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SCIENTIFIC NOTE

IMPACT OF THREE SPECIES OF AQUATIC PLANTS ON *ANOPHELES QUADRIMACULATUS* AND ITS EFFECT ON THE EFFICACY OF BORIC ACID SUGAR BAITS

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ABSTRACT. The purpose of this study was to investigate the sugar-feeding behavior of *Anopheles quadrimaculatus* by measuring the impact of different aquatic plants on its survival. At the same time, the potential use of boric acid in toxic sugar bait (TSB) applications to the leaves of these plants was also evaluated. Mean survival rates of mosquitoes after 120 h feeding on 3 common aquatic plant species—*Thalia geniculata*, *Pontederia cordata*, and *Limnobium spongia*—were 10.55%, 1.86%, and 6.21%, respectively. No significant difference in mortality between mosquitoes feeding on separate plant species was detected ($P = 0.05$). The TSB efficacy was evaluated by leaf dip bioassay to compare 24-h mortality of mosquitoes feeding on leaves treated with TSB formulation (1% boric acid, 10% sucrose) and leaves dipped in 10% sucrose. Mortality was significantly higher for TSB-treated leaves for *T. geniculata* ($t = 12.5$, df $= 8$, $P < 0.0001$) and *P. cordata* ($t = 5.42$, df $= 8$, $P = 0.0006$) than for *L. spongia* ($t = 1.4003$, df $= 8$, $P = 0.199$). One-way ANOVA analysis showed no significant difference in efficacy between TSB-treated leaves of the 3 plants.

KEY WORDS *Anopheles quadrimaculatus*, aquatic vegetation, boric acid, toxic sugar bait survival

The mosquito species *Anopheles quadrimaculatus* Say is a nuisance biter and important malaria vector widely distributed throughout the eastern USA (Reinert et al. 1997). Historically known as the primary malaria vector within North America, *An. quadrimaculatus* is also a capable vector for Cache Valley virus, West Nile virus, eastern equine encephalitis, and St. Louis encephalitis (O'Malley 1992; Blackmore et al. 1998; Moncayo et al. 2000a, 2000b). Despite its public health significance, there are few studies in the existing literature on *An. quadrimaculatus* sugar-feeding behavior and none examining specific mosquito–plant interactions (Burkett et al. 1999, Qualls et al. 2014). This mosquito species is frequently associated with aquatic vegetation and can be found breeding in a wide range of habitats such as rice fields, swamps, freshwater marshes, and the vegetated margins of lakes, ponds, and water reservoirs (Orr and Resh 1989, O'Malley 1992). However, the exact nature of interaction between aquatic plants and the mosquito is not fully understood. Investigating the impact of different aquatic plant species on adult *An. quadrimaculatus* survival may gather critical insight into its sugar-feeding behavior, which can be used to develop and implement new control strategies against this species.

One promising technology that may be suitable for targeted application against aquatic vegetation is attractive toxic sugar bait (ATSB) (Fiorenzano et al. 2017), which works by targeting the need for mosquitoes to regularly sugar feed (Yuval 1992, Foster 1995). The ATSB and toxic sugar bait (TSB) (without attractant) function as an oral insecticide by incorporating a toxin and attractant into a sugar solution, which can be administered as either a bait station or a foliar surface spray. These have been successfully field-tested in Israel, Mali, Morocco, and Florida, demonstrating significant reductions in mosquito populations across arid, semiarid, and subtropical environments (Xue et al. 2006, Muller and Schlein 2008, Beier et al. 2012, Naranjo et al. 2013, Qualls et al. 2014). Boric acid sugar baits sprayed on plants in the laboratory and field have demonstrated significant control of *Aedes albopictus* (Skuse) (Xue et al. 2006) and caused high toxicity to *An. quadrimaculatus* evaluated in the laboratory (Xue and Barnard 2003). However, there is a lack of documentation on the survival of this species of mosquitoes fed on common aquatic plants sprayed with boric acid sugar baits against *An. quadrimaculatus*.

The purpose of this study was to test the impact of different aquatic vegetation as the sole source of a sugar meal on adult *An. quadrimaculatus*. At the same time, the potential use and efficacy of boric acid mixed with sugar without attractant as TSB foliar application was also evaluated.

Laboratory studies were conducted using colonized laboratory-reared *An. quadrimaculatus* Say supplied by US Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. The mosquitoes were reared following standard protocol, maintained at 27–28°C, 80–85% RH, and sustained on a 10% sucrose solution. Larvae were hatched and reared in the

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Table 1. Percent mortality (± SE) of adult *Anopheles quadrimaculatus* ($n = 20$) in treatment and control cages. Mortalities (%) at 48, 72, 96, and 120 h were corrected by using Abbott's formula due to high mortality presented in control group.

Plant species	Mosquito exposure (% mortality ± SE)				
	24 h	48 h	72 h	96 h	120 h
Thalia geniculata (fireflag)	15.00 ± 3.33	54.37 ± 6.43	60.98 ± 6.15	76.40 ± 6.92	89.45 ± 6.07
Pontederia cordata (pickerel weed)	10.00 ± 4.41	46.70 ± 5.71	70.12 ± 4.06	93.17 ± 4.41	98.14 ± 4.34
Limnobium spongia (frogsbit)	6.67 ± 2.76	40.12 ± 3.34	71.96 ± 3.77	83.23 ± 4.12	93.79 ± 3.61
Control (10% sucrose)	2.78 ± 1.21	7.22 ± 2.65	8.89 ± 2.86	10.56 ± 2.27	10.56 ± 2.27

insectary of Anastasia Mosquito Control District (AMCD), St. Augustine, FL.

Laboratory tests were conducted to evaluate the survival duration of adult *An. quadrimaculatus* mosquitoes feeding on 3 different aquatic plants—*Thalia geniculata* L. (fireflag), *Pontederia cordata* L. (pickerel weed), and *Limnobium spongia* Humb. and Bonpl. (frogsbit)—collected from local waterways and ponds surrounding AMCD, 120 EOC Drive, St. Augustine, FL 32092. Plants were pruned of flowers, potted into 500-ml plastic containers, and placed inside either BUGDORM 2120 Insect Rearing Tents (BioQuip, Rancho Dominguez, CA) (60 cm long × 60 cm wide × 60 cm high) or converted 5-gal Home Depot (St. Augustine, FL) insect rearing buckets (*L. spongia*) due to availability. Treatment cages were exposed only to the potted plant species. Control cages were exposed only to a 10% sucrose solution administered through a cotton wick from a sealed plastic cup. Each plant had 3 treatment cages and 1 control for a total of 12 cages. Twenty mosquitoes (10 male and 10 female; 5–7 days old) were aspirated into each cage. Number of dead mosquitoes was recorded every 24 h for 5 days, and the survival was calculated by comparing with control group. The experiment was repeated 3 times.

For foliar TSB application, leaf bioassays were conducted to compare mortality of mosquitoes feeding on TSB-treated leaves with those feeding on 10% sucrose–treated leaves. Ten mosquitoes (5–7 days old) were aspirated into paper cages (24 cm^3) covered with a mesh fabric. Treatment 1 had leaves dipped in a 1% boric acid, 10% sucrose TSB solution. Treatment 2 had leaves dipped into a 10% sucrose solution. Control cages were exposed to cotton balls dipped in a 10% sucrose solution. The dried leaves or cotton balls were placed directly onto the mesh cover for the mosquitoes to feed on. Each of the 3 plants for both the TSB and sucrose solution had 3 treatments and 1 control for a total of 24 cages (12 cages each). Cages were held in the insectary with optimal humidity. Mortality was recorded at 24 h; mosquitoes were considered dead if they were unable to stand and had no wing movement. The experiment was repeated 3 times.

Data were analyzed using JMP statistical software (SAS Institute Inc., Cary, NC). Data collection and methods were standardized with an $\alpha = 0.05$ Type 1 error to assess significance. A 1-way ANOVA analysis was conducted to assess differences in survival rates at 24-h intervals for 120 h after the data were corrected by using the Abbott's formula (Abbott 1925). Also, a 1-way ANOVA analysis was conducted to assess differences in mortality at 24 h for plants dipped in TSB solution. A paired *t*-test was conducted to assess differences in mortality for leaves dipped in the TSB solution to leaves dipped in the 10% sucrose solution.

As shown in Table 1, mortality of *An. quadrimaculatus* separately fed on all 3 species of aquatic plants for 120 h was 89.45% for *T. geniculata*, 98.14% for *P. cordata*, and 93.79% for *L. spongia*. There was a significant difference in mortality between *An. quadrimaculatus* feeding on *T. geniculata*, *P. cordata*, and *L. spongia* compared with the control group. However, there was no significant difference in mosquito mortality among the 3 plant species. Moreover, mosquitoes on all 3 plant species showed a gradual declines then a sharp drop-off.

Mean mortality of mosquitoes exposed for 24 h to TSB on *T. geniculata*, *P. cordata*, and *L. spongia* was 95.6%, 98.9%, and 83.3%, respectively (Table 2). Mortality of mosquitoes exposed to TSB-treated leaves of *T. geniculata* and *P. cordata* was significantly higher than that of *L. spongia,* as well as mortality in control. The difference in mean mortality between TSB- and 10% sucrose solution–treated leaves leaf dips was significantly higher for TSB-treated leaves of *T. geniculata* ($t = 12.5$, df $= 8$, $P < 0.0001$) and *P. cordata* ($t = 6.095$, df $= 8$, $P = 0.0003$) than for *L. spongia* ($t = 1.4003$, df $= 8$, $P =$

Table 2. Mean mortality (% ± SE) of *Anopheles quadrimaculatus* exposed for 120 h to toxic sugar bait (TSB)– and sugar solution–dipped leaves for 3 aquatic plant species under laboratory conditions. The table shows *t*-values and *P*-values for data comparison.

Plant species	TSB	10% sucrose	Difference	*t*-value	*P*-value
Thalia geniculata (fireflag)	95.6 ± 0.24	4.00 ± 0.50	91.6 ± 0.44	12.5	<0.0001
Pontederia cordata (pickerel weed)	98.9 ± 0.11	0.56 ± 0.75	98.3 ± 0.80	5.42	0.0006
Limnobium spongia (frogsbit)	83.3 ± 0.50	6.67 ± 0.78	76.63 ± 1.19	1.4	1.199
Control	0.44 ± 0.24	0.10 ± 0.11	0.34 ± 0.29	1.15	0.2815

0.199). However, 1-way ANOVA analysis showed no significant difference in efficacy between TSB-treated leaves of all 3 plants.

Survivorship of *An. quadrimaculatus* at 120-h feeding exposure to the 3 common species of aquatic plants ranged between 1.86% and 10.55%, indicating limited sugar-feeding activity (Table 1). One potential explanation for this may be due to difficulty feeding on plant stems and leaves. Previous studies have shown mosquitoes predominantly acquire their nutrition and energy resources from floral nectar, honeydew, and fruit (Yuval 1992). Furthermore, no significant difference in adult *An. quadrimaculatus* survival outcomes between plant species may indicate that plants only minimally provide a sugar resource. These results are consistent with the report that common species of Florida landscaping plants did not significantly impact survival of *Ae. albopictus* under laboratory conditions (Seeger et al. 2017).

In contrast, TSB laboratory results demonstrated high efficacy, with mortality exceeding 83% for all plant species. There were significant differences in mortality of TSB-treated leaves compared with the 10% sugar solution for *T. geniculata* and *P. cordata*, but not *L. spongia*. The discrepancy for *L. spongia* was due to a high mortality for 10% sucrose–treated leaves, which may be due to difficulty in feeding on the leaf. This could potentially be explained by reduced points of contact due to bending as the leaf dried.

This study demonstrated no significant differences in the survival of *An. quadrimaculatus* feeding on 3 common species of aquatic plants in addition to the potential effectiveness of targeted foliar TSB application. Aquatic plants have been suggested to play an important role in anopheline breeding cycles by providing a stable habitat and refuge from potential predators such as *Gambusia affinis* Baird & Girard, 1853 (Orr and Resh 1989). Water with well-developed beds of floating leaf or rooted vegetation may also offer enhanced larval food resources and favorable oviposition sites for adults (Orr and Resh 1989). This study attempted to evaluate if emergent adults may benefit from the immediate proximity to these same plants as a food source. Though study results suggest that *An. quadrimaculatus* does not depend much on aquatic plants for sugar feeding, more in-depth studies may reveal important mosquito–plant interaction that can improve mosquito control efforts. Future research should consider examining the effects flowering bodies have on *An. quadrimaculatus* survival as well as plant impact on larvae.

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