Larval habitats of potential mosquito vectors of West Nile virus in the Florida Keys

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ABSTRACT

The occurrence of larvae of two potential vectors of West Nile virus, *Culex nigripalpus* and *Culex quinquefasciatus*, was examined in the Florida Keys. About half of the aquatic habitats examined contained larvae of either one or both of the species. *Culex quinquefasciatus* was the most frequently encountered species, whereas only 9% of habitats sampled contained *Culex nigripalpus*. Over half of those samples that contained *Culex nigripalpus* also contained *Culex quinquefasciatus*. The two species utilize similar larval habitats in the Florida Keys, Monroe County, Florida, USA.

Key words | aquatic insects, Florida, mosquitoes, West Nile virus

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INTRODUCTION

Mosquitoes in the genus *Culex* are the most important vectors of West Nile (WN) virus worldwide (Hayes 1989). Adult females of the mosquito species *Culex nigripalpus* Theobald and *Culex quinquefasciatus* Say have been found naturally infected with WN virus in the Florida Keys (Hribar *et al.* 2004*b*). Both of these species are competent laboratory vectors of WN virus (Sardelis *et al.* 2001; Turell *et al.* 2005). Although their larvae may be collected in water-filled containers near human habitation (Nayar 1982; Hribar *et al.* 2001, 2004*a*), risk of infection with WN is focal and varies in space and time (Rutledge *et al.* 2003). Therefore, this study was initiated to identify the habitats where the larvae of these two potential vector species occur in the Florida Keys, Monroe County, Florida, USA.

METHODS

Larval mosquitoes were collected daily from 1 January until 31 December 2002 (exclusive of weekends and holidays). Natural and artificial containers, septic tanks, sewage treatment plants and standing ground waters were examined visually for presence or absence of mosquito larvae. When larvae were encountered they were collected either by use of a 250 ml (half-pint) dipper or a 45 ml turkey baster, depending on the size of the larval habitats. Narrow-mouthed containers (e.g. drinks bottles) were ignored because they usually do not produce significant numbers of mosquito larvae (Focks *et al.* 1981). One larval sample was removed from each larval habitat; no attempt was made to remove all larvae. Larval samples were placed into 150-ml plastic jars and returned to the laboratory daily for identification. Specific identification and habitat type were recorded for each larval sample. All larvae removed from the same container during the same inspection event were considered to be one sample. Number of larval samples per species per habitat was tabulated. Results for each species are reported as number of collections, not number of larvae.

Possible association between *C. nigripalpus* and *C. quinquefasciatus* was investigated by use of Cole's index of interspecific association (Cole 1949). Cole's coefficient of interspecific association utilizes only numbers of collections in which two species occur together, and can vary from +1, complete association, to −1, complete dissociation. Significance of Cole's index of interspecific association was tested via a χ^2 analysis (Service 1993). Differences in rank order of

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larval habitats exploited by both species were investigated via Kendall's τ statistic. This statistic was calculated for the 16 most commonly utilized larval habitats for both species. Larval habitats with less than 20 total samples were not included in the calculations of Kendall's τ statistic because 'rare' categories may skew the calculation of Kendall's τ and obscure patterns among the more common categories (Bullock 1971). Statistical analyses were calculated with the aid of SPSS computer software (SPSS, Inc. 2001). Samples lacking habitat data or specific identification of larvae were not included in statistical analyses.

RESULTS

A total of 4,777 mosquito larval collections were examined, 4,589 of which were included in the statistical analyses. Of these, 2,289 (49.9%) contained neither *C. nigripalpus* nor *C. quinquefasciatus* larvae. The remaining 2,300 samples consisted mostly of *C. quinquefasciatus* (2,093; 91%). *Culex nigripalpus* was found in 9% of the larval samples. The co-occurrence of *C. nigripalpus* with *C. quinquefasciatus* was detected in 58% of samples containing *C. nigripalpus* (Table 1). The coefficient of interspecific association was 0.190 ± 0.001, indicating a slightly positive association between the two species. This association was significant at the 0.05 level, χ^2 (7.206) $> \chi^2_{0.05}$ (3.841).

There was a significant correlation of rank-order of occurrence of larvae in the 16 most commonly used larval habitats ($\tau = 0.513$, $P = 0.007$, two-tailed test). Septic tanks and sewage treatment plants were common habitats for *C. quinquefasciatus* larvae, but much less so for *C. nigripalpus*. Buckets and boats were common larval habitats for both species, as were ornamental ponds, tyres and garbage receptacles. Discarded plastic containers, especially food containers, were also utilized by both species. Interestingly, vases, flowerpots and plant trivets were only rarely used by *C. nigripalpus*, whereas *C. quinquefasciatus* larvae were often collected from these containers (Table 2).

The diversity of containers utilized by *Culex* species mosquitoes was impressive. In addition to the larval habitats listed in Table 2, ornamental plants such as bromeliads were found to serve as larval habitats for these species, as well as less commonly encountered larval habitats such as abandoned household fixtures (bathtubs, toilets, sinks), discarded cooking utensils (broiler pans, cups, pots), pet dishes, automobiles and golf carts,

Table 1 | Contingency Table for presence or absence of *Culex* species mosquitoes in larval samples

	C. quinquefasciatus	
C. nigripalpus	**Present**	**Absent**
Present	120	87
Absent	2,093	2,289

Table 2 | Larval habitats most commonly utilized by *Culex* species in the Florida Keys

Larval habitat	*C. quinquefasciatus*	*C. nigripalpus*
Septic tanks and sewage treatment plants	323	8
Buckets and rooting pails	241	20
Boats	217	14
Ornamental ponds	196	20
Tyres	177	14
Rubbish bins and dumpsters	141	13
Plastic containers	105	14
Vases, flower pots and plant trivets	85	3
Fountains	82	5
Swimming pools	75	4
Hot tubs	49	1
Wheelbarrows	36	2
Tarpaulins	26	3
Metal containers	24	0
Standing water and cisterns	20	17
Bird baths	14	2

children's toys and swimming pools, Styrofoam® coolers, and holes in street kerbs.

DISCUSSION

A major source of *Culex* mosquitoes in the Florida Keys appears to be larval development in water-filled containers near homes and businesses. This agrees with the study of Heidt (1964), who found buckets, tyres, fish ponds, rooting pails and pools to be major sources of *C. nigripalpus* larvae. The degree of association of larvae of both *Culex* species was slight, however, and may be due to some factor other than type of container (Marcondes & Paterno 2005). Kendall's τ statistic revealed that a significant correlation exists for larval habitat use by *C. quinquefasciatus* and *C. nigripalpus*. This at first appears surprising, since *C. nigripalpus* and *C. quinquefasciatus* often use different larval habitats and water quality. Female *C. nigripalpus* oviposit more often in larger water bodies, whereas *C. quinquefasciatus* is more often found in smaller containers; *C. quinquefasciatus* will also oviposit into water of lesser quality than will *C. nigripalpus* (Root 1922; Provost 1969; Frank 2000; Vinogradova 2000). However, O'Meara & Evans (1983) collected large numbers of *C. nigripalpus* from a nutrient-rich sewage lagoon on a diary farm in Okeechobee County, Florida. It may be that differences in seasonal activity between *C. nigripalpus* and *C. quinquefasciatus* in south Florida account for some of the variation seen in this study (O'Meara & Evans 1983).

Water-filled containers are a developmental site for vector mosquitoes that residents can remove on their own initiative, without need for mosquito control personnel. In spite of public education efforts by the District, including visits to elementary schools, a weekly television programme, and public service announcements placed in newspapers, some residents persist in maintaining suitable larval habitat near their homes and businesses. Perhaps there is a difference in tolerance to mosquitoes among residents. Hribar *et al.* (2001) found more containers holding water, and more containers with mosquito larvae, in the rural areas of the Florida Keys than in the urban ones. From the standpoint of nuisance reduction and disease prevention, this is one aspect of domestic mosquito production that should be investigated more thoroughly.

CONCLUSIONS

1. About half of the aquatic habitats examined contained *Culex nigripalpus* or *Culex quinuefasciatus* larvae.
2. *Culex quinquefasciatus* larvae are more commonly collected in aquatic habitats than are larvae of *Culex nigripalpus*.
3. There is a positive association in the co-occurrence of larvae of the two species in aquatic habitats in the Florida Keys.
4. The two *Culex* species utilize similar larval habitats in the Florida Keys.

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