Reproductive Biology and Susceptibility of Florida *Culex coronator* to Infection with West Nile Virus

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Abstract

Ornithophilic *Culex* species are considered the primary amplification vectors of West Nile virus (WNV) in bird hosts as well as vectors responsible for epidemic transmission. *Culex coronator* was first collected from Okaloosa, Santa Rosa, Walton, and Washington Counties in Florida in 2005 and has since spread throughout the state. The vector competence of *Cx. coronator* for WNV, known to be infected in nature, has not been assessed. Without this knowledge, we are unable to assess this species' potential as an enzootic and epidemic vector of WNV in Florida. In the current study, we investigate the reproductive biology and susceptibility to WNV infection, dissemination, and transmission by *Cx. coronator*. We show that *Cx. coronator* is capable of delaying oviposition for several weeks after blood feeding and that the number of eggs laid is greater for avian than mammalian hosts. *Cx. coronator* were highly susceptible to infection (~80–100%) and dissemination (~65–85% by 18 days since exposure) with lower rates of transmission (0–17% at 25°C and 28–67% at 28°C), suggesting that it is a competent vector of WNV under some conditions. The proportion of mosquitoes with disseminated infections related to the time since exposure and was higher at 28°C than at 25°C. The rapid and statewide distribution of *Cx. coronator* throughout Florida poses as a potential public health risk. This baseline knowledge is essential information for mosquito control and public health agencies to assess current and future disease risk to Southeastern United States.

Key Words: Nonnative species—Reproduction—Adult survival—West Nile virus infection and transmission.

Introduction

THE RANGE EXPANSION OF *Culex coronator* Dyar and Knab has the potential to alter the dynamics of West Nile virus (WNV) in North America, directly by becoming a novel vector and indirectly by interacting with native vectors. The primary vectors of WNV in Florida are *Cx. nigripalpus* Theobald and *Cx. quinquefasciatus* Say (Rutledge et al. 2003, Colton and Nasci 2006). The first collections of *Cx. coronator* in Florida were from four northwest Florida counties (Okaloosa, Santa Rosa, Walton, and Washington) in 2005 (Smith et al. 2006). Since then, *Cx. coronator* has rapidly expanded its statewide distribution in a north to south direction, similar to the spread of *Aedes albopictus* (Skuse) a decade earlier (O'Meara et al. 1995). To date *Cx. coronator* has been collected in 64 of the 67 Florida counties (Connelly and O'Meara, unpublished). *Cx. coronator* have been found co-occurring in larval habitats with other WNV vector species, including *Cx. nigripalpus* and *Cx. quinquefasciatus* in Florida, suggesting species overlap in

habitat use (O'Meara, unpublished data). Additionally, we have found larvae in used tires and other types of aquatic habitats in southern and central Florida throughout the year in 2011. For the few counties where *Cx. coronator* has not been reported, it is likely that the species is there, but has not been detected due in most cases to a lack of primarily active mosquito surveillance programs.

Host-feeding patterns for field-collected blood-fed *Cx. coronator* in Louisiana suggest that this species is primarily a mammalian blood feeder, although a proportion of blood meals are from birds (Mackay et al. 2010). However, WNV has been found to occur in *Cx. coronator* in nature (Mackay et al. 2008). This apparent contradiction in feeding pattern and infection with WNV that has an enzootic cycle between birds and mosquitoes may be explained by plasticity in host feeding patterns. For example, blood meal analyses have demonstrated that the proclivity of *Cx. tarsalis* Coquillett, *Cx. pipiens* Linnaeus, *Cx. nigripalpus*, and *Cx. erraticus* (Dyar and Knab) to feed on mammals and birds varies geographically and seasonally,

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although these species tend to feed primarily on birds (Edman and Taylor 1968, Kilpatrick et al. 2006a, Kent et al. 2009, Burkett-Cadena et al. 2012). Variability in feeding patterns may be due to genetic makeup, host composition and availability, physiological status or age of the vector, seasonal changes in environmental conditions, variation in host defense behavior, and foraging experience (Niebylski and Meek 1992, Kilpatrick et al. 2006b, Kilpatrick et al. 2007, Lyimo and Ferguson 2009).

The vertebrate source of blood may also influence other aspects of *Cx. coronator* biology relevant to its role as a potential WNV vector. It has been suggested that nucleated erythrocytes of birds, reptiles, and amphibians provide greater nutrition than anucleated mammal erythrocytes (Bennett 1970, Downe and Archer 1975). Thus, fecundity may be higher in mosquitoes that derive blood meals from these formerly mentioned vertebrates than mammals. Additionally, the blood meal may be used for synthesis of energy reserves, especially among anthropophilic species (*Ae. aegypti* Linnaeus, Clements 1992, Day et al. 1994, Harrington et al. 2001; *Anopheles gambiae* sensu stricto Giles, Beier 1996, Gary and Foster 2001) and starved females (Nayar and Sauerman 1975), which may have direct effects on parameters of vectorial capacity (e.g., adult survival).

The vector competence of *Cx. coronator* for WNV has not been assessed. Without this knowledge, we are unable to assess this species' potential as an enzootic and epidemic vector of WNV in Florida. In the current study, we investigate the reproductive biology, adult female survival, and susceptibility to WNV infection, dissemination, and transmission of *Cx. coronator*. The rapid and statewide distribution of *Cx. coronator* throughout Florida poses a potential public health risk. This baseline knowledge is essential information for mosquito control and public health agencies to assess current and future disease risk to areas of the southeastern United States where *Cx. coronator* is established.

Materials and Methods

Reproductive biology and adult survival

Blood feeding trials were used to determine the influence of the blood meal type on timing of oviposition, fecundity, and survival. An established colony of *Cx. coronator* originating from Indian River County, Florida, in 2011 was used for all experimental trials. Larvae were reared in 1.0 liters of tap water in plastic photo trays (25 cm width, 30 cm length, 5 cm height; Richard MFG Co. Fernandina Beach, FL) on a diet of liver powder. Approximately 150 larvae were cultured together with food additions being provided at ~0.2 gram every 3 days. Adult *Cx. coronator* male and females were held together for 23 days in a cage (0.3³ meter) in an outdoor insectary at the Florida Medical Entomology Laboratory and provided with 10% sucrose solution. Females were transferred to 0.5 L cages with mesh screening 2 days prior to feeding trials and provided with water but not sucrose. Females aged for 25 days were then given an opportunity to feed for 1 h on sources of blood via an artificial membrane feeding system (Hemotek, Lancashire, United Kingdom). We deliberately used older mosquitoes because we were concerned whether sufficient numbers of *Cx. coronator* females would successfully blood feed from an artificial membrane feeding system. Some studies have demonstrated higher host-seeking and feeding rates among older mosquitoes (Davis 1984, Alto

et al. 2003). We completed feeding trials using blood sources comprised of five mammals (bovine, guinea pig, horse, pig, and rabbit) and two birds (chicken and turkey). Our rationale for choice of vertebrates was based on making use of more mammals than birds since *Cx. coronator* frequently feeds on mammals (Mackay et al. 2010). We used a range of mammal hosts (five orders) that were commercially available. Due to limited availability of bird hosts, we used hosts from a single order, Galliformes. Feeding trials were performed in a dark climate-controlled room at 24°C and 66% humidity. Mosquitoes were acclimated for 2 h to the climate-controlled room before the start of feeding trials. After feeding trials, we enumerated unfed and fully engorged adult females.

Following feeding trials, blood-fed *Cx. coronator* females were housed individually in 0.5 L cages and maintained in the outdoor insectary and provided with 10% sucrose solution, renewed weekly. Mosquitoes were monitored daily for dates of oviposition and death, which were recorded. The dry mass and wing length of *Cx. coronator* females were measured of blood-fed mosquitoes. Egg rafts were collected within 24 h after oviposition, and the number of eggs was enumerated with the use of light microscopy at 40× magnification. We used regressions to relate adult dry mass to the number of eggs laid and wing length to the number of eggs laid. Differences in blood feeding rates were tested by maximum likelihood (ML) categorical analyses of contingency tables (ML ANOVA, PROC CATMOD; SAS 2002). Significant treatment effects were followed by pairwise contrasts using similar methods correcting for multiple comparisons using the sequential Bonferroni method. A total of 286 adult females were offered blood during the feeding trials. Treatment effects of blood source on timing of oviposition was compared using a nonparametric analysis (PROC LIFETEST; SAS 9.22). Treatment effects of blood source on number of eggs was assessed using analysis of variance (ANOVA). When significant treatment effects were found, we followed up with pairwise comparisons of means, adjusting for multiple comparisons using the Tukey–Kramer method (PROC GLM; SAS 9.22). Treatment effects of blood source on survival were compared using nonparametric survival analysis (PROC LIFETEST; SAS 9.22) followed by log-rank test statistics to compare pairwise estimates of survival adjusting for multiple comparisons using the Sidak method (Sidak 1967).

Infection, dissemination, and transmission of WNV

Nine- to 17-day-old adult *Cx. coronator* females were provided with WNV-infected chicken blood using an artificial membrane feeding system, as described in the study on reproductive biology and adult survival. To prepare fresh virus for mosquito infection, monolayers of African green monkey kidney (Vero) cells in two T-175-cm² flasks were inoculated with 200 µL of stock WNV and incubated for 1 h at 37°C and 5% CO₂ atmosphere, after which 24 mL of medium (Medium 199 supplemented with 10% fetal bovine serum, penicillin/streptomycin, mycostatin) was added to each flask and incubated for an additional 5 days. Subsequently, medium from the two flasks was combined, and aliquots were frozen at –80°C for later determination of working stock virus by plaque assay. Virus stock that had not been previously frozen was used to prepare the infectious blood. The working stock virus (log₁₀ 8.1 plaque-forming units [pfu]/mL) were combined with chicken

blood containing Alsever's anticoagulant (Hemostat, Dixon, CA) in a 1:10 ratio for log₁₀ 7.1 pfu/mL WNV-infected blood. We used the WN-FL03p2-3 strain of WNV (GenBank accession no. DQ983578) that was isolated from a pool of *Cx. nigripalpus* from Indian River County, Florida, in 2003. After the feeding trials, fully engorged females were held in 0.5 L cages at 25°C and 28°C, approximating the average temperature observed in geographic regions of Florida historically associated with high WNV transmission (Richards et al. 2007, <http://cdo.ncdc.noaa.gov/>). Mosquitoes were maintained in temperature-controlled incubators set with a 14:10 h light:dark cycle during the incubation period and provided with 10% sucrose solution and an oviposition substrate.

Females were stored at -80°C after 4, 9, 14, and 18 days postfeeding (time since exposure) and subsequently dissected to remove the legs from the bodies. At the same time, the saliva of a subsample of mosquitoes was used as an approximation of transmission. Saliva was collected from *Cx. coronator* in capillary tubes with immersion oil using methods by Anderson et al. (2010a). The bodies and legs were homogenized separately in 0.9 mL of BA-1 diluent. A 250-μL sample of mosquito body, legs, and saliva homogenate was used for RNA extraction using the MagNA Pure LC total nucleic acid isolation kit (Roche, Indianapolis, IN) and eluted in 50 μL of buffer according to the manufacturer's protocol. WNV RNA was measured using the Superscript III One-Step qRT-PCR Kit with a Platinum[®] Taq Kit (Invitrogen, Carlsbad, CA,) as described previously (Lanciotti et al. 2000, Richards et al. 2007). Quantitative RT-PCR was performed with the LightCycler[®] 480 system (Roche, Mannheim, Germany) using WNV-specific primers and probes (Chisenhall and Mores 2009). The LightCycler program for qRT-PCR was as follows: 48°C for 30 min, 95°C for 2 min, 45 cycles at 95°C for 10 s, and 60°C for 15 s, and 50°C for 30 s. The WNV titer present in samples was expressed in pfu equivalents (pfue)/mL using a standard curve method that compared cDNA synthesis for a range of serial dilutions of WNV in parallel with plaque assays using the same dilutions of virus (Richards et al. 2007).

Assays of mosquito bodies, legs, and saliva determined infection, dissemination, and transmission, respectively. Rates were calculated as the number of infected bodies, legs, and saliva from the total tested, expressed as a percent. Tests of infection, dissemination, and transmission enabled us to determine the vector competence of *Cx. coronator* for WNV and barriers to infection (e.g., midgut infection and escape barriers, salivary gland barrier [infection + escape]).

Temperature-related and temporal patterns in infection and dissemination were tested using separate ML categorical analyses of contingency tables (PROC CATMOD; SAS 2002) based on counts of individual mosquitoes being categorized as 1 or 0 for the presence or absence of virus, respectively. A similar approach was used to estimate transmission, except that the temporal patterns only included 9, 14, and 18 days postfeeding. The infection experiment with *Cx. coronator* and WNV was not repeated multiple times. We used individual mosquitoes as our replicates and analyzed infection responses by analysis of frequency distribution. ANOVA was used to test for differences in virus titers in the individual mosquito bodies for each temperature treatment and time since exposure. To account for the assumption of normality, virus titer was log₁₀ transformed. Nine to 73 mosquitoes were individually analyzed at each time point for each temperature for a

total of eight time point-temperature pairs (mean ± standard deviation [SD] number of mosquitoes tested; 25 ± 20). Significant effects were followed by Tukey-Kramer multiple comparisons among treatment least-squares means for pairwise comparisons.

Results

Reproductive biology and adult survival

Cx. coronator had significantly higher feeding rates on mammal (median ± SD percent fed of total offered blood; horse, 66.2 ± 21.6; rabbit, 30.8 ± 25.4; bovine, 38.1 ± 13.9; guinea pig, 47.2 ± 13.6; porcine, 50.0 ± 17.3) than avian hosts (median ± SD, chicken, 23.3 ± 20.8; turkey, 35.7 ± 5.0) using an artificial membrane feeding system (Hemotek[®]) ($\chi^2 = 6.9$, degrees of freedom [df] = 1, $p = 0.0083$). Pairwise comparisons of host blood feeding rates showed that *Cx. coronator* had significantly lower blood feeding rates on chicken than horse blood ($\chi^2 = 10.2$, df = 1, $p = 0.0014$). All remaining pairwise comparisons were not significantly different from one another. No significant differences were observed in the timing of oviposition from *Cx. coronator* fed on mammalian and avian blood sources ($\chi^2 = 10.3$, df = 6, $p = 0.1097$; Fig. 1A). Significantly greater numbers of eggs were laid by female adults fed on avian than on mammalian blood ($t = 4.9$, df = 106, $p < 0.0001$). Analyzing each blood type separately, ANOVA showed a significant effect of blood source on number of eggs laid ($F_{6, 101} = 5.89$, $p < 0.0001$). Significantly more eggs were laid by *Cx. coronator* fed on turkey blood than all other sources of blood, except rabbit and chicken (Fig. 1B). Regression analyses showed no significant relationship between *Cx. coronator* mass (mg) and number of eggs laid ($r^2 = 0.03$, $n = 40$, $p = 0.96$; Fig. 2A) or wing length (mm) and number of eggs laid ($r^2 = 0.02$, $n = 43$, $p = 0.87$; Fig. 2B); the latter was log₁₀-transformed to meet the assumption of normality. Non-parametric survival analyses showed significantly greater survival of female adults fed on avian than mammalian blood ($\chi^2 = 9.6$, df = 1, $p = 0.0019$). Comparison of survival over time showed significantly steeper declines for *Cx. coronator* previously fed on bovine blood compared to chicken ($\chi^2 = 9.3$, df = 1, $p = 0.04$) and turkey blood ($\chi^2 = 18.0$, df = 1, $p = 0.0005$) (Fig. 3). Marginally nonsignificant steeper declines in survival were observed for *Cx. coronator* fed on rabbit compared to turkey blood ($\chi^2 = 8.7$, df = 1, $p = 0.06$; Fig. 3).

Infection, dissemination, and transmission of WNV

Virus titers of freshly fed mosquitoes were 6.8 ± 0.1 and 6.7 ± 0.1 log₁₀ pfue of WNV/mL for *Cx. coronator* at 25°C and 28°C, respectively. WNV infected approximately 80–100% of *Cx. coronator* with no significant effect of temperature, time since exposure, or their interaction (all $\chi^2 < 2.2$, $p > 0.10$; Fig. 4A). Temperature ($\chi^2 = 7.92$, df = 1, $p = 0.0049$) and time since exposure ($\chi^2 = 36.54$, df = 3, $p < 0.0001$) significantly affected disseminated infections. The interaction of treatment factors on disseminated infections was marginally nonsignificant ($\chi^2 = 7.63$, df = 3, $p = 0.0542$). There were significantly more *Cx. coronator* with disseminated infections at 28°C than 25°C (Fig. 4B). On days 9, 14, and 18, there were significantly more *Cx. coronator* with disseminated infections than on day 4 (all $\chi^2 > 12.8$, $p \leq 0.0003$; Fig. 4B). Similarly on day 18, there were significantly more disseminated

FIG. 1. Differences in timing of oviposition (**A**) and number of eggs laid (**B**) after feeding on mammal and avian blood. Symbols associated with different letters show significant differences between blood sources. No error bars are shown for timing of oviposition for *Cx. coronator* fed on chickens because oviposition occurred on the same day.

infections than on days 9 and 14 (all $\chi^2 > 7.4$, $p \leq 0.0065$; Fig. 4B). Temperature ($\chi^2 = 3.82$, $df = 1$, $p = 0.0508$) significantly affected transmission, but not time since exposure ($\chi^2 = 0.74$, $df = 1$, $p = 0.6896$) or their interaction ($\chi^2 = 2.67$, $df = 1$, $p = 0.2632$). Significantly more *Cx. coronator* transmitted WNV at 28°C than 25°C (Fig. 4C).

WNV titer

Body titer varied significantly as a function of temperature ($F_{1, 189} = 46.89$, $p < 0.0001$), days since exposure ($F_{3, 189} = 51.62$, $p < 0.0001$), and their interaction ($F_{3, 189} = 4.36$, $p = 0.0054$). Body titer was significantly greater for *Cx. coronator* at 28°C than 25°C (Fig. 5). Body titer was significantly higher at later times since exposure (Fig. 5). For the interaction, body titer was significantly higher at 14 and 18 days than at 4 and 9 days since exposure at 25°C (Fig. 5). Body titer was significantly higher at 9, 14, and 18 days than at 4 days since exposure at 28°C (Fig. 5).

Discussion

Host-feeding patterns indicate that *Cx. coronator* is primarily a mammalian blood feeder (Mackay et al. 2010). We show under laboratory conditions that the source of the blood meal does not alter the timing of oviposition, but it does influence the number of eggs laid and the survival of adults. The number of eggs laid tended to be greater for avian than mammalian hosts, and survival was enhanced after having fed on avian-derived blood compared to some but not all mammalian blood sources, suggesting greater nutrition (or lower energetic cost of digestion) and corresponding reproductive fitness for feeding on avian hosts over some mammals. Although survival and fecundity of *Cx. coronator* appears to be higher when fed on avian blood, there seems to be a trade-off, because the feeding success on avian hosts was lower compared to mammalian hosts. Differences in avian and mammal blood have been demonstrated for other mosquito species. For example, *Cx. tarsalis* digests chicken blood

FIG. 2. Relationships of dry mass with number of eggs laid (A) and wing length and number of eggs laid (B) in *Cx. coronator* fed on mammalian and avian blood.

more rapidly than rodent blood (Downe and Archer 1975). Birds tend to be the most productive hosts in terms of fecundity for several *Culex* species (*Cx. quinquefasciatus*, Richards et al. 2010, Hamer et al. 2008; *Cx. nigripalpus*, Gomes et al. 2003; *Cx. salinarius*, Cupp and Stokes, 1976), although not always different from the mammal hosts surveyed (reviewed in Lyimo and Ferguson 2009). Similar results have been observed for *Ae. aegypti* (Bennett 1970). It has been hypothesized that nucleated erythrocytes of birds provide greater nutrition than anucleated mammal erythrocytes (Bennett 1970, Downe and Archer 1975), although there are exceptions, e.g., *Ae. albopictus* Skuse blood fed on guinea pig and human hosts had higher fecundity than those fed on chickens (Xue et al. 2009). Differences in the observed outcomes of studies suggest that both nutrition and evolutionary history with the host influence mosquito fecundity (Lyimo and Ferguson 2009, Takken and Verhulst 2012).

Although the source of blood did not alter the timing of oviposition, we observed that *Cx. coronator* had the capability to lay eggs several weeks after blood feeding. Plasticity in the timing of oviposition may influence mosquito reproductive cycles and arbovirus transmission. During periods of drought, the availability of suitable oviposition habitats may

be limited and so *Cx. coronator* may delay oviposition. Following rainfall, *Cx. coronator* will lay eggs and enter a host-seeking behavior. Extended periods of drought may enhance the potential of *Cx. coronator* as a WNV transmitter because the rate of dissemination was related to time since exposure. As a consequence, risk of WNV transmission by *Cx. coronator* may be highest after rainfall events that follow a warm drought period. For instance, heavy rainfalls synchronize *Cx. nigripalpus* blood feeding (Day and Curtis 1989) and oviposition (Day et al. 1990) and also are associated with epizootic and epidemic transmission of WNV (Shaman et al. 2005) and St. Louis encephalitis virus (Shaman et al. 2002, Day and Curtis 1999). The mechanism for amplification and epizootic transmission of these arboviruses seems to be due to drought-induced congregation of birds and mosquitoes in refuges allowing for amplification. Rainfalls subsequently increase availability of oviposition habitats and arbovirus-infected *Cx. nigripalpus* and birds disperse, allowing for the possibility of epidemic transmission.

The lack of a significant relationship between body size and the number of eggs laid deviates from the well-established notion that larger mosquitoes are more fecund. This result was surprising given that the range in sizes

FIG. 3. Survival of adult female *Cx. coronator* ($n=254$) fed on mammalian and avian blood (left y axis). Mean temperature in Indian River County, Florida, during the experiment (right y axis).

between mosquitoes in our study was similar to those in other mosquito species (*Ae. albopictus*, Lounibos et al. 2002; *Cx. quinquefasciatus*, McCann et al. 2009). However, we did not dissect mosquitoes to examine unladen (stage five) eggs, which may obscure relationships between mosquito size and fecundity. Another possibility is that, unlike several other studies, we did not deliberately manipulate the nutrient environment of the larvae and this may have resulted in a more pronounced size–fecundity relationship. Alternatively, the relationship between size and fecundity may vary with mosquito age. A comparison of *Cx. quinquefasciatus* categorized by age demonstrated that fecundity positively related to size of females aged 5–13 days, whereas no relationship between size and fecundity was found for females aged 17–25 days (McCann et al. 2009). This study demonstrated a decline in fecundity with age over a span of 25 days. These authors suggested that larger, but not smaller, mosquitoes may invest fewer lipids into their yolk mass with increasing gonotrophic age, as observed with *Ae. aegypti* (Briegleb et al. 2002), and this may account for age-dependency when body size is used to predict fecundity. This conclusion is supported in the current study because we used mosquitoes within this age range and we did not observe a relationship between size and fecundity. Along the same lines, because we did not include mosquito age as a treatment, we cannot determine whether these mosquitoes respond similarly to younger mosquitoes for the responses that were measured.

Viral titers used in the WNV infection study, $6.7\text{--}6.8 \log_{10}$ pfue WNV/mL, were comparable to viremia profiles for several orders of avian hosts (Komar et al. 2003, Guerrero-

Sánchez et al. 2011). *Cx. coronator* were highly susceptible to infection with WNV at both 25°C and 28°C, with similar rates of infection as observed for Florida *Cx. quinquefasciatus* (Anderson et al. 2010b) and *Cx. nigripalpus* (Richards et al. 2011) at 25°C and 28°C using the same WNV strain (WN-FL03p2-3). Increases in WNV titer with time since exposure indicated maximum virus population size in *Cx. coronator* by day 14 for 25°C and by day 9 for 28°C. These observations are comparable to those body titers observed in *Cx. nigripalpus* held at 28°C and tested at 6 and 12 days since exposure to WNV (Richards et al. 2011) and *Cx. quinquefasciatus* held at 25°C and 28°C and tested between 4 and 21 days since exposure to WNV (Anderson et al. 2010b, Richards et al. 2012). However, observed virus titers in the bodies of mosquitoes are somewhat higher than those observed in other studies (Reisen et al. 2006, Styer et al. 2007a, Ciota et al. 2011), which may be, in part, attributable to differences in testing methodologies (tissue culture versus qRT-PCR).

West Nile virus escape from the midgut occurred only at very low rates ($\sim 4\%$) at or before 4 days since exposure. Between 4 and 9 days since exposure, disseminated infections increased by ~ 10 -fold for *Cx. coronator* at 25°C and 28°C. Similar rates of dissemination were observed for *Cx. quinquefasciatus* fed $7.0 \log_{10}$ pfue WNV/mL (strain WN-FL03p2-3) at 25°C and 28°C (Anderson et al. 2010b). In contrast, rates of dissemination for *Cx. coronator* were lower than those observed for *Cx. nigripalpus* at 28°C (Richards et al. 2011). Percent of disseminated infections related to time since exposure with peak dissemination reached by day 14 for

FIG. 4. West Nile virus infection (A), dissemination (B), and transmission (C) maintained at 25°C and 28°C. Numbers above the bars show the total number of *Cx. coronator* females assayed for West Nile virus RNA.

Cx. coronator at 28°C and at day 18 for mosquitoes at 25°C, an indication of a time-sensitive midgut escape barrier (Kramer et al. 1981, Lutomiah et al. 2011). The percent of *Cx. coronator* infected and with disseminated infections tended to be equal to or higher than those estimates for known WNV vectors *Cx. pipiens* and *Cx. restuans* Theobald held at comparable environmental conditions fed similar amounts of WNV (Turell et al. 2001, Ebel et al. 2005). Rates of transmission were lower than those observed for dissemination, an

FIG. 5. Body titer ($\pm$ standard error [SE]) log₁₀ plaque-forming units equivalents/mL for *Cx. coronator* infected with West Nile virus. Body titers include individuals with nondisseminated and disseminated infections. Lowercase and uppercase letters show significant differences between body titer since time of exposure for *Cx. coronator* at 25°C and 28°C, respectively.

indication of a salivary gland infection and/or escape barrier for *Cx. coronator*. Additionally, studies have indicated that transmission rates detected with the hanging blood drop or capillary tube methods are lower than those detected when feeding on an actual susceptible animal (Akhter et al. 1982, Turell et al. 2006, Styer et al. 2007b). Observed transmission rates were similar or higher than those observed for known vectors *Cx. pipiens* (Ebel et al. 2005), *Cx. restuans* (Ebel et al. 2005), *Cx. nigripalpus* (Richards et al. 2011), and *Cx. quinquefasciatus* (Richards et al. 2012) under similar environmental conditions, and in some cases, using the same WNV isolate (e.g., Richards et al. 2011, 2012). Increases in temperature enhance the competence of *Cx. coronator* for WNV by shortening the time for viral dissemination and enhancing transmission. Although the mechanism(s) responsible for enhanced competence require further investigation, higher virus replication at warmer conditions may, in part, be responsible (Watts et al. 1987, Dohm et al. 2002) and is consistent with our observations on body titer.

Although it is not known whether *Cx. coronator* plays a role in the natural transmission of WNV in Florida, in some instances there may be a delay of years after a mosquito enters a new geographic region before it becomes involved in the transmission cycle (e.g., *Ae. albopictus* and La Crosse encephalitis virus; Gerhardt et al. 2001). Similar concerns have been suggested for newly established *Ae. japonicus* (Theobald) in North America (Sardelis et al. 2002). The results of this study combined with our knowledge of host-feeding patterns indicate that *Cx. coronator* could serve as a bridge vector for WNV between the enzootic avian cycle and mammals.

Conclusions

This is the first study evaluating the susceptibility to WNV infection and transmission by *Cx. coronator*. These data support the notion that the vector competence of *Cx. coronator* for WNV is similar to, and in some instances exceeds,

those rates observed for other known vectors of WNV. The rapid spread of *Cx. coronator* in Florida and other parts of the southeastern United States poses a potential public health risk.

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Author Disclosure Statement

No competing financial interests exist.

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