Increased Human Incidence of West Nile Virus Disease near Rice Fields in California but Not in Southern United States

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Abstract. Anthropogenic land use change, including agriculture, can alter mosquito larval habitat quality, increase mosquito abundance, and increase incidence of vector-borne disease. Rice is a staple food crop for more than half of the world’s population, with ~1% of global production occurring within the United States (US). Flooded rice fields provide enormous areas of larval habitat for mosquito species and may be hotspots for mosquito-borne pathogens, including West Nile virus (WNV). West Nile virus was introduced into the Americas in 1999 and causes yearly epidemics in the US with an average of approximately 1,400 neuroinvasive cases and 130 deaths per year. We examined correlations between rice cultivation and WNV disease incidence in rice-growing regions within the US. Incidence of WNV disease increased with the fraction of each county under rice cultivation in California but not in the southern US. We show that this is likely due to regional variation in the mosquitoes transmitting WNV. Culex tarsalis was an important vector of WNV in California, and its abundance increased with rice cultivation, whereas in rice-growing areas of the southern US, the dominant WNV vector was Culex quinquefasciatus, which rarely breeds in rice fields. These results illustrate how cultivation of particular crops can increase disease risk and how spatial variation in vector ecology can alter the relationship between land cover and disease.

INTRODUCTION

Human land cover change can alter the spatial and temporal risk of vector-borne disease.1–3 Anthropogenic land use changes commonly associated with increased mosquito-borne disease include deforestation, urbanization, and agricultural development.4 Agricultural cropland covers ~12% of the earth’s surface and flooded rice fields make up ~11% of these areas.5 Rice fields can provide an extensive larval habitat for particular mosquito species, increasing local mosquito populations and disease risk in surrounding regions.5,6

West Nile virus (WNV) is a widespread mosquito-borne pathogen that was introduced to the Americas in 1999 and causes yearly epidemics in the United States (US) with an average of ~1,400 neuroinvasive cases and 130 deaths.7,8 In addition, WNV has caused widespread mortality and substantial declines in populations of several bird species.9–11 Culex mosquitoes are considered to be the most important vectors for WNV transmission,12,13 and the abundance of infected Culex mosquitoes is strongly correlated with the number of human WNV cases.14,15

Several studies have previously examined the effect of land use on several aspects of WNV transmission. Urbanization has been associated with higher WNV seroprevalence in wild bird and mammal populations,16–17 and higher human disease incidence on a county scale in the eastern and central regions of the US.18–21 This is thought to be due to urbanization increasing larval habitat for container-breeding vectors of WNV including Culex pipiens and Culex quinquefasciatus.22–24 In the western US, grassland and agricultural land covers have been associated with higher human WNV disease incidence.19–21,25,26 Grassland and agricultural habitats are thought to increase the abundance of another important WNV vector, Culex tarsalis.22,28 Several studies at the national scale have argued that regional differences in land covers associated with increased WNV disease incidence roughly correspond to the distributions of major Culex vectors.19–21,29 However, the broad classification of grassland and agriculture land cover encapsulates a wide diversity of crop types, each with variable effects on mosquito abundance and WNV risk.27 In addition, none of the broad-scale studies correlating land cover with WNV disease incidence include quantitative data on mosquito abundance or infection to support the different regional correlations and conclusions.

We examined the influence of a particular agricultural land cover, rice fields, that provides larval habitat for some but not all mosquito species. We tested the following hypotheses: rice cultivation would increase the abundance of C. tarsalis mosquitoes; human WNV disease incidence would increase with C. tarsalis abundance; and as a result, human WNV disease incidence would increase with rice cultivation in regions where C. tarsalis was a key vector of WNV but not in areas where C. tarsalis is rare or absent and other mosquito species are the dominant WNV vectors.

MATERIALS AND METHODS

Land use, climate, and WNV disease incidence. We obtained 30 m land cover data from the United States Department of Agriculture National Agricultural Statistics Service.30 For each county, we summed the area of several land cover classes that could potentially be important for mosquitoes (rice fields, developed areas, wetlands, open water, and forested areas). We averaged data from seven different years available with 30 m resolution (2008–2014) and calculated the percent cover of each land cover class using the area of each land cover class divided by total county area. Developed areas included a combination of low, medium, and high intensity, as well as open space developed. Wetland areas included a combination of woody and herbaceous wetlands and forested areas included all deciduous, mixed, and evergreen forest types.

We calculated the percent of each county that was “irrigated agriculture” (including rice fields) from United States Geological Survey Moderate Resolution Imaging Spectroradiometer at 250 m resolution.31 We estimated “non-rice irrigated
areas" by subtracting the rice-growing areas from the total irrigated area within each county. In addition, we calculated mean annual temperature and precipitation (2003–2011) for each county using data from the North America Land Data Assimilation System.32

We compiled reported human cases of WNV for each county from Centers for Disease Control and Prevention (CDC) ArboNET program for the years 2004–2015.7 Average human WNV disease incidence was calculated as the mean number of all cases (fever and neuroinvasive cases combined) per year divided by the county’s population.33

**West Nile virus vector identification.** We estimated the role of different *Culex* species mosquitoes in the transmission of WNV in each county using the fraction of *Culex* WNV-positive pools reported to the CDC from 2004 to 2009. We used the fraction of positive pools to account for differences in sampling effort among counties; as long as each pool of *Culex* mosquitoes is equally likely to be tested and reported to the CDC, these data should provide relatively accurate estimates of the relative abundance of infected mosquitoes of each *Culex* species. We also examined the importance of *Culex* species in human WNV disease incidence across the US, by calculating a human population-weighted average of the county values of the fraction of WNV-positive mosquito pools attributed to each species. These estimates do not take into account the differences in feeding preferences or the fraction of WNV-infected mosquitoes that transmit WNV between mosquito species.12

**Mosquito abundance.** We obtained mosquito trapping data from the California Vectorborne Disease Surveillance Gateway vector-borne disease surveillance system, which includes trapping data from vector control districts across California. We used New Jersey light trap (NJLT) data from the summer months (June–September) from the years 2000–2015. This dataset consisted of > 100,000 unique site visits across 1,284 locations spanning 34 counties in California. We estimated relative

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**Figure 1.** Rice cultivation and West Nile virus (WNV) incidence in rice-growing regions of the United States (US). (A) Average percent of each county growing rice over the period 2008–2014. (B) Average yearly human WNV incidence in reported cases per 100,000 people over the period in rice-growing counties 2004–2015. (C) Yearly average WNV incidence plotted against average rice cover in California: Log₁₀ WNV incidence = 0.35 + 0.21 (±SE = 0.039) × Log₁₀ percent rice cultivation; $R^2 = 0.41$, $N = 46$; general least squares model including spatial autocorrelation, $P = 0.04$. (D) Average yearly WNV incidence plotted against average rice cover in the rest of the US: Log₁₀ WNV incidence = −0.11 + 0.04 (±SE = 0.014) × Log₁₀ percent rice cultivation; $N = 413$; $R^2 = 0.02$; general least squares model including spatial autocorrelation, $P = 0.88$. For panels (C) and (D) counties with no WNV cases are shown with an incidence of 0.1. This figure appears in color at www.ajtmh.org.
summer abundance of *C. tarsalis* in each county by taking the mean number of mosquitoes caught per trap location and then averaging across all trap locations within each county over the period 2000–2015. We further estimated the mean summer (June–September) abundance of *Culex* mosquitoes at particular trap sites located within 10 km of rice fields. This distance is well above the estimated average dispersal distance for *C. tarsalis*.34 For these estimates, each trap site was included only if it had at least 5 years of NJLT data with at least 10 visits per year.

Statistics. We summarized geographic data using ESRI ArcMap 10, and performed all statistical analyses using program R, version 3.1.3. We used generalized least squares (gls) to build least squares regression models to predict the mean WNV disease incidence in each county using land cover and climate data (developed, water, wetland, rice, forest, irrigated areas, mean temperature, and mean rainfall data). West Nile virus disease incidence, land cover variables, and *Culex* abundance data were log10 transformed to equalize leverage and maintain adequate homogeneity of variance (see Supplemental Table 1). We accounted for spatial autocorrelation in WNV disease incidence data using exponential correlation structure within the gls models. We used piecewise regression models (R package “segmented”) to examine relationships between mosquito abundance and distance to nearest rice field.35 Piecewise regression uses an iterative process to reduce the residual sum of squares by fitting linear line segments across different rice distance intervals and comparing models with multiple segments to models with fewer segments.35

### RESULTS

The main rice-growing regions of the US were in California, the Mississippi river delta, and southern Texas, with small additional areas in South Carolina and Florida (Figure 1A). A total of 459 counties in the US had rice-growing regions during the years 2008–2014. The amount of rice grown in each county varied widely with some rice-intensive counties having up to 30% of the county area covered in rice fields. Mean WNV disease incidence (2004–2015) in rice-growing counties was 1.25 people/100,000 per year (95% confidence interval = 1.08–1.42, standard error (SE) = 0.09) and ranged from 0 to 31 cases/100,000 people/year (Figure 1B).

In California, incidence of WNV disease increased with the percent of the county growing rice (Figure 1C). No other
climate or land use variables contributed to an increase in WNV disease incidence (Supplemental Table 2). By contrast, outside California, incidence of WNV disease was uncorrelated with rice cover, and increased with developed area and decreased with open water cover (Figure 1D; Supplemental Table 3).

Of 13 different Culex species found to test positive for WNV across the US, the vast majority (> 93%) of WNV-positive mosquito pools came from only four species: C. pipiens (29%, SE = 1.1), C. tarsalis (28.3%, SE = 1.4), Culex restuans (18.7%, SE = 0.9), and C. quinquefasciatus (16.6%, SE = 1.2) (Supplemental Figure 1). The population weighted analysis also identified the same four species: C. pipiens (33%, SE = 0.7), C. quinquefasciatus (27%, SE = 1.5), C. restuans (17%, SE = 0.5), and C. tarsalis (15%, SE = 0.5) (Supplemental Figure 1). However, the importance of each mosquito species differed among counties and regions (Figures 2 and 3). In rice-growing regions of California, approximately 65% (SE = 5.0) of all reported WNV-positive mosquito samples were from C. tarsalis, 14% (SE = 3.6) were C. pipiens, and 14% (SE = 4.3) were C. quinquefasciatus (Figure 3). In rice-growing regions outside of California, the most important species were C. quinquefasciatus 66.8% (SE = 3.7), C. pipiens 20% (SE = 2.8), and C. restuans 7.3% (SE = 1.6), whereas C. tarsalis made up very few of the WNV pools 0.02% (SE = 0.01) (Figure 3).

In California, rice cultivation was linked to mosquito abundance, and mosquito abundance was correlated with WNV disease incidence. The relative abundance of C. tarsalis per NJLT-week increased with rice cover (Figure 4A) and WNV disease incidence increased with C. tarsalis abundance (Figure 4B). Culex tarsalis abundance increased at trap sites (N = 388) located near rice fields (Figure 5). By contrast, we found no significant relationship between C. pipiens abundance and distance to rice fields (P = 0.74, Supplemental Figure 2). In addition, zero C. quinquefasciatus were caught in > 97% of trap sites (377/388) located within 10 km of rice fields.

DISCUSSION

The larval ecology of mosquito vectors appears to play a key role in determining the effect of land use on mosquito-borne disease. Previous studies had found correlations between WNV disease incidence and agricultural land cover in the western US, and urban land cover in the eastern regions.19–21 These studies attributed these regional differences in which land cover increased WNV disease incidence to differences in the distributions of mosquito vectors. We extend these results by showing that an important agricultural crop, rice, appears to play a key role in WNV transmission in the western US by increasing the abundance of an important WNV vector in this

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**FIGURE 3.** Relative contribution to West Nile virus (WNV)-infected Culex species in rice-growing areas across two different regions of the United States (US), 2004–2009. Columns show average fraction of WNV-positive pools attributed to each mosquito species from California counties with rice fields (CA, N = 44) and other rice field counties not in California (Not CA, N = 106).
region, C. tarsalis. We further showed that the effect of rice fields on WNV disease incidence depended on the relative importance of C. tarsalis in that region. Of the four Culex species that make more than 93% of reported WNV-positive mosquito pools in the US (C. pipiens, C. quinquefasciatus, C. restuans, and C. tarsalis), only C. tarsalis breeds in flooded agricultural fields and grasslands, whereas the other three species breed in container habitats. As a result, in rice fields and grasslands, whereas the other three species breed in container habitats. As a result, in rice fields was uncorrelated with the abundance of C. tarsalis and was instead correlated with urban land cover, as in other studies. These results provide a more detailed understanding of the mechanisms underlying some previous correlations with land use and land cover along with an evidentiary basis for the previously proposed hypotheses. It is worth noting that these findings are limited to rice-growing regions in California and the US. In other regions of North America, other species of mosquitoes are more important WNV vectors, including C. pipiens and C. restuans. Our study further shows how rice fields specifically increased C. tarsalis abundance whereas having no effects on other important WNV vectors such as C. pipiens or C. quinquefasciatus. Previous studies in California have found C. tarsalis larva to be abundant in rice fields and the rice-growing region of northern California to have the highest overall abundance of adult C. tarsalis of anywhere in the state. Other studies outside of the US have also found that the extent of rice fields was uncorrelated with the abundance of C. pipiens and C. quinquefasciatus. We also show that the increased abundance of C. tarsalis in rice field areas extended outward 2 km from rice field sites, well within the dispersal distance associated with this mosquito species. We observed a 7-fold increase in C. tarsalis abundance within 2 km of rice fields. This suggests that residential neighborhoods located within 2 km of rice fields are likely to have higher WNV disease risk. Although rice cultivation is clearly important for C. tarsalis, other factors, such as blood meal hosts and anthropogenic sources of light, also influence mosquito abundance or abundance estimates using NJLT.

Rice fields also appear to be important for other mosquito-borne diseases. Results from this study are similar to findings in another disease system, Japanese encephalitis (JE). Japanese encephalitis is an important emerging infectious disease, endemic to many regions of Southeast Asia resulting in widespread morbidity (30,000–50,000 annual cases) and mortality (10,000–15,000 annual deaths). The abundance of one potentially important mosquito vector of JE, Culex tritaeniorhynchus, closely tracks rice-growing in space and time, and C. tritaeniorhynchus abundance is correlated with JE disease incidence. Rice is grown in more than 100 countries worldwide, with extensive cultivation in Southeast Asia (Supplemental Figure 3). Our results illustrate how certain crops can increase disease risk and how spatial variation in vector ecology can alter the relationship between land cover and disease. Efforts to mitigate this increased disease risk while supporting production of this key agricultural crop are needed to maximize human health and well-being.

Received February 9, 2018. Accepted for publication March 12, 2018. Published online April 30, 2018.

Note: Supplemental figures and tables appear at www.ajtmh.org.

Acknowledgments: We would like to thank members of the Kilpatrick Lab at University of California Santa Cruz for feedback. Mosquito trapping data used in this study were obtained from the California Vector-borne Disease Surveillance (CalSurv) System, and we acknowledge its contributors, including the member agencies of the Mosquito and Vector Control Association of California, California Department of Public Health, and the Davis Arbovirus Research and Training (DART) laboratory at the University of California, Davis.

Financial support: Funding was provided by National Science Foundation grants DEB-1115069 and EF-0914866, National Institutes of Health grant 1R01AI090159, and a postgraduate doctoral scholarship from National Sciences and Engineering Research Council of Canada.

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