted epizootic levels

of WNV transmission when increased temperatures and decreased precipitation co-occurred.

Another aspect of drought conditions that could amplify WNV transmission is an increase in avian abundance at limited water sources, where *Culex* mosquitoes are likely to be present. This hypothesis is thought to apply to the amplification and transmission risk of the closely related St. Louis encephalitis virus (Shaman et al. 2002, Wang et al. 2010).

A unique feature of Clay County is that it lies within the Rainwater Basin Area of Nebraska. This area, managed by the United States Fish and Wildlife Service, incorporates 61 waterfowl production areas (WPAs) throughout 21-counties in south-central Nebraska. Sixteen of the 61 WPAs are within Clay County. Waterfowl production areas are managed mainly for migratory waterfowl and consist of wetland and upland areas. The goal of these areas is to support plant communities that decompose quickly, reproduce from seed, produce abundant seed, support higher aquatic invertebrate levels, and attract migrating waterfowl. These WPAs are often artificially flooded by Fish and Wildlife Service personnel utilizing groundwater to offset the loss of surface runoff to roadside ditches and pits used for agriculture. Fish and Wildlife Service research has shown that most species of migratory waterfowl and shorebirds prefer shallow water, with six inches (152.4 mm) or less during the height of migration season (FWS 2014).

Management practices of WPAs can produce sites conducive for mosquito larval development. *Culex tarsalis* oviposition sites include permanent or semi-permanent pools

that are surrounded by or include vegetation (Reisen, 1993). Larvae are tolerant to a variety of water conditions such as agricultural runoff, alkaline lake beds, freshwater and saltwater wetlands, and secondary treated sewage effluent, but are intolerant to excessive organic pollution and areas of permanent water with a fixed depth (Reisen, 1993). During drought years, such as 2012 and 2013, as WPAs dry, waters become more eutrophic, and could be potential *Cx. pipiens* larval developmental sites as well (Bowden et al. 2011).

Waterfowl production areas were not sampled during this survey. Future studies should look into the influence of WPAs in mosquito diversity and abundance within the Rainwater Basin Area of Nebraska.

This study has shown the diversity and abundance of mosquitoes, to include WNV vector species, in irrigated agro-ecosystems of Clay County, Nebraska. However, only irrigated fields were sampled and to get true insight into the effect of irrigation practices on vector mosquito production, researchers should sample mosquitoes from dry land fields, to act as a control, within the same relative area if possible.

The intensity of WNV transmission is determined primarily by the abundance of competent mosquitoes and the prevalence of infection in mosquitoes (Hayes et al. 2005). Familiarity of mosquito biology, abundance, and diversity, within a region, are vital in mosquito-borne disease surveillance and for developing effective mosquito control campaigns (Janousek and Kramer 1999). Knowledge of the relationship between precipitation, temperature, and *Culex* vector species, is important for determining the risk of human WNV outbreaks (Wang et al. 2010). This information may be utilized in global

climate change scenarios to predict future outbreaks (Wang et al. 2010). An understanding of ecological factors that impact WNV transmission can also help predict human WNV risk and improve effectiveness of control programs and measures (Miramontes et al. 2006, Wang et al. 2010). Information derived from this study can aid in public health outreach campaigns in rural settings, and assist in designing vector control programs.

To evaluate the entomological risk in Clay County, the following formula could be used: Risk=A x F_m x P x C_v, where A is mosquito abundance, F_m is the fraction of blood meals taken from mammals, P is WNV infection prevalence, and C_v is an index of vector competence) (Kilpatrick et al. 2005). Future research could include WNV testing of *Culex* species collected and blood meal analysis of blood-fed mosquitoes to determine hosts. These studies should be conducted to be able to determine the seasonality of WNV in the area.

Lastly, since 2012 and 2013 were drought years, several more years, should be sampled to determine how varying meteorological factors affect mosquito diversity and abundance of irrigated agro-ecosystems of Clay County.

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Table 2.1 Description and GPS coordinates of mosquito traps at South Central Agricultural Laboratory, 2012 & 2013

Trap No.	Crop	Irrigation	GPS Coordinates	Trap No.	Crop	Irrigation	GPS Coordinates
1	Corn	Linear Overhead	40° 34' 57.468" N 098° 8' 47.65" W	14	Corn	Linear Overhead	40° 34' 31.655" N 098° 8' 01.06"W
2	Corn	Linear Overhead	40° 34' 57.503" N 098° 8' 33.54" W	15	Corn	Linear Overhead	40° 34' 31.727" N 098° 7' 47.13"W
3	Corn	Linear Overhead	40° 34' 57.468" N 098° 8' 17.19" W	16	Corn	Linear Overhead	40° 34' 19.379" N 098° 7' 46.92" W
4	Corn	Sub-surface	40° 34' 57.468" N 098° 8' 03.33" W	17	Corn	Linear Overhead	40° 34' 06.347"N 098° 7' 46.74" W
5	Soy	Center Pivot	40° 34' 57.468" N 098° 7' 44.90" W	18	Corn	Linear Overhead	40° 34' 06.384" N 098° 8' 0.70" W
6	Soy	Center Pivot	40° 34' 43.608" N 098° 7' 45.12" W	19	Corn	Linear Overhead	40° 34' 17.795" N 098° 8' 0.88" W
7	Soy	Center Pivot	40° 34' 43.391" N 098° 8' 02.47" W	20	Corn	Linear Overhead	40° 34' 17.724" N 098° 8' 14.31" W
8	Soy	Furrow Surface	40° 34' 44.543" N 098° 8' 16.54" W	21	Corn	Linear Overhead	40° 34' 06.456" N 098° 8' 14.17" W
9	Soy	Furrow Surface	40° 34' 44.580" N 098° 8' 31.63" W	22	Corn	Linear Overhead	40° 34' 06.239" N 098° 8' 31.59" W
10	Soy	Furrow Surface	40° 34' 44.580"N 098° 8' 49.05" W	23	Corn	Linear Overhead	40° 34' 17.039" N 098° 8' 31.88" W
11	Corn	Linear Overhead	40° 34' 32.051" N 098° 8' 48.98"W	24	Corn	Linear Overhead	40° 34' 17.004" N 098° 8' 48.80" W
12	Soy	Linear Overhead	40° 34' 31.836" N 098° 8' 33.39" W	25	Corn	Linear Overhead	40° 34' 06.384" N 098° 8' 48.69" W
13	Corn	Furrow Surface	40° 34' 31.908" N 098° 8' 17.12" W				

Table 2.2 Description and GPS coordinates of mosquito traps in privately owned fields, 2012

Trap No.	Crop	Irrigation	GPS Coordinates	Trap No.	Crop	Irrigation	GPS Coordinates
1	Soy	Center Pivot	40° 27' 34.29" N 098° 3' 55.20" W	13	Corn	Center Pivot	40° 30' 02.45" N 098° 3' 40.66" W
2	Soy	Surface	40° 27' 45.57" N 098° 4' 12.98" W	14	Corn	Center Pivot	40° 29' 45.13" N 098° 4' 13.34" W
3	Soy	Surface	40° 27' 59.58" N 098° 4' 01.06" W	15	Corn	Surface	40° 29' 44.56" N 098° 6' 13.40" W
4	Soy	Center Pivot	40° 28' 00.01" N 098° 3' 27.79" W	16	Corn	Surface	40° 29' 25.96" N 098° 6' 29.74" W
5	Corn	Surface	40° 28' 26.71" N 098° 4' 04.96" W	17	Soy	Center Pivot	40° 30' 11.42" N 098° 6' 29.96" W
6	Soy	Surface	40° 28' 26.64" N 098° 3' 55.16" W	18	Soy	Center Pivot	40° 30' 36.95" N 098° 5' 57.40" W
7	Corn	Surface	40° 28' 47.40" N 098° 3' 56.16" W	19	Corn	Surface	40° 30' 11.28" N 098° 5' 56.35" W
8	Soy	Surface	40° 28' 47.06" N 098° 3' 40.29" W	20	Corn	Surface	40° 30' 24.18" N 098° 5' 48.60" W
9	Corn	Center Pivot	40° 28' 52.72" N 098° 4' 47.51" W	21	Corn	Surface	40° 34' 58.73" N 098° 10' 23.96" W
10	Corn	Center Pivot	40° 29' 18.35" N 098° 4' 14.18" W	22	Corn	Surface	40° 35' 13.27" N 098° 10' 12.77" W
11	Corn	Center Pivot	40° 29' 18.49" N 098° 4' 37.54" W	23	Soy	Center Pivot	40° 35' 49.49" N 098° 9' 56.08" W

12	Corn	Center Pivot	40° 29' 39.19" N 098° 4' 14.29" W	24	Soy	Center Pivot	40° 35' 23.79" N 098° 10' 29.21" W

Table 2.3 Description and GPS coordinates mosquito traps in privately owned fields, 2013

Trap No.	Crop	Irrigation	GPS Coordinates	Trap No.	Crop	Irrigation	GPS Coordinates
1	#Corn	Center Pivot	40° 27' 34.29" N 098° 3' 55.20" W	13	Corn	Center Pivot	40° 30' 02.45" N 098° 3' 40.66" W
2	#Corn	Surface	40° 27' 45.57" N 098° 4' 12.98" W	14	Corn	Center Pivot	40° 29' 45.13" N 098° 4' 13.34" W
3	#Corn	Surface	40° 27' 59.58" N 098° 4' 01.06" W	15	Corn	Surface	40° 29' 44.56" N 098° 6' 13.40" W
4	#Corn	Center Pivot	40° 28' 00.01" N 098° 3' 27.79" W	16	Corn	Surface	40° 29' 25.96" N 098° 6' 29.74" W
5	Corn	Surface	40° 28' 26.71" N 098° 4' 04.96" W	17	Soy	Center Pivot	40° 30' 11.42" N 098° 6' 29.96" W
6	#Corn	Surface	40° 28' 26.64" N 098° 3' 55.16" W	18	#Corn	Center Pivot	40° 30' 36.95" N 098° 5' 57.40" W
7	Corn	Surface	40° 28' 47.40" N 098° 3' 56.16" W	19	*Soy	Surface	40° 30' 11.28" N 098° 5' 56.35" W
8	#Corn	Surface	40° 28' 47.06" N 098° 3' 40.29" W	20	*Soy	Surface	40° 30' 24.18" N 098° 5' 48.60" W
9	Corn	Center Pivot	40° 28' 52.72" N 098° 4' 47.51" W	21	*Soy	Surface	40° 34' 58.73" N 098° 10' 23.96" W
10	Corn	Center Pivot	40° 29' 18.35" N 098° 4' 14.18" W	22	*Soy	Surface	40° 35' 13.27" N 098° 10' 12.77" W

11	Corn	Center Pivot	40° 29' 18.49" N 098° 4' 37.54" W	23	#Corn	Center Pivot	40° 35' 49.49" N 098° 9' 56.08" W
12	Corn	Center Pivot	40° 29' 39.19" N 098° 4' 14.29" W	24	#Corn	Center Pivot	40° 35' 23.79" N 098° 10' 29.21" W

= Soybean in 2012
* = Corn in 2012

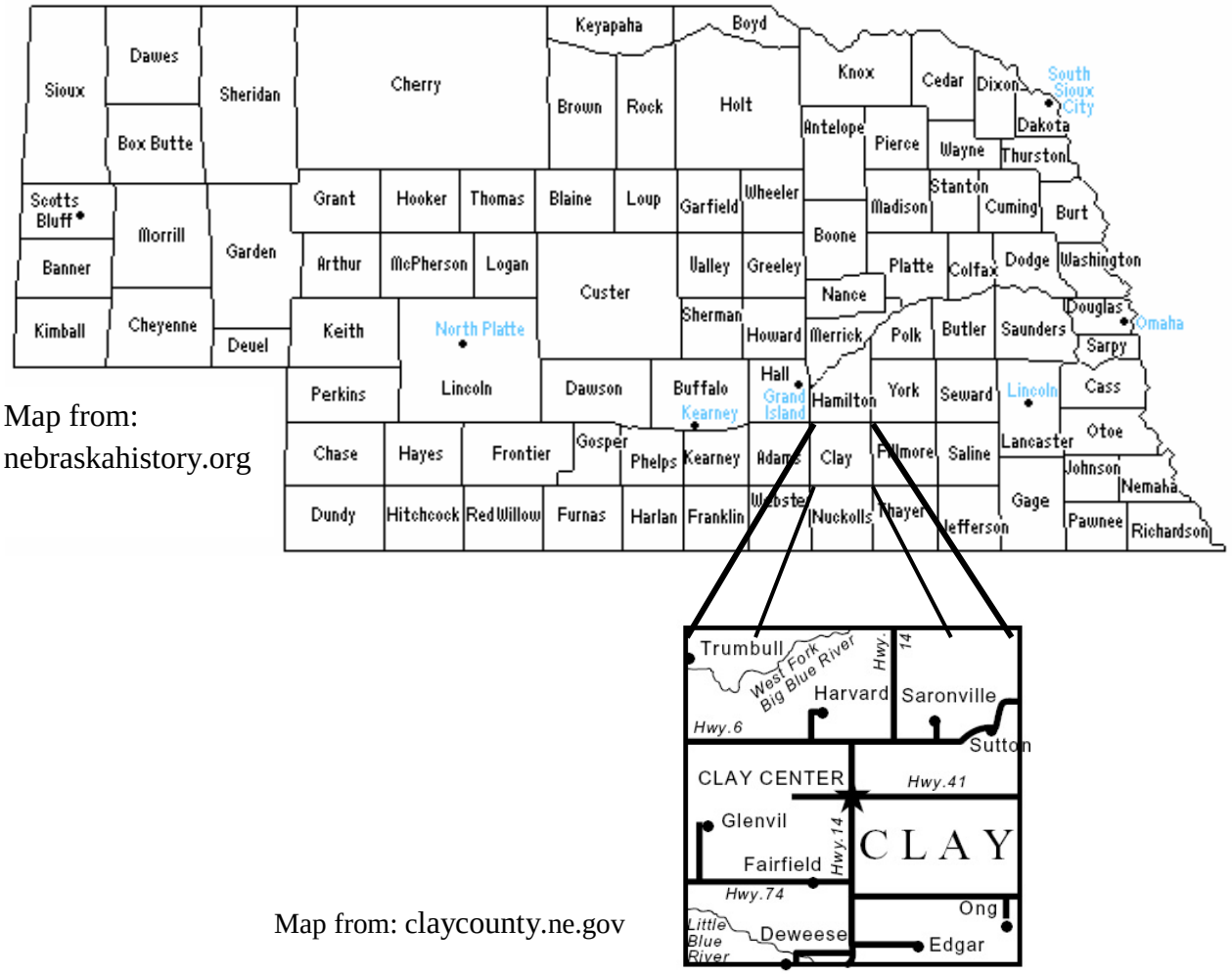
Table. 2.4 Mosquito species and abundance at South Central Agricultural Laboratory, 2012 & 2013

	Abundance				
	2012			**2013**	
Species	**Total**	**%**		**Total**	**%**
Aedes vexans	2,360	16.17		15,741	14.30
Anopheles quadrimaculatus	146	1.00		86	0.08
Coquillettidia perturbans	6	0.04		25	0.02
Culiseta impatiens	425	2.91		1,185	1.08
Culiseta inornata	47	0.32		305	0.28
Culex pipiens	5,749	39.40		672	0.61
Culex tarsalis	4,579	31.38		86,511	78.57
Ochlerotatus dorsalis	2	0.01		48	0.04
Ochlerotatus sollicitans	527	3.61		4,004	3.64
Ochlerotatus trivittatus	4	0.03		800	0.73
Psorophora ciliata	181	1.24		229	0.21
Psorophora columbiae	513	3.52		388	0.35
Psorophora cyanescens	54	0.37		107	0.10
Total	**14,593**	**100.00**		**110,101**	**100.00**

Table 2.5 Mosquito species and abundance at privately owned fields, 2012 & 2013

	Abundance				
	2012			**2013**	
Species	**Total**	**%**		**Total**	**%**
Aedes vexans	123,875	90.57		45,964	52.01
Anopheles punctipennis	8	0.01		6	0.01
Anopheles quadrimaculatus	854	0.62		2,307	2.61
Coquillettidia perturbans	160	0.12		22	0.02
Culiseta impatiens	472	0.35		644	0.73
Culiseta inornata	1	0.0007		22	0.02
Culex pipiens	1,476	1.08		603	0.68
Culex tarsalis	5,191	3.80		35,424	40.08
Ochlerotatus dorsalis	3	0.0022		10	0.01
Ochlerotatus sollicitans	1,468	1.07		2,167	2.45
Ochlerotatus trivittatus	36	0.03		21	0.02
Psorophora ciliata	1,340	0.98		249	0.28
Psorophora columbiae	1,577	1.15		714	0.81
Psorophora cyanescens	315	0.23		224	0.25
Total	**136,776**	**100.00**		**88,377**	**100.00**

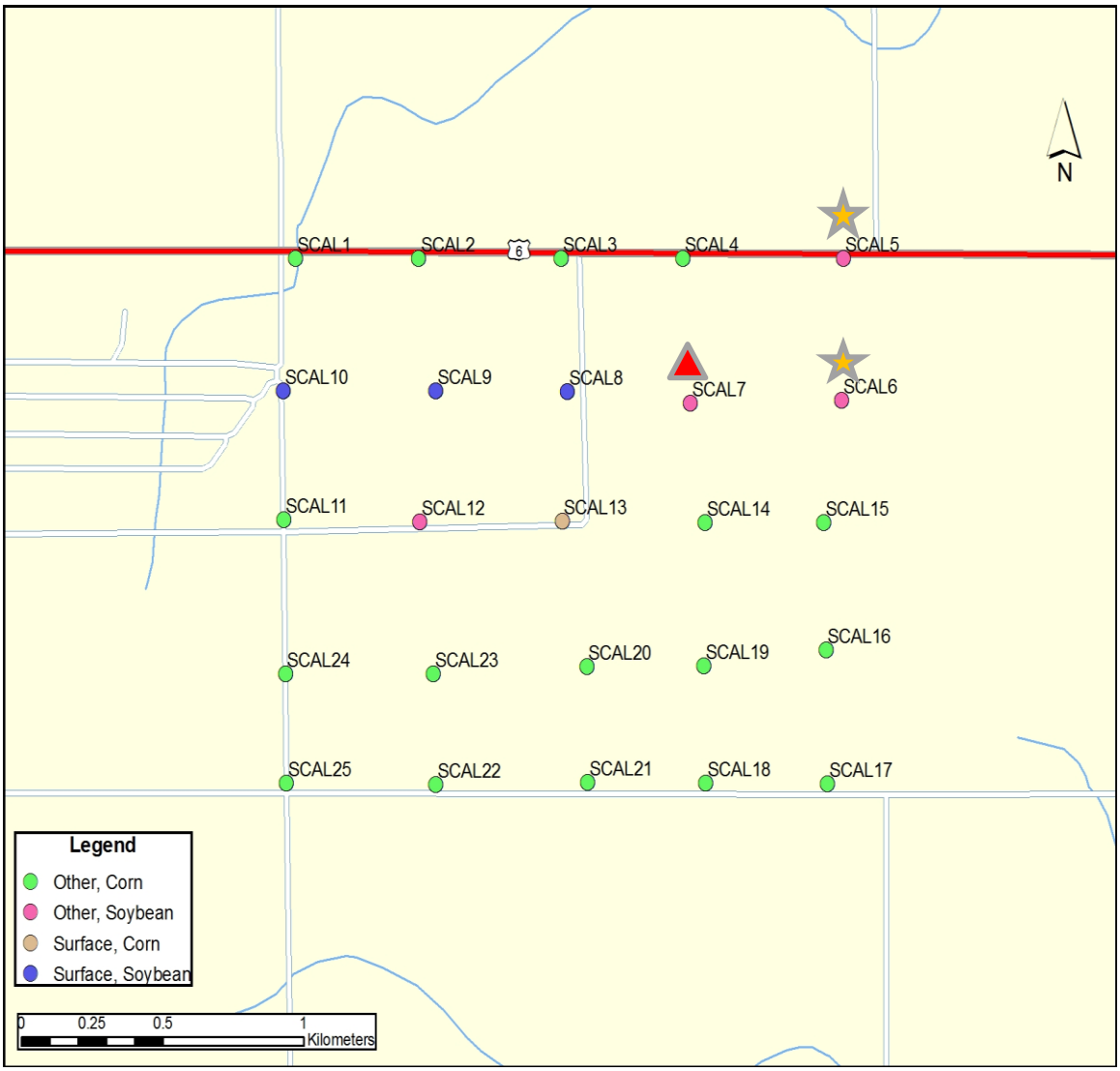
Figure 2.1 Map of Nebraska with Clay County highlighted



Map from: nebraskahistory.org

Map from: claycounty.ne.gov

Figure 2.2 South Central Agricultural Laboratory trap setup, 2012 & 2013



Other in the legend denotes linear overhead, center pivot, or subsurface drip irrigation.

= center pivot

=subsurface drip

Figure 2.3 Privately owned field locations, 2012 & 2013

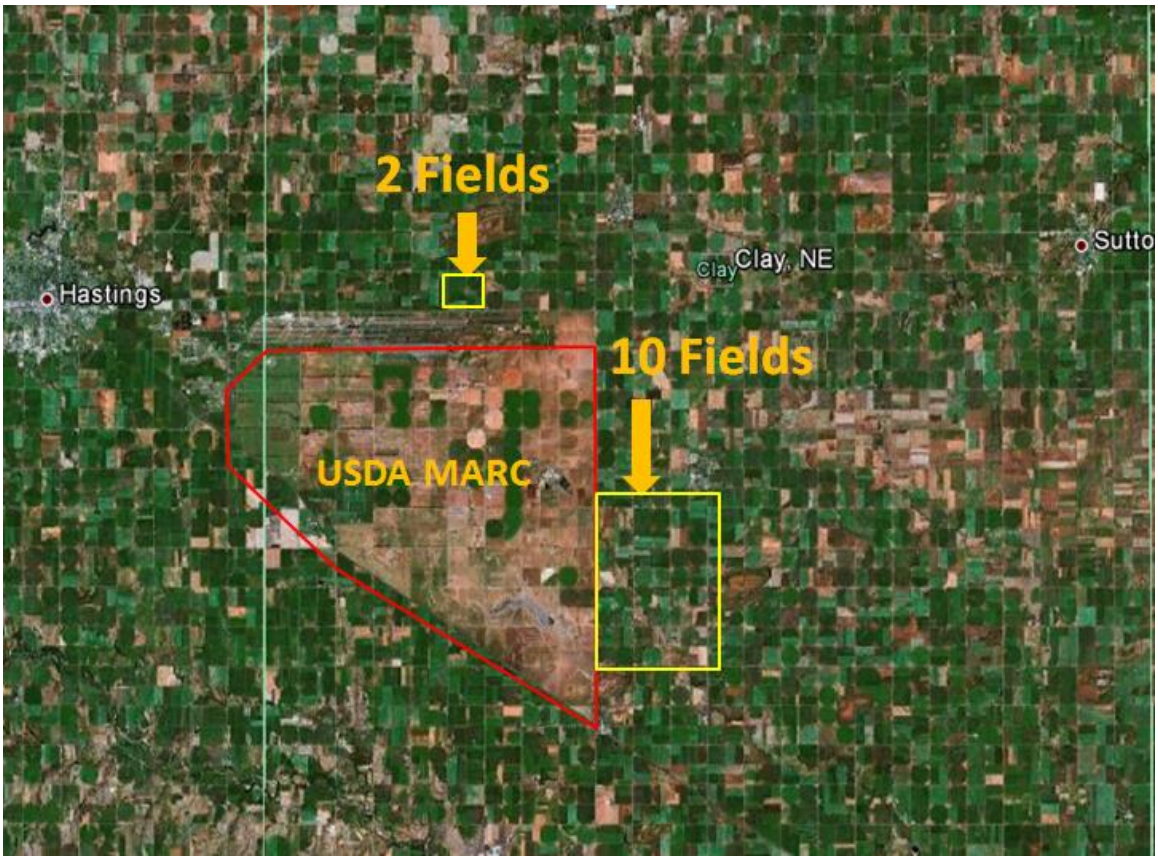
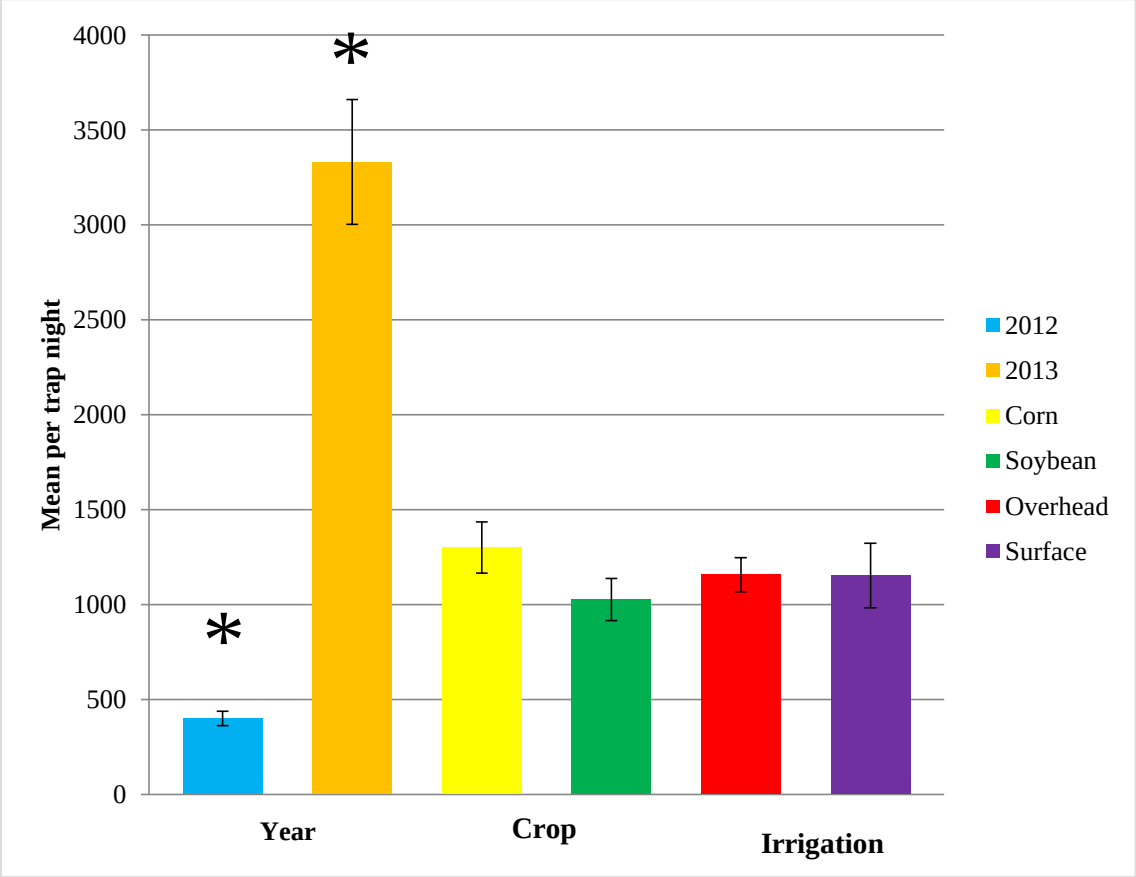


Image from Google Earth©

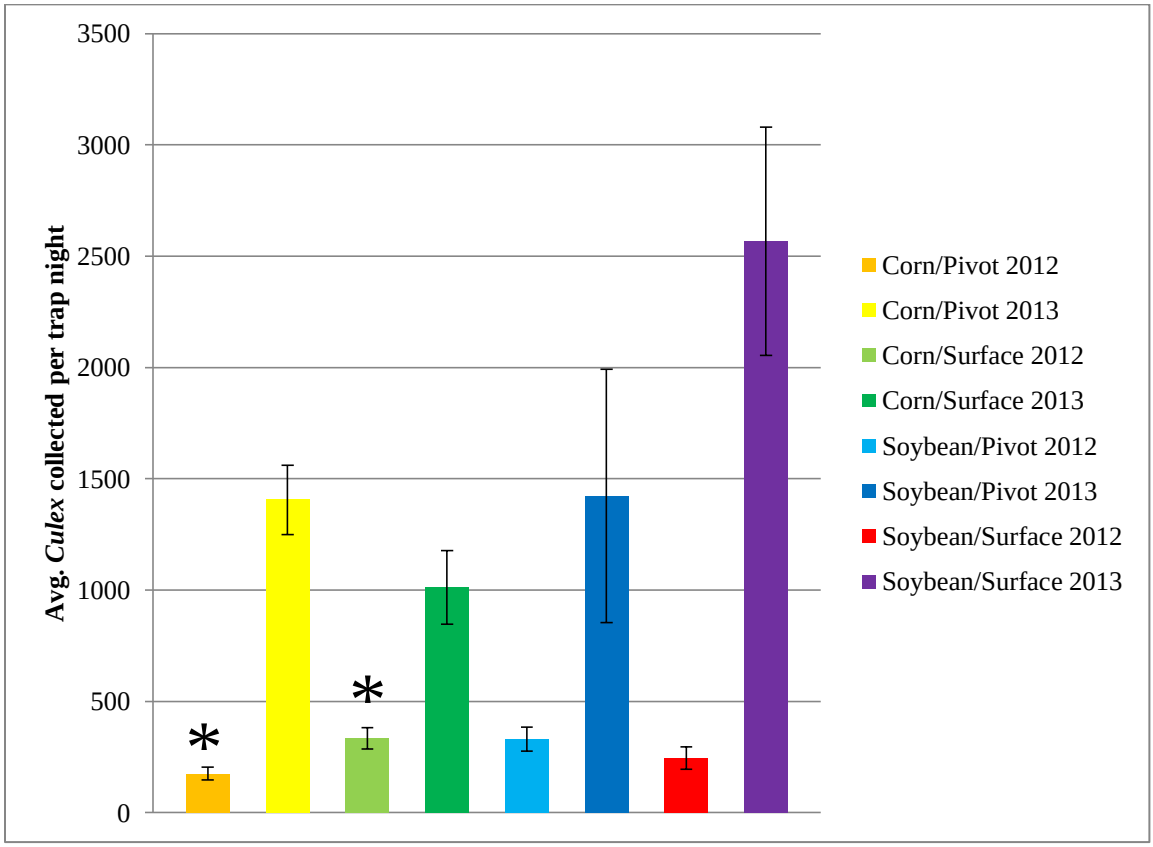
Figure 2.4 CO_2-baited CDC miniature light trap assembly

Figure 2.5 South Central Agricultural Laboratory Least Squares Means for main effects for *Culex* species collected



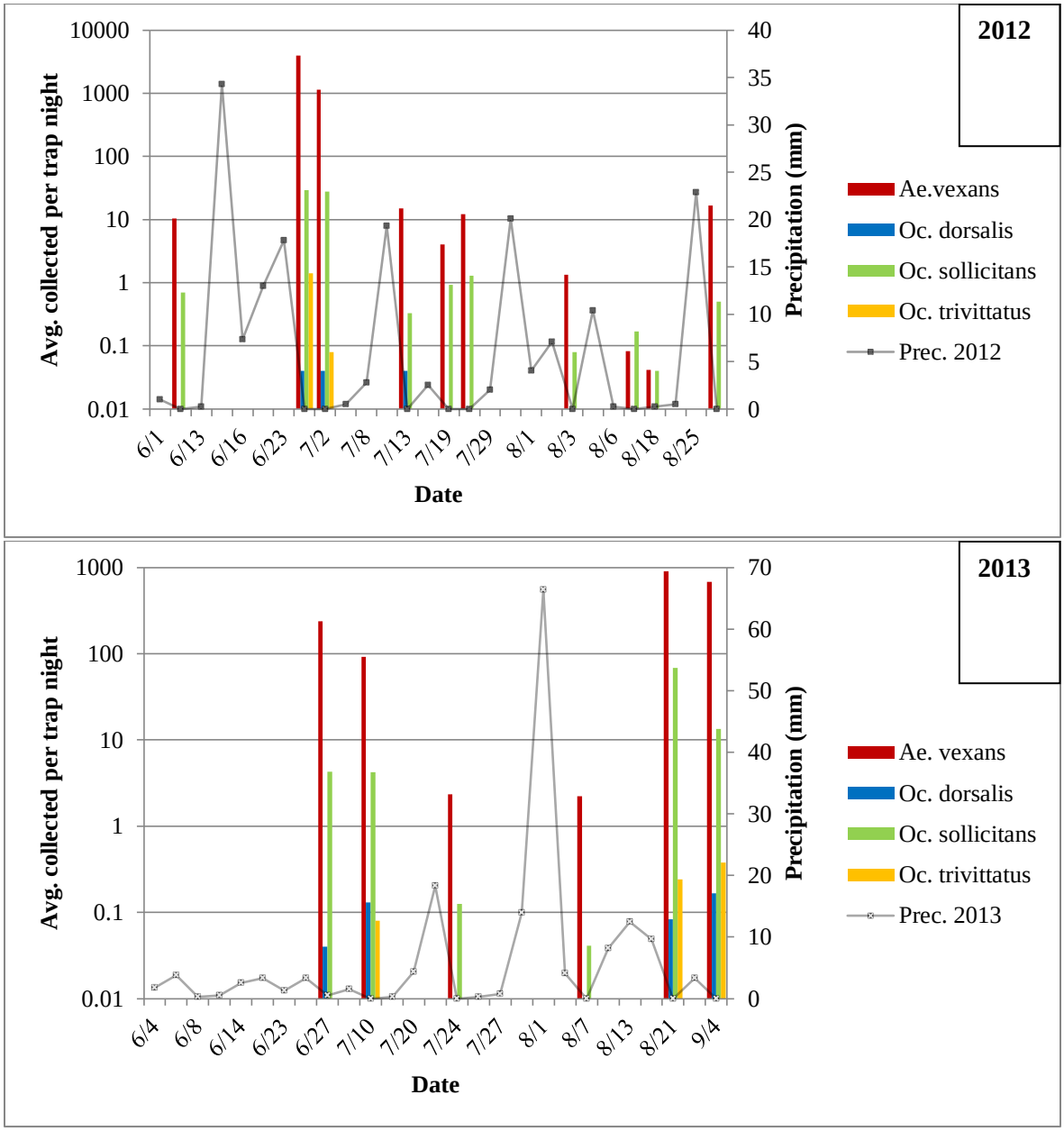
*****Year was the only main effect of significance (F=333.53, P<.0001).
Crop (F=2.70, P=0.1077).
Irrigation (F=0.00, P=0.9792)

Figure 2.6 Privately owned fields estimates for Crop*Irrigation*Year interaction for *Culex* total abundance



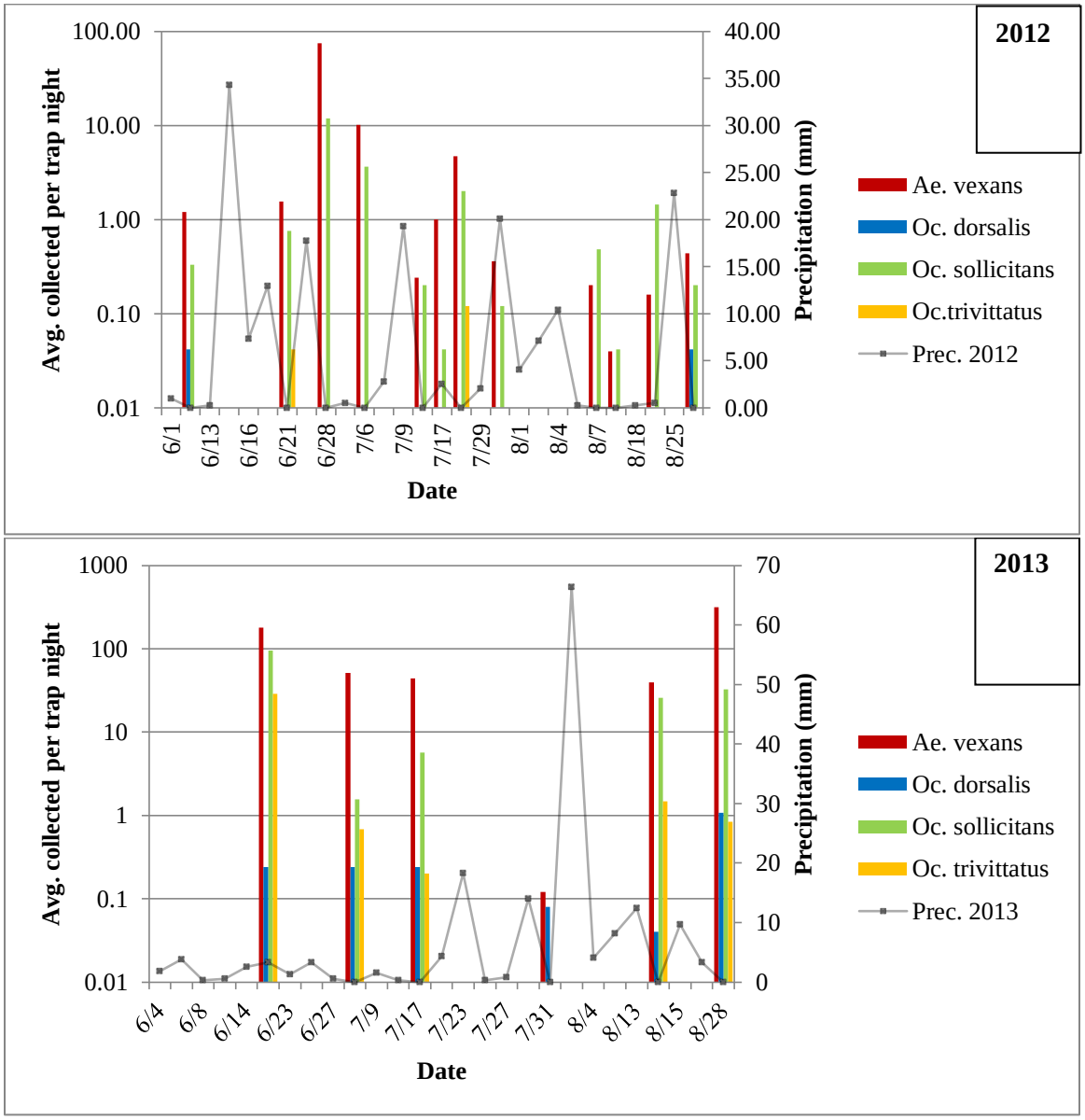
*Three-way interaction when comparing *Culex* total means from corn irrigated with center pivot versus furrow surface irrigation in 2012 (P=0.0053) .

Figure 2.7 *Aedes* & *Ochlerotatus* averages per trap night, privately owned fields, 2012 & 2013



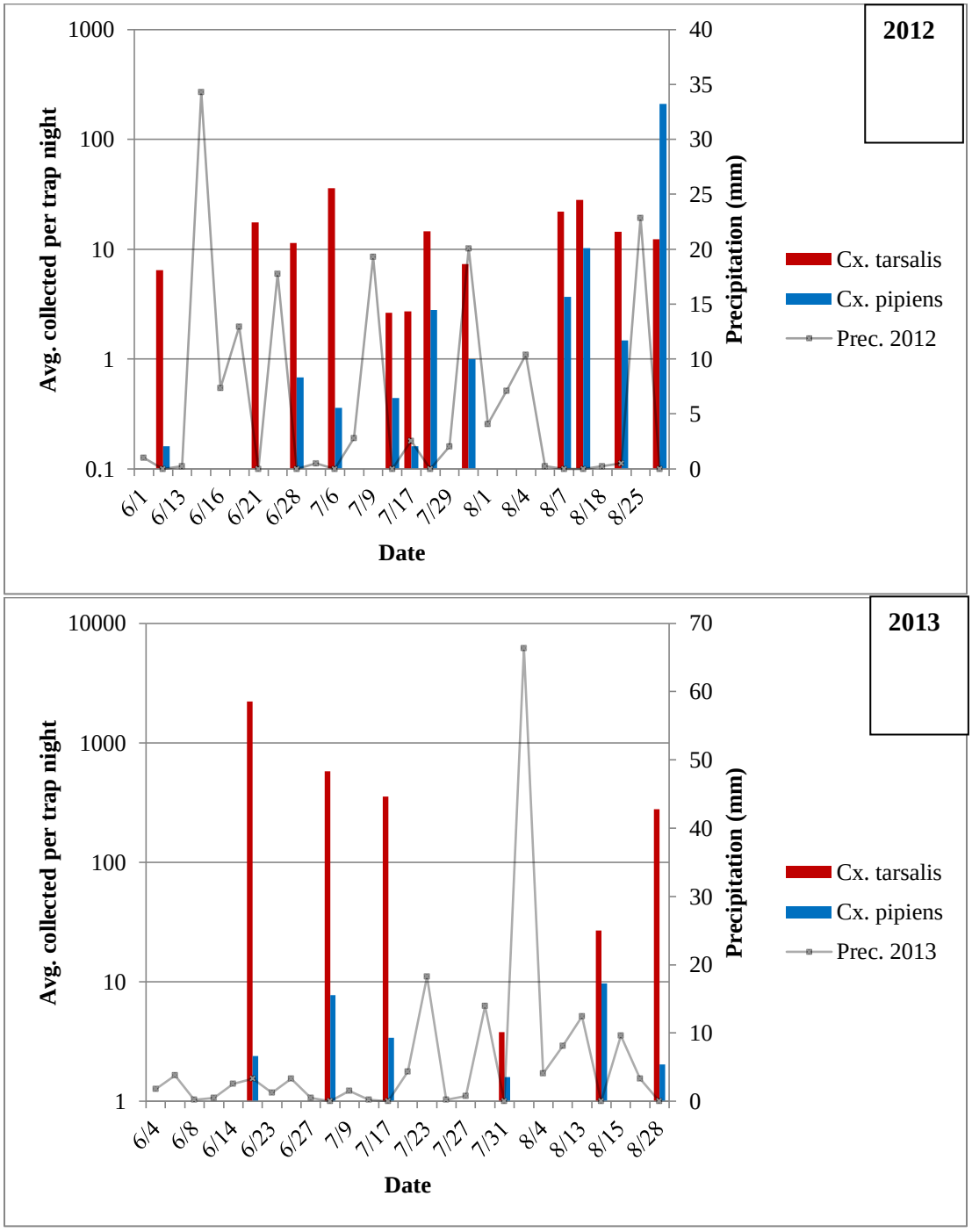
Note: Left Y-axis have different logarithmic scales

Figure 2.8 *Aedes* & *Ochlerotatus* collected per trap night at South Central Agricultural Laboratory, 2012 &2013



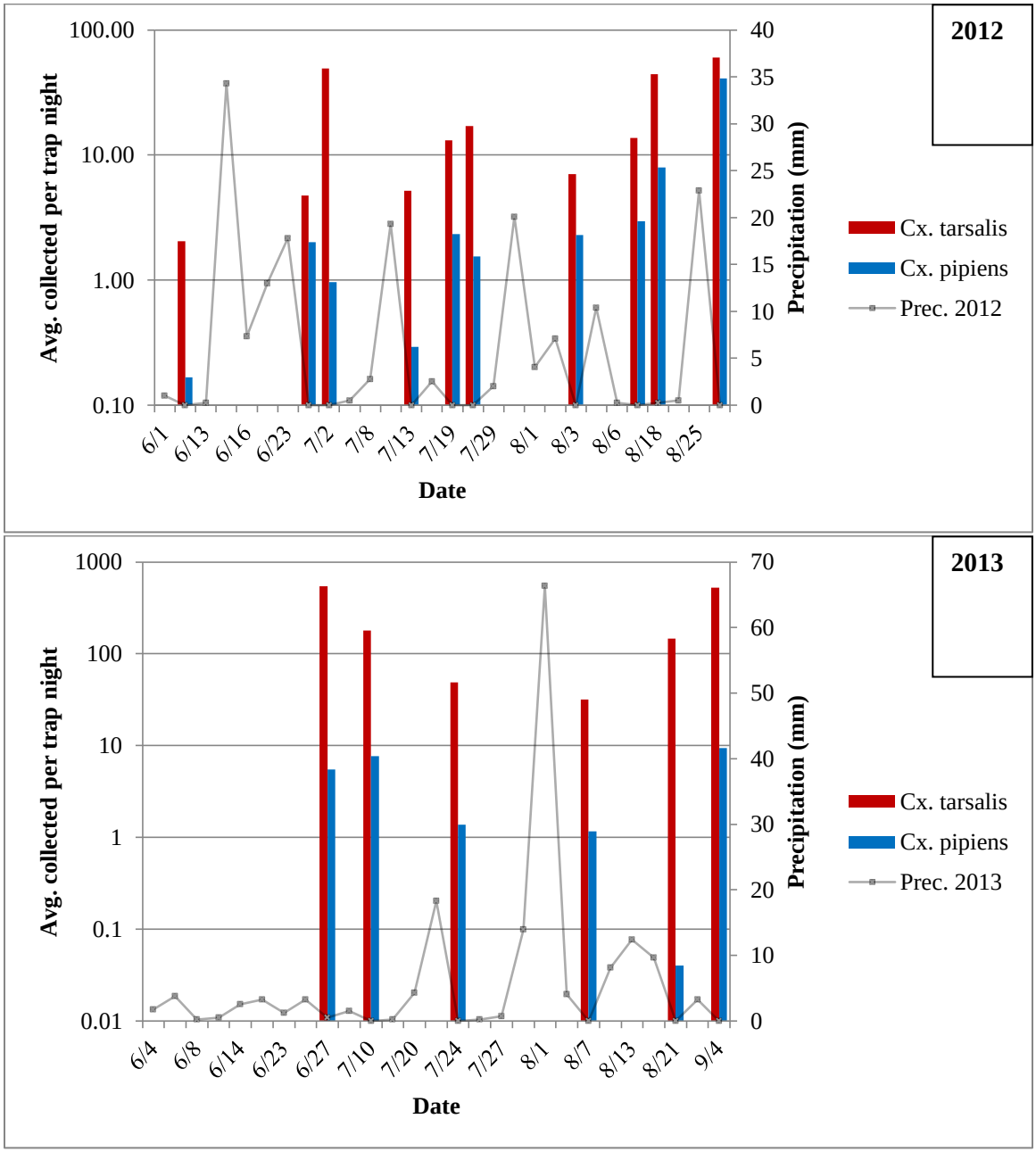
Note: Left Y-axis have different logarithmic scales

Figure 2.9 *Culex* species collected per trap night, South Central Agricultural Laboratory, 2012 & 2013



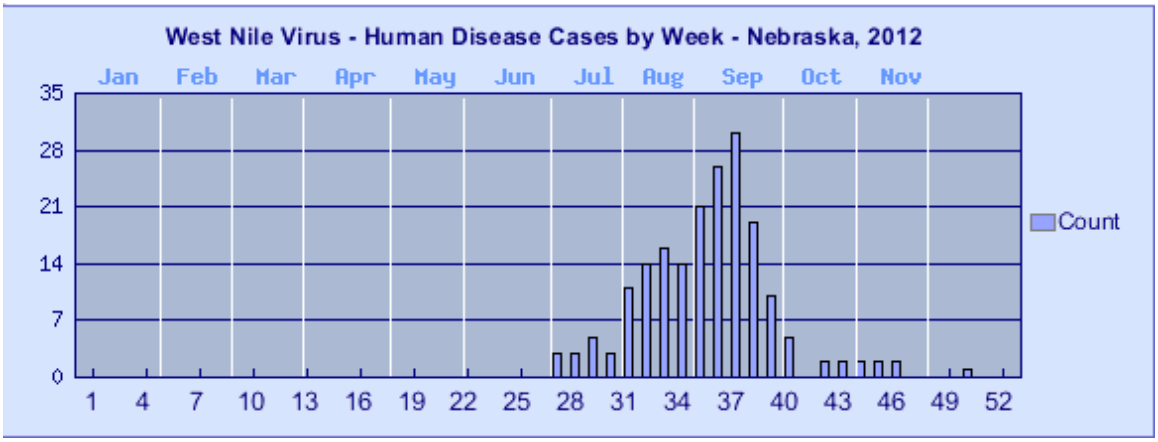
Note: Left Y-axis have different logarithmic scales.

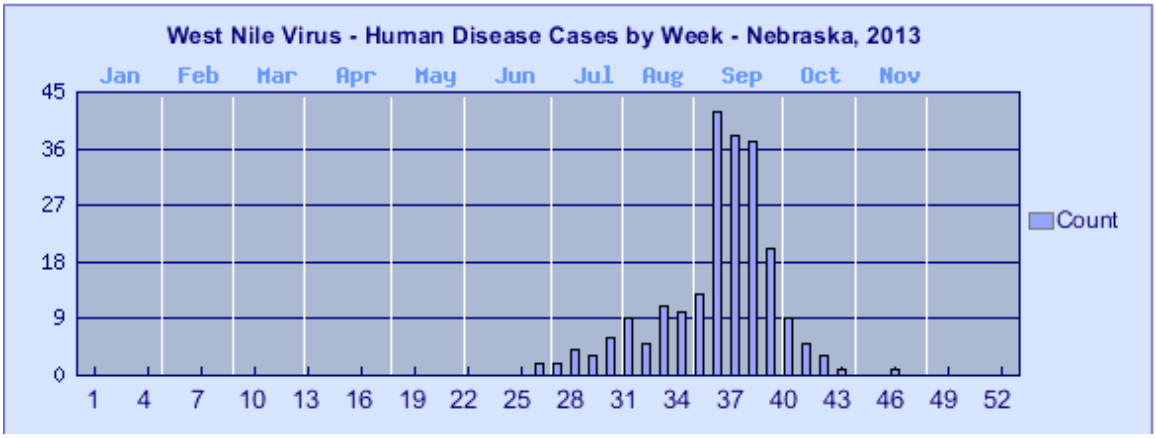
Figure 2.10 *Culex* species collected per trap night at privately owned fields, 2012 & 2013



Note: Left Y-axis have different logarithmic scales

Figure 2.11 Human WNV case reports per week for Nebraska, 2012 & 2013





*Data from United States Geological Service WNV disease maps (USGS 2014)

ADDENDUM

Mosquito immature stage, larva and pupa, sampling was conducted throughout the field study; however, those collected were not used in statistical analysis. A total of 67 mosquito immatures were collected from 555 dips. In 2012, 321 samples were taken with 60 immatures collected. In 2013, 234 samples were taken with seven immatures collected. The numbers collected did not reflect the totals of adults collected. Low collection numbers of mosquito immatures may be a reflection of inadequate sampling, improper sampling technique, or adults coming from locations outside of the fields sampled.

Larval sampling was accomplished utilizing a larval dipper which was constructed of a telescoping aluminum rod (0.91 m-1.83 m) with a 350 ml cup (Bioquip, Rancho Dominguez, CA) attached at the end of the rod. Areas with standing water were sampled during each trapping day. Samples were transferred from the dip to 355 ml plastic jars, labeled with date, location, and sample number, and transported to the University of Nebraska-Lincoln. Each sample was carefully examined for aquatic insect life and logged. Mosquito larvae and pupae collected were transferred to 9 mm diameter plastic petri dishes and allowed to develop to the adult stage for easier identification. Larvae that did not survive were still identified to species (Darsie and Ward 2005).

Of the 60 immature mosquitoes collected in 2012, 16 were *Aedes vexans,* one *Anopheles quadrimaculatus,* seven *Culiseta impatiens,* three *Culex pipiens*, 10 *Cx. tarsalis,* 12 *Psorophora ciliata*, nine *Ps. columbiae,* and two *Ps. cyanscens.* Most of the mosquitoes (37/60) were collected in or near privately owned fields. Of the 37, 32 were

collected in flooded ditches and five were collected from reuse pits. The other 23 were collected from SCAL. Thirteen were collected from puddles around the property, eight were collected from flooded ditches from irrigation runoff, and two were collected from the reuse pits.

Of the seven immature mosquitoes collected in 2013, four were *Ae. vexans*, one *Cx. tarsalis*, one *Oc. sollicitans*, and one *Ps. columbiae*. All immature stages for 2013 were collected at SCAL, in flooded ditches from irrigation runoff, and a low depression near the center of the acreage. Reuse pits were not as conducive to mosquito larval development as was originally expected at the beginning of the field study. These pits often fluctuated in water depth, emergent vegetation was non-existent, and predators were frequently sampled. Additionally, it is possible that pesticides, if applied to fields, may have runoff the fields, in subsequent irrigations or precipitation events, and accumulated in the pits and affected mosquito larval communities.

Other aquatic insects collected included beetles from the families Gyrinidae, Dytiscidae, and Hydrophilidae; Odonata naiads from Anisoptera and Zygoptera; Dipteran larvae from the families Stratiomyidae, Syrphidae, Psychodidae, and Chironomidae; and Hemipteran adults from the families Hebridae, Corixidae, and Notonectidae (Triplehorn et al. 2005). Small fish and frogs were often seen in reuse pits associated with surface irrigated fields.

Literature Cited

Triplehorn, C. A., and N. F. Johnson. 2005. Borror and DeLong's introduction to the study of insects (seventh edition). Thomson Brooks Inc. Belmont, CA.