Introduction

Oviposition behaviour is a plastic trait and can be affected by a variety of cues (Bentley & Day, 1989; Isoe & Millar, 1996; Yu et al., 2004). The ability of the parent to distinguish among oviposition sites is important for many insects because microhabitat quality is often the major determinant of larval survival (Thompson, 1988). Oviposition behaviour is especially influential when larval movement is limited such that the habitat of larval development is determined wholly by oviposition site (Rausher, 1979; Resetarits, 1996). Consequently, oviposition behaviour can influence the density and distribution of insect populations.

Oviposition decreased in response to enriched water: a field study of the pitcher-plant mosquito, *Wyeomyia smithii*

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Abstract. 1. Environmental cues are known to influence oviposition behaviour in mosquitoes, with important consequences for larval survival and insect population dynamics. Enriched microhabitats have been shown to be preferred oviposition sites.

2. In a field experiment designed to determine whether ovipositing mosquitoes are sensitive to different levels of nutrient enrichment, new pitcher-plant (*Sarracenia purpurea*) leaves were opened and enriched with 0, 2, or 20 dead ants, and the number of pitcher-plant mosquito (*Wyeomyia smithii*) larvae resulting from subsequent oviposition were measured.

3. Oviposition rates were higher in leaves with low levels of enrichment (0 and 2 ants per leaf), although larval development was enhanced at the highest enrichment level.

4. Results suggest that, although these mosquito larvae are nutrient limited, ovipositing females preferentially avoid highly enriched leaves. This counterintuitive result may be due to low oxygen concentrations or a masked cue in enriched leaves, and contrasts with other oviposition studies.

Key words. Inquilines, mosquitoes, oviposition, oviposition behaviour, oviposition cues, pitcher-plants, *Sarracenia purpurea*, *Wyeomyia*.

Both abiotic and biotic factors affect mosquito oviposition behaviour (Bentley & Day, 1989). Abiotic factors known to influence oviposition behaviour include water temperature, depth, color, and salinity (Dhileepan, 1997). Biotic factors that can affect mosquito oviposition include the presence and density of conspecific larvae (Allan & Kline, 1998), predators (Tietze & Mullar, 1991; Fry et al., 1994; Eitam & Blaustein, 2004), parasites (Lowenberger & Rau, 1994), pathogens (Reeves, 2004), bacteria (Nguyen et al., 1999; Trexler et al., 2003), and nutrients (Reiskind & Wilson, 2004). Multiple studies have demonstrated that mosquitoes preferentially oviposit in water enriched with organic matter and bacteria, presumably because these microhabitats provide more nutrients for developing larvae (Beehler & Mulla, 1995; Dhileepan, 1997; Du & Millar, 1999; Nguyen et al., 1999). Many synthetic and naturally-occurring chemical cues have also been shown to influence mosquito oviposition (Millar et al., 1994; Olagbemiro et al., 1999; Geetha et al., 2003).

The pitcher-plant mosquito, *Wyeomyia smithii* (Coq.), oviposits exclusively in the water-holding leaves of the
Pitcher-plant leaves with more prey have been shown to attract oviposition sites for a variety of mosquito species (Dhileepan, 1997; Du & Millar, 1999; Nguyen et al., 1999). Few studies have examined the oviposition behaviour of pitcher-plant mosquitoes. Gravid pitcher-plant mosquitoes are thought to be drawn to pitcher-plant leaves by a water-soluble chemical cue (Istock et al., 1983). Leaf characteristics may also act as cues. For example, younger leaves are strongly preferred over older leaves as oviposition sites (Bradshaw, 1983; Miller & Kneitel, 2005). In a manipulative experiment using newly opened leaves, Heard (1994) found that pitcher-plant mosquitoes prefer to oviposit in larger leaves containing either conspecific or midge larvae. Nastase et al. (1995) found that larval abundance was positively correlated with size and youth of leaves. Miller and Kneitel (2005) have also shown that younger leaves are preferred oviposition sites, that most oviposition probably occurs in the first few weeks, when the leaf also captures the majority of its prey (Fish & Hall, 1978), and that higher numbers of prey lead to higher abundances of bacteria and protozoa, which may be consumed by mosquitoes (Kneitel & Miller, 2002; Miller et al., 2002; Hoekman, 2007). Taken together, these studies suggest that pitcher-plant mosquitoes choose oviposition sites on the basis of their potential for prey abundance, as higher prey abundance provides more nutrition for larval mosquitoes. Prey abundance, in turn, is affected primarily by leaf age; younger and, to a lesser degree, larger leaves attract more prey (Wolfe, 1981). The current study was conducted to determine the direct effects of prey number on oviposition behaviour in the pitcher-plant mosquito.

Gravid female mosquitoes should oviposit in what they perceive to be the best quality environment for larval development (Heard, 1994). Previous work has demonstrated that enriched environments, with higher bacterial levels, are favored oviposition sites for a variety of mosquito species (Dhileepan, 1997; Du & Millar, 1999; Nguyen et al., 1999). Pitcher-plant leaves with more prey have been shown to have higher bacterial and protozoan densities (Kneitel & Miller, 2002; Hoekman, 2007), and mosquito larvae in enriched leaves (those with more per capita resources) have been shown to develop into larger and consequently more fecund adults (Bradshaw & Holzapfel, 1992). Furthermore, total mosquito biomass and number of pupae emerging have been positively correlated with prey capture (Bradshaw & Holzapfel, 1986). Female pitcher-plant mosquitoes might therefore be expected to oviposit preferentially in leaves containing higher densities of dead ants, on the expectation that such leaves will provide a better environment for larval development. This hypothesis was tested by manipulating ant density in naturally occurring pitcher-plant leaves.
of ants (0 vs. 2 + 20 contrast) did not have a significant effect ($F_{1,68} = 2.96, P = 0.09$).

Overall, ant treatment had a significant effect on the number of each instar in each leaf (Wilks' lambda $F_{3,132} = 2.58, P = 0.012$). As expected after recent oviposition, the majority of the mosquito larvae were 1st instar; in fact, the 0-ant treatment contained no 3rd- or 4th-instar larvae. The proportion of 1st- and 2nd-instar larvae declined with prey treatment (Fig. 1B). As indicated by product-moment correlations, the density of 1st instars was most influential in determining treatment differences (0.860), followed by the 3rd ($-0.507$), 4th ($-0.365$) and 2nd (0.184) instar density. The sign of these correlations reveals that high numbers of early instars are associated with low numbers of late instars.

Discussion

These results are contrary to the expectation that female pitcher-plant mosquitoes would oviposit preferentially in leaves with more ants. Instead, female mosquitoes preferred to oviposit in leaves with lower levels of enrichment, including leaves that had not received any added nutrients (Fig. 1A).

Nutrient levels did affect larval development in an expected manner. In the low-nutrient treatment, larval abundance was high, but no larvae developed past the 2nd instar (Fig. 1B). In the medium-nutrient treatment, larval abundance was equally high, but a small number of 3rd and 4th instars were able to develop. These results suggest that nutrient limitation prevented larvae from developing under low-nutrient conditions. Larvae in the high-nutrient treatment achieved levels of development similar to those of larvae in the medium-nutrient treatment (Fig. 1B). Though they were less abundant, eggs laid in the high-nutrient leaves appeared to develop normally and perhaps even more rapidly than eggs in other treatments. Their success can be attributed to high per-capita resources, due to high nutrients, low density, or their combination.

Although the pattern of larval development can be explained according to a nutrient or density effect, the abundance pattern is still counterintuitive: why were fewer eggs laid in high nutrient leaves? There may be an intermediate maximum in the relationship between prey availability and oviposition somewhere between 0 and 20 ants that was unable to be observed with our treatments. If so, we would have expected there to be an increase in oviposition between the 0- and 2-ant treatments; however, the possibility of an intermediate maximum cannot be excluded.

Another explanation is that the addition of 20 ants caused some pitcher microhabitats to become anoxic. Dissolved oxygen and number of ants are negatively correlated in leaf fluid (T. E. Miller, unpublished data). Extreme anoxic leaves are occasionally observed in the field and are characterised by a pungent odor that may repel gravid female mosquitoes. As mosquito larvae can breathe at the water surface, anoxia may not directly affect mosquito growth or survival but may affect the abundance of protozoa and bacteria, the primary mosquito prey.

One final possibility is that high densities of decomposing ants mask the olfactory cue that attracts ovipositing pitcher-plant mosquitoes. Because both oviposition rate and prey capture rate decline exponentially with leaf age (Bergland et al., 1996; Dhileepan, 1997; Du & Millar, 1999), ovipositing females must frequently encounter young pitchers with high prey densities. Perhaps their apparent aver- sion to highly enriched pitchers helps them identify the youngest possible leaves, i.e. those that have just opened and have not yet accumulated prey. Pitcher-plant mosquitoes have been observed ovipositing in freshly opened leaves with no prey or standing water (Bradshaw & Holzapfel, 1986), displaying the anticipatory nature of their oviposition behaviour.

No study to the authors’ knowledge has reported the unexpected pattern of lower oviposition in response to enriched water. In fact, previous studies have shown the opposite; other mosquito species preferentially oviposit in enriched environments (e.g., Beehler & Mulla, 1995; Isoe et al., 1995; Lampman & Novak, 1996; Dhileepan, 1997; Du & Millar, 1999; Nguyen et al., 1999; Reiskind & Wilson, 2004). Unfortunately, most of these studies included no measure of bacterial density and used only an enriched and an unenriched treatment rather than a gradient, so they could not document any threshold where attraction was gained or lost. In a lab bioassay experiment, however, Du and Millar (1999) did find both a monotonic and an intermediate response to enrichment by two different species of mosquitoes. In general, females preferred to oviposit in more enriched water, but Culex quinquefasciatus (Say) preferred the highest

Fig. 1. Total number of mosquito larvae (A) and number per instar (B) found per leaf (± SE). Oviposition frequency depends on prey treatment (ants added per leaf).
concentration of bulrush infusion, whereas Culex tarsalis (Coq.) preferred intermediate concentrations.

One difference between prior studies and ours is the unique relationship between W. smithii and its host plant, S. purpurea. The leaf habitat may differ from the larval habitat of other mosquito species in that it goes through a predictable pattern of nutrient availability (Miller & Kneitel, 2005) and potential competition (Bradshaw & Creelman, 1984), which likely gives a very large benefit to oviposition in very young leaves. In a recent field experiment with pitcher-plant mosquitoes in a northern bog, however, Bergland et al. (2005) found that per leaf oviposition was positively correlated with larval resources (captured prey). It may be that latitude or seasonality also plays a role in the relationship between resources and oviposition behaviour.

A surprising, but ultimately unexplained, pattern is documented in this experiment. Clearly, oviposition rates strongly decline at high prey levels, although high prey levels appear to allow higher developmental rates. The consequence of this complex oviposition behaviour for the mosquitoes requires further study.

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References


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