Behavioral responses of gravid *Culex quinquefasciatus, Aedes aegypti,* and *Anopheles quadrimaculatus* mosquitoes to aquatic macrophyte volatiles

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ABSTRACT: Mosquitoes use many cues to assess whether a habitat is conducive for reproduction, possibly including the presence of stimuli from aquatic macrophytes. The effect of water infusions of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pista stratioles*), parrotfeather *(Myriophyllum aquaticum)*, and water pennywort (*Hydrocotyle umbellata*) on mosquito oviposition and attraction was investigated. Gravid *Culex quinquefasciatus* deposited significantly more egg rafts in water hyacinth, water lettuce, or Bermuda hay (positive control) infusions compared to water, while water pennywort and parrotfeather infusions did not differ from water. In-flight attraction responses of *Cx. quinquefasciatus*, *Aedes aegypti,* and *Anopheles quadrimaculatus* were evaluated. The strongest attraction of gravid *Cx. quinquefasciatus* and *Ae. aegypti* occurred in the presence of volatiles from infusions of water hyacinth and water lettuce, which were equal in attractiveness to hay infusion. Water pennywort and parrotfeather infusions were not attractive. Gravid *An. quadrimaculatus* were not attracted to aquatic plant volatiles. The results suggest that water hyacinth and water lettuce emit volatile chemicals that attract two of three mosquito species tested and stimulate oviposition by Cx. *quinquefasciatus*, demonstrating that the level of attraction of aquatic plant volatiles varies among species in ways that may have relevance to bait-based detection and control methods. ***Journal of Vector Ecology*** 43 (2): 252-260. **2018.**

Keyword Index: Aquatic weed, water lettuce, water hyacinth, hay infusion, oviposition, Diptera, Culicidae, volatiles.

INTRODUCTION

The association of plants with mosquito oviposition and larval habitats has been characterized for a wide range of mosquito species with plants providing habitat (phytotelmata), air, shelter, or nutrition associated with microbial activity (Bentley and Day 1989, Clements 1999, Day 2016, Rejmánková et al. 2013). Chemosensory cues used for location of resources may be influenced by the presence of conspecific eggs and immatures (Allan and Kline 1998, Zahiri and Rau 1998), mosquito predators and parasites (Angelon and Petranka 2002), microbial fauna often interacting with plant material (Hazard et al. 1967, Millar et al. 1992, Ikeshoji et al. 1975, Poonam et al. 2002, Sumba et al. 2004, Ponnuswany et al. 2008), pheromones (Bentley and Day 1989), or plant odors (Nyasembe and Torto 2014, Wondwosen et al. 2016, Asmare et al. 2017). Oviposition site selection is mediated by a variety of environmental, tactile, visual, and olfactory cues (Bentley and Day 1989, Clements 1999, Afify and Galizia 2015, Day 2016), of which olfactory cues appear to be the most important and best characterized. Many studies examining the involvement of plant odors with attraction for oviposition relate to the microbial degradation of plant materials, not odors associated directly with living plants (Bentley and Day 1989, Millar et al. 1992, Sumba et al. 2004, Navarro-Silva et al. 2009, Afify and Galizia 2015). For instance, attraction to hay infusion is related to microbial

production of chemicals such as indole and 4-methyl phenol (Millar et al.1992, Ponnusamy et al. 2008). The potential role of volatiles from living aquatic plants remains unexamined. Of the cues of plant origin related to mosquito oviposition listed by Afify and Galizia (2015), none consisted of aquatic plants. Similarly, while there are recent reports of the attraction of gravid *Anopheles* to grass, rice, and maize pollen volatiles (Wondwosen et al. 2016, 2017, Asmare et al. 2017), investigations of the possible role of aquatic plants remain underrepresented.

A wide range of aquatic macrophytes are associated with mosquito habitats, including plants that are emergent (e.g., cattails *Typha* spp.; Typhaceae) and floating (e.g.,water lettuce, *Pistia stratiotes* L.; Pontederiaceae) (Walton and Workman 1998, Russell 1999). Native aquatic plants constitute important components of ecosystems (Carpenter and Lodge 1986, Engelhardt and Ritchie 2001) and some invasive species have potential use as biofuel or livestock feed (Kaur et al. 2017), and in water remediation efforts (Muradov et al. 2014, Sharma et al. 2015). However, invasive, mostly non-native floating aquatic weeds can cause great environmental and economic harm due to their ability to proliferate and obstruct waterways (Getsinger et al. 2014). In the Sacramento-San Joaquin River Delta region of northern California, floating plants such as water hyacinth (*Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.; Haloragaceae), and native water

pennywort (*Hydrocotyle ranunculoides* L. f; Araliaceae), among others, cover the surface of canals and sloughs, impeding water conveyance for agriculture and domestic use, hindering recreational navigation, and degrading aquatic habitat quality (Sytsma and Anderson 1993, Santos et al. 2009, Boyer and Sutula 2015, Ta et al. 2017). Water hyacinth and water lettuce pose similar threats in Florida (Tipping et al. 2014, Allen and Keith 2015) even though water lettuce is native to Florida (Evans 2013). Aquatic macrophytes can provide both habitat and nutritional support for mosquito larvae resulting in enhanced fitness of adults (Orr and Resh 1992, Duguma et al. 2013). The role of volatile cues from aquatic plants that impact mosquito oviposition is poorly understood. Recently, Webb et al. (2012) reported enhanced oviposition by *Culex annulirostris* Skuse but not *Culex quinquefasciatus* Say in the presence of giant salvinia, *Salvinia molesta* D. Mitch (Salviniaceae). Neither species exhibited increased oviposition in the presence of water hyacinth or dwarf papyrus sedge, *Cyperus* haspan L. (Cyperaceae).

The objective of the study was to examine the response of gravid females of several mosquito species (Diptera: Culicidae) present in Florida to volatiles associated with several species of native and invasive aquatic plant species. Initially, plant infusions were screened against gravid *Cx. quinquefasciatus* to determine if oviposition responses occurred in the presence of the plant volatiles. Secondly, the effects of volatiles from infusions on in-flight orientation of gravid *Cx. quinquefasciatus*, *Aedes aegypti* (L.) and *Anopheles quadrimaculatus* Say were evaluated.

MATERIALS AND METHODS

Biological materials

Adults of *Cx. quinquefasciatus, Ae. aegypti,* and *An. quadrimaculatus* were obtained from laboratory colonies at the USDA Center for Medical, Agricultural, and Veterinary Entomology facility in Gainesville, FL and were reared as described in Gerberg et al. (1994). While laboratory colonies and field populations may differ in levels of response (Hoffman and Ross 2018), the same stimuli can elicit responses in both (Clark et al. 2011). Gravid mosquitoes for assays were prepared by blood-feeding (defibrinated bovine blood) adults 2.5 days before the day of assays for *Ae. aegypti* and *An. quadrimaculatus* and four days before the start of assays for *Cx. quinquefasciatus*. Mosquitoes were maintained at 27-29° C under a 14:10 h L:D photoperiod and adults were continuously provided with a 5% sugar solution. Invasive plant species used for comparisons included water hyacinth and rooted parrotfeather. Native aquatic plants included rooted/floating water pennywort and floating water lettuce. All plants were collected from ponds near Gainesville, FL and rinsed in well water to remove associated debris and arthropods. All plants were collected within a three-day period and used within two weeks of collection. Plants were held prior to making infusions in plastic wading pools (ca. 1 m diam) filled with well water (20 cm deep) in a greenhouse under natural lighting conditions. Hay infusion, known to be attractive to many gravid mosquito species (Polson et

al. 2002, Allan and Kline 2004), was included as a positive control. Hay infusion was prepared with Bermuda grass hay, *Cynadon dactylon* (L.) Pers. (Poaceae) and prepared following Millar et al. (1992) (450 g dried hay, 5 g brewer's yeast, 20 g lactalbumen hydrolysate, 75 liters water, fermented 7-12 days). Infusions of aquatic plants were prepared by placing equivalent amounts of of each taxon of aquatic vegetation to cover the surface area of water in 2 liters of well water (non-chlorinated) in 2 liter glass beakers for 48 h. Plant roots were rinsed thoroughly under running tap (well water) water for at least 3 min before being placed in glass beakers. Rinsing was done to remove associated arthropods and organic debris associated with the roots. Immediately prior to assays, plants were removed and the water was used for assays. Plants were held under laboratory conditions at 27-29° C under a 14:10 h L:D photoperiod.

Two-choice cage assays

Two-choice cage assays were used to evaluate the effect of plant infusions from invasive (i.e., water hyacinth, parrotfeather) and native (water lettuce, pennywort) plant species on mosquito oviposition site selection. A 10% hay infusion solution was included as a positive control and water served as a negative control. Research was conducted in the laboratory at 27-29° C, 70-85% relative humidity, and a 14:10 L:D photoperiod at the USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology laboratory in Gainesville, FL. Assays were conducted in standard screened mosquito rearing cages (30x30x30 cm, BioQuip, Rancho Dominguez, CA) that contained two clear glass 295.7 ml Pyrex° bowls (Corning, NY) containing 266 ml of either unchlorinated water (control) or plant infusions. Each pair of bowls was placed in the center of the cage with at least 4 cm between the bowls. Before and after use, bowls were washed with hot soapy water, rinsed with water, methanol and acetone, and dried overnight at 80º C. Treatments included a water control tested with either a water control, or infusion of water hyacinth, parrotfeather, water pennywort, water lettuce, or hay. A total of 12 replicates were run per treatment. Assays were conducted over a 24-h period with the paired bowls of control and treatment solutions placed in a cage along with 50 gravid *Cx. quinquefasciatus* that had been blood-fed four days previously. Female mosquitoes were chilled for counting and verification of gravid status prior to the assays. Mosquitoes in each cage were observed for 15 min after release to verify that they were alive and moved normally following handling. Mosquitoes were discarded after each assay and not reused. After 24 h, bowls were removed from cages, egg rafts were counted, and the proportion of rafts deposited in each bowl was determined for each cage. The position of the treatment and control in each cage were switched between replicates.

Olfactometer assays

Responses of gravid *Cx. quinquefasciatus, Ae. aegypti,* and *An. quadrimaculatus* to plant volatiles were evaluated using a two-choice triple-cage olfactometer (Posey et al. 1998). The olfactometer consisted of a large, rectangular acrylic arena partitioned into three stacked chambers. Each

chamber contained mosquitoes with moist (RH 60-62%), warm (27-29° C), charcoal-filtered air entering into the chamber through a pair of ports. Air was removed from the chamber from the opposite screened end. Upwind of the ports were shelves where treatments could be placed such that their volatiles could enter the chambers and stimulate attracted mosquitoes to fly upwind and enter the ports. Ports were screened with funnels so that mosquitoes that entered the ports would be trapped and could be counted at the end of the assay. Ports could be accessed through iris closures to remove mosquitoes and replace treatment and control solutions. Air flow through the ports was 28.0 cm/s. Within the middle chamber, 50 gravid mosquitoes were released and allowed to acclimate for 1 h. Female mosquitoes were chilled for counting and verification of gravid status prior to the assays. Mosquitoes were observed for 15 min after release to verify that specimens were alive and responsive. After 1 h, a glass bowl that contained 266 ml of either water (negative control), hay infusion (positive control), or plant infusion was added to each port. All materials used in the olfactometer were handled with gloves to avoid potential contamination with skin. Assays were conducted for 1 h, during which time mosquitoes had the option to remain in the chamber or follow an upwind current to either the treatment port or control port. At the end of the test, mosquitoes collected in each port were counted and proportions of responding mosquitoes calculated for each port. Each test was replicated ten times.

Data analysis

Means for treatment and control comparisons of egg raft or female mosquito counts for two-choice bioassays and olfactometer assays were evaluated for normality using Shapiro-Wilk tests and equality of variance test and differences among treatments examined with paired t-tests if data were normal and by Mann-Whitney Rank Sum test if data were not normal. Comparisons also were made among treatment means of final egg rafts or number of mosquitoes for data from two-choice assays and olfactometer data, respectively. First, data were tested for normality and if normal, a one-way ANOVA was conducted. If data were not normal, a Kruskal-Wallis one-way ANOVA on ranks was conducted. Differences between means were detected using Student-Newman-Keuls test ($P < 0.05$). Analyses were conducted using SigmaStat (Systat, San Jose, CA).

RESULTS

Two-choice cage assays

Oviposition responses (number of egg rafts deposited) of gravid *Cx. quinquefasciatus* to various plant infusions and controls are presented in Figure 1. There was no difference between water presented as a treatment or control ($t = 0.19$; df = 1,22; $P < 0.85$). Hay infusion elicited strongly positive responses in oviposition compared to the water control ($U = 0$; N = 12; $P < 0.001$). Parrotfeather infusion did not affect oviposition choice ($t = 1.54$; df = 1,22; $P = 0.13$). In contrast,
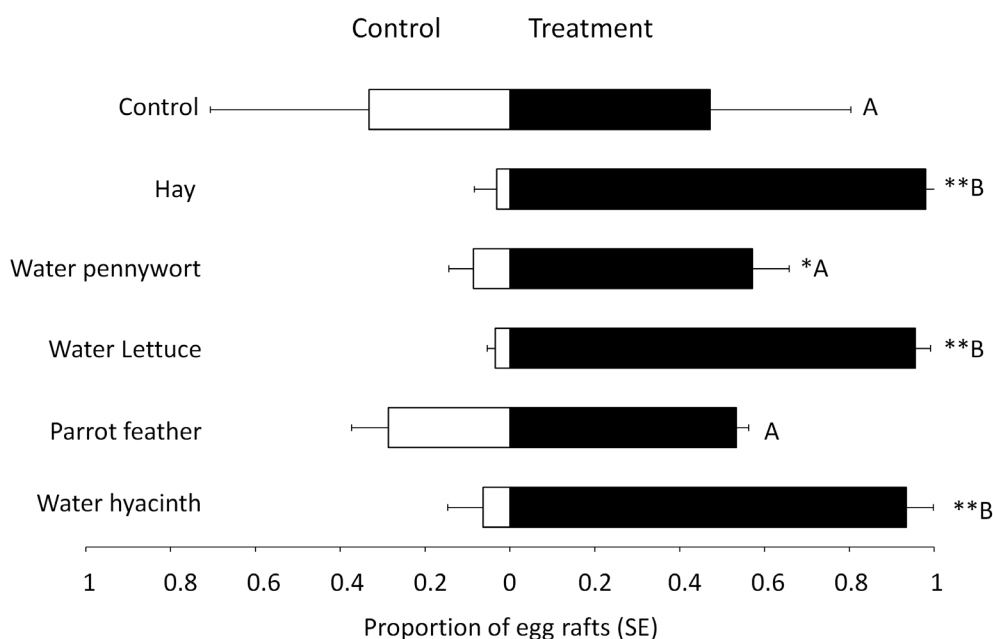

Figure 1. Proportion of *Culex. quinquefasciatus* egg rafts deposited in treatment (different plant infusions) or control (water, hay infusion) bowls in a laboratory cage two-choice bioassay. Assays were conducted for 24 h and replicated 12 times. Responses significantly different from those observed in controls are designated by asterisks (* $P < 0.05$, ** $P < 0.001$). Treatment bars (SE) with different letters are significantly different in a one-way ANOVA ($P < 0.05$).
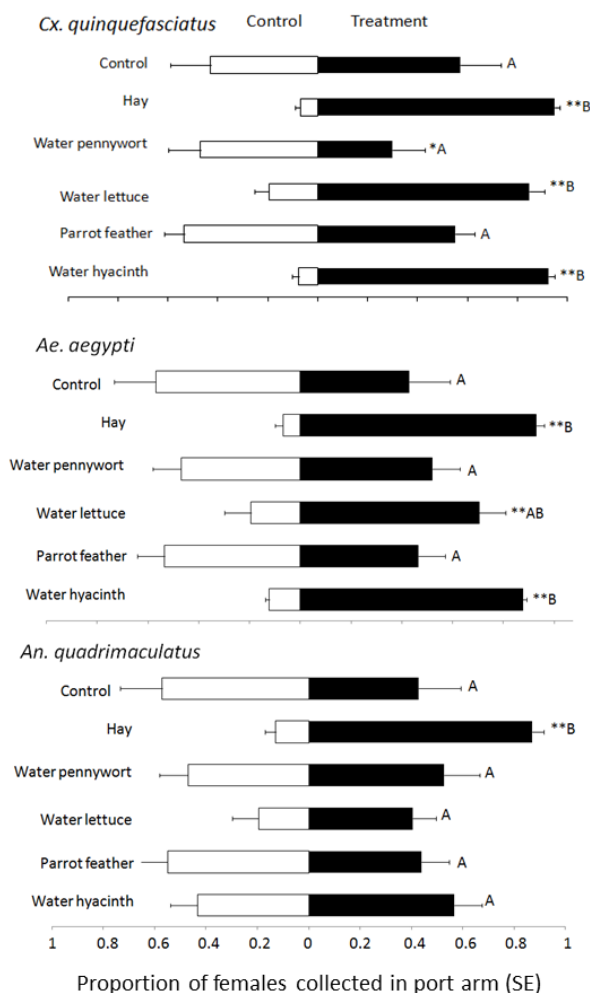
Figure 2. Response of gravid mosquitoes (*Culex quinquefasciatus, Anopheles quadrimaculatus, Aedes aegypti*) in two-choice olfactometer assays to control (water) or treatments consisting of different plant infusions. Assays were conducted for one h and replicated ten times. Responses to treatments significantly greater than controls are designated by asterisks (* $P < 0.05$, ** $P < 0.001$). Treatment bars with different letters are significantly different in a one-way ANOVA for each mosquito species ($P < 0.05$).

strong positive oviposition responses occurred in response to water lettuce ($U = 0$; df = 1,12; $P < 0.001$) and water hyacinth ($U = 0$; N = 1,12; $P < 0.01$) infusions. Although not as strong a difference, significantly more egg rafts were laid in the presence of water pennywort infusions compared to water controls ($t = 2.24$; df = 1,22; $P = 0.035$). Comparison of treatment means was significant (H = 45.952; df = 5; $P < 0.001$) with hay, water lettuce, and water hyacinth treatments being similarly attractive for oviposition (Figure 1), and egg raft counts for those treatments were greater than those associated with water pennywort and parrotfeather infusions. Mean numbers of egg rafts laid in response to controls, hay infusion, water hyacinth, parrotfeather, water lettuce, and pennywort were 1.70 ± 0.23, 13.41 ± 0.64, 11.16 ± 0.63, 2.41 ± 0.28, 10.16 ± 0.52, and 5.91 ± 0.86, respectively. Responses to infusions differed significantly (F = 50.44; df = 4, 59; $P < 0.001$) with egg rafts laid in response to hay infusion and water hyacinth similar and highest, lowest on parrotfeather and similar to the water control, and intermediate and similar on water lettuce and pennywort.

Olfactometer assays

In-flight orientation towards volatiles of plants and controls by gravid females of three mosquito species are presented in Figure 2. Gravid *Cx. quinquefasciatus* were more strongly attracted to volatiles from hay (U = 0, $P < 0.0001$), water lettuce ($t = 7.38$; df = 1,18; $P < 0.001$), and water hyacinth ($t = 21.86$; df = 1,18; $P < 0.001$) infusions compared to corresponding water controls. There was no difference in response among water (U = 45, $P = 0.68$), water pennywort (U = 32, $P = 0.15$), and parrotfeather (U = 20.5, $P = 0.08$) infusions compared to their controls. Similarly, gravid *Ae. aegypti* responded more strongly to volatiles from hay (U = 0, $P < 0.0001$), water lettuce (U = 12, $P < 0.001$), and water hyacinth (U = 0, $P < 0.001$) than corresponding water controls, with no significant attraction to water itself (U = 40, $P = 0.30$), water pennywort ($t = 0.33$; df = 1,18; $P = 0.74$), or parrotfeather (U = 36.5, $P = 0.31$) infusions compared to water controls. In contrast, gravid *An. quadrimaculatus* only responded to volatiles of hay infusion (U = 0, $P < 0.001$) compared to the water controls. There were no significant differences in attraction to water alone (U = 44, $P = 0.301$), water pennywort (U = 41, $P = 0.501$), water lettuce ($t = 1.39$; df = 18; $P = 0.80$), parrotfeather (U = 36.5, $P = 0.316$), or water hyacinth ($t = 0.89$; df = 18; $P = 0.386$) infusions compared to their corresponding water controls.

When responses to plant treatments by gravid *Cx. quinquefasciatus* were compared, significant differences in

response were detected (H = 20.63; df = 5; *P* < 0.001). High levels of attraction (> 92%) were obtained in the presence of hay infusion, water hyacinth, and water lettuce with no difference in response levels among those three volatile sources (Figure 2). Attraction to parrotfeather and water pennywort infusions were similar to the water control. The strongest attraction of gravid *Ae. aegypti* was to hay, water hyacinth, and water lettuce infusions with no difference among these treatments (Figure 2). Responses to the water control were similar to those to water pennywort and parrotfeather infusions. Among the four aquatic weed infusions, only water hyacinth differed specifically from the water control. Gravid *An. guadrimaculatus* females, in ANOVA on final means, showed no difference in attraction to any of the four aquatic plants compared to water control, with only the hay infusion attracting more females than water (Figure 2).

DISCUSSION

Gravid female *Cx. quinquefasciatus* were attracted to volatiles from multiple aquatic plant species and detected volatile infusions from attractive plants as demonstrated by assays for oviposition site choice and orientation in flight. Another species, *Ae. aegypti,* showed a similar pattern of attraction to volatiles of specific aquatic plants. These two mosquito species responded positively to water hyacinth volatiles with similar levels of response as to the hay infusion positive control. Additionally, volatiles from water lettuce attracted gravid *Ae. aegypti* and *Cx. quinquefasciatus* in pairwise comparisons with water and, in the case of *Cx. quinquefasciatus*, attraction was as strong as that of hay infusion in final female counts, with also strong attraction as measured in egg raft deposition. Water hyacinth and water lettuce odors failed to attract *An. quadrimaculatus.* For all species, volatiles from water pennywort and parrotfeather were no more attractive than the water controls to gravid females, although egg raft deposition by *Cx. quinquefasciatus* was greater on pennywort infusion than on water control bowls in oviposition choice tests.

Results from the two-choice oviposition bioassay with *Cx. quinquefasciatus* were consistent with results from in-flight attraction observed in the olfactometer. Water hyacinth, water lettuce, and hay infusion in both experiments enhanced oviposition in bowls and orientation in the olfactometer of this mosquito species. Interestingly, water pennywort did not significantly attract *Cx. quinquefasciatus* mosquitoes in the olfactometer relative to the control even though water pennywort infusion attracted mosquito females for oviposition relative to the control. This may indicate that compounds from the water pennywort infusion attract mosquitoes from very close range and possibly entail contact cues but not from further ranges; the olfactometer chamber was larger in size compared to the bioassay cages and odor concentration may have been lower.

The results indicate that aqueous infusions from water hyacinth and water lettuce possess attractive properties that facilitate *Cx. quinquefasciatus* and *Ae. aegypti* mosquito oviposition more than the other two macrophytes tested.

These attractive volatiles may include specific attractive chemicals or a blend of chemicals in a ratio and concentration that gravid female mosquitoes associate with preferred oviposition sites (Pichersky and Gershenzon 2002, Bruce et al. 2005, Bruce and Pickett 2011). Attractive plants, such as water hyacinth and water lettuce, as well as hay infusions, presumably produce concentrations and mixtures of volatile chemicals optimal for attraction of mosquitoes. Less attractive plants, such as parrotfeather and pennywort and their associated biofilms, may produce some of the same chemicals but in different ratios or concentrations or may produce some compounds that are repellent.

Attraction to hay infusion has been documented for many species of mosquitoes (Bentley and Day 1989, Afify and Galizia 2015, Day 2016) and chemicals associated with the attraction are well characterized (Afify and Galizia 2015). Fermentation of the hay infusion contributes to its attraction due to the microbial degradation of plant material that ultimately produces the attractive volatile chemicals (Hazard et al. 1967). Other studies of gravid female attraction have reported results similar to those in the present study (Benzon and Apperson 1988, Hasselschwert and Rockett 1988, Pavlovich and Rockett 2000, Ponnusamy et al. 2008, 2010) with well documented attraction by gravid *Cx. quinquefasciatus* and *Ae. aegypti*. Hay infusions were used as a positive control for attraction of gravid females to verify that assays were effective in eliciting oviposition choice and in-flight attraction, and that gravid females were responsive to oviposition cues. In contrast to the hay infusion, infusions of aquatic macrophytes produced in our study were not fermented and represented chemicals produced and accumulated over a 48 h period. Contributions of microbial fauna to the volatiles produced cannot be excluded; however, as the period of volatile collection was short, opportunities for microbial contributions would be limited. Thus volatiles produced could represent chemicals emitted by the plants with possible contributions from microbial fauna associated with the plants. Future studies could focus on identification of attractive chemicals and the determination of their origin, i.e., if they are plant or microbe-produced.

As a common invasive floating macrophyte, water hyacinth has been associated with a range of mosquito species. Species of *Mansonia* and *Coquillettidia* require access to roots to supply air for larvae and are associated with both water hyacinth and water lettuce (Slaff and Haefner 1985, Clements 1999). Facultative association with water hyacinth has been documented for some species of *Anopheles* (Slaff et al. 1984, Ofulla et al. 2010, Minakawa et al. 2012) and *Culex* (Galindo and Adames 1973, Slaff et al. 1984, Easton 1994, Mottram and Kettle 1997) but rarely with *Aedes* spp. Extracts of water hyacinth leaves have been reported to have larvicidal activity to *Cx. quinquefasciatus* (Jayanthi et al. 2012, Annie et al. 2015) and this may reflect higher levels of compounds that are either toxic or repellent to larvae in the concentrated extracts used in those studies than in the infusions used in this study. While water hyacinth and some other aquatic macrophytes may elicit oviposition and enhance larval development by mosquitoes due to the provision of protection

from predators, little is known about the actual attraction of mosquitoes to these plants for oviposition, especially in the absence of physical plant structures. In a study in Australia with *Culex annulirostris* Skuse and *Cx. quinquefasciatus*, there was no preference for water hyacinth over *Salvinia molesta or Cyperus haspens* when all three plants were present in assay cages compared to open water controls (Webb et al. 2012). Differences between that study and our study may be related to additional visual cues provided by use of entire plants and potential interaction of plant odors in the three-choice assay in the former study. Container-breeding species such as *Ae. aegypti* often have larvae present in containers associated with human residences (Bentley and Day 1989, Day 2016) and as such, these environments rarely contain any of the aquatic macrophytes evaluated in this study. However, these habitats do often contain decaying leaves which provide a substrate for microbial degradation and production of potentially attractive volatiles (Hasselschwert and Rockett 1988, Ponnusamy et al. 2008, 2010). Both hay and water hyacinth infusions accumulated more *Ae aegypti* females than water controls and presumably responses to these infusions may be related to the release to similar volatiles present in larval container habitats, or reflect sensitivity to plants present in natural habitats.

While the majority of anopheline species are not selective for a particular type of vegetation for oviposition (Rejmánkova et al. 2013), aquatic macrophytes may be associated with habitats of larvae. There can be a strong association of larval abundance with vegetative structure (Hall 1972), particularly at the intersection with the water line, and larval habitats can be categorized on the basis of vegetation characteristics including plant species (Rejmánkova et al. 1992). In a study on vegetation structure, more *An. quadrimaculatus* larvae were associated with lengths of downed plant stems (Walker et al. 1988), although no specific plants have been associated with attraction to an oviposition site. Rejmánkova et al. (1992) characterized larval habitats of *Anopheles albimanus* Weideman in Mexico and found that habitats dominated by water hyacinth were moderately productive for larvae. Water hyacinth has been positively associated with populations of *Anopheles rivulorum* Leeson (Minakawa et al. 2012), and *Anopheles funestus* Giles (Ofulla et al. 2010; Minakawa et al. 2002) within and around Lake Victoria in Africa, in contrast to the present study, in which water hyacinth was not attractive to *An. quadrimaculatus*

Attraction responses to volatiles from water hyacinth by gravid *Cx. quinquefasciatus* and *Ae aegypti* were similar to responses to hay infusion and may reflect the presence of similar compounds in both infusions that in turn reflect volatiles from preferred natural habitats. While these species are not often associated with water hyacinth, the attraction of gravid females to this invasive plant, which frequently invades backwater habitats near human populations (Getsinger et al. 2014) may facilitate associations.

Water lettuce is strongly associated with *Mansonia* and *Coquillettidia* mosquitoes as an air source for larvae (Lounibos and Escher 1985, Clements 1999). Lounibos and Escher (1985) reported consistent but low numbers of *Culex*

erraticus Dyar, *Culex nigripalpus* Theobald, and *Anopheles crucians* Weidemann in association with water lettuce in man-made habitats in Florida. Rejmankova et al. (1992) reported that habitats containing water lettuce were moderately productive for *An. albimanus* larvae. Water lettuce also has been associated with some anopheline larvae (Leeson 1937). The present study indicates that water lettuce is equivalent to water hyacinth for attraction of *Cx. quinquefasciatus* and *Ae. aegypti* females and oviposition responses, while not attractive to *An. quadrimaculatus* .

Both water pennywort and parrotfeather are only incidentally associated with mosquito populations. While the presence of parrotfeather was reported to affect abundance of larval *Anopheles* spp. and oviposition, with more larvae and eggs associated with higher densities of stems (Orr and Resh 1992), this is not believed to reflect specific preference for the plant species. Hall (1972) indicated that anopheline production was related to the amount of plant/surface interaction rather than specific plant species and, therefore, association of parrotfeather with anopheline larvae may reflect structural attributes rather than chemical attraction, as suggested by the lack of attraction to parrotfeather by gravid females of any of the three mosquito species tested here.

Recently, volatile attractants for *Anopheles arabiensis* Patton and *Anopheles coluzzii* Coetzee & Wilkerson from rice, grass, and pollen were obtained from headspace volatile collections. These collections contained only above-ground plant material excluding roots, soil, or water, thus contributions from microbes associated with those substrates were not included (Asmare et al. 2017; Wondwosen et al. 2016, 2017), indicating the presence of attractive volatile chemicals of plant origin. These reports comprise the first indication of plant-produced volatiles influencing oviposition responses of mosquitoes. In our study, attraction of gravid *Cx. quinquefasciatus* and *Ae. aegypti* females to water hyacinth and water lettuce represent a direct influence of aquatic macrophyte volatiles, derived from plants directly or from microbial-root interactions, on mosquito oviposition.

Novel methods for suppressing mosquitoes remain a priority as diseases with which these pests are associated continue to exist in different parts of the world. Effective management of mosquito populations relies on an integration of approaches that include the application of larvicides, adulticides, and source reduction guided by effective surveillance. Attractive plant infusions could be used to develop more effective mosquito traps for surveillance of gravid females in the field (Reiter et al.1991, Chadee et al. 1993, Polson et al. 2002, Sant'ana et al. 2006, Ponnusamy et al. 2010). Additionally, based on known plant-based chemicals associated with nectar feeding behaviors of mosquitoes (Nyasembe and Torto 2014), targeted control approaches are being considered (Ferguson et al. 2010, Govella and Ferguson 2012). Such an approach has been demonstrated with toxic sugar baits, which incorporate volatiles that attract nectar-seeking mosquitoes (Foster 2008, Stone and Foster 2013, Fiorenzano et al. 2017, Tenywa et al. 2017). Similarly, attractive volatile chemicals from aquatic plant leaves and stems may be exploited for their attractive properties for use in ovitraps

for surveillance or as a mixture with pesticides or biological agents for an attract-and-kill approach (Chandel et al. 2016, Day 2016, Schorkopf et al. 2016). Future studies examining the potential of these plant species and extracts in the attraction of field populations of mosquitoes would provide further insight into the potential of these for surveillance or targeted management. Identification of new volatile attractant sources, which guide oviposition behavior of gravid mosquitos, could provide the basis for the discovery of new attractive chemicals that can be utilized for enhancement of surveillance and management of nuisance and disease vector mosquitoes.

Acknowledgments

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