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Short communication

Evaluation of larviposition site selection of *Glossina brevipalpis*

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ABSTRACT

Tsetse species (Diptera: Glossinidae) are vectors of trypanosome parasites which cause disease in both humans and livestock. In South Africa *Glossina austeni* Newstead, 1912 and *G. brevipalpis* Newstead, 1911 are responsible for the cyclical transmission of animal trypanosomes causing African animal trypanosomiasis also referred to as nagana. Gravid tsetse females deposit a single larva in specific sites but little information is available on biotic and abiotic factors that govern site selection. This study therefore aimed to characterize some of the substrate conditions that may influence selection of larviposition sites. Colonised, gravid female *G. brevipalpis* were presented with a choice of four larviposition sites. Sites differed in qualities of pH (5, 7, 9), salinity (0, 1.3, 4 g/L) and the presence of other tsetse pupae (*G. brevipalpis* or *G. austeni*). These trials indicated no significant selection by gravid females with regard to pH and salinity. Females selected significantly more often for sites with pupae ($P < 0.05$), but also favored sites containing conspecific over heterospecific pupae ($P < 0.05$). These results present the first indication of an aggregation effect of tsetse pupae in *G. brevipalpis*. This may imply that *G. brevipalpis* larvae produce a pheromone during pupation as seen in *G. morsitans morsitans*. Isolation of such semio-chemicals would allow the development of larviposition traps to attract gravid females.

1. Introduction

Of the 23 *Glossina* species (Diptera: Glossinidae) endemic to Africa only *Glossina brevipalpis* Newstead, 1911 and *Glossina austeni* Newstead, 1912 are currently present in the north eastern parts of the Kwazulu-Natal Province in South Africa (Kappmeier Green, 2002). These populations represent the southernmost distribution of tsetse in Africa. These two species transmit *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei brucei* which cause nagana, a fatal disease in cattle, in South Africa with *G. austeni* indicated as the more competent vector (Motloang et al., 2012). Whilst these two species do occur largely sympatrically, *G. austeni* seems restricted to indigenous forest habitats in its distribution (Esterhuizen et al., 2005; Motloang et al., 2012). The more abundant *G. brevipalpis* has also been collected from open grassland and, to a lesser extent, from exotic blue gum plantations surrounding the indigenous forests (Esterhuizen et al., 2005). Whether the presence of *G. brevipalpis* in these open grassland areas and plantations is linked to a greater mobility in adult flies or if they breed and complete a life cycle in these areas is unknown (Esterhuizen et al., 2005). In either case, this species may have greater opportunity to encounter susceptible animals as a result of their presence in such areas. If *G. brevipalpis* can complete its life cycle in these atypical situations, there are implications in terms of both species distribution as well as vector control.

Uncommon to most insects, tsetse are truly viviparous. The female, once she has selected an appropriate site, deposits a single fully developed third instar larva which burrows into the substrate and pupates within an hour after larviposition (Phelps and Lovemore, 1994). Thus, two stages occur in association with the substrate, the initial selection of site by the female fly and the subsequent development of the pupa. It has been shown that moisture, soil texture, pH and salinity can determine oviposition site choice in insects (Choudhuri, 1958; Emmens and Murray, 1983; Huang et al., 2005; Cornelisse and Hafernik, 2009). The extent to which each of these factors will influence oviposition site selection depends on the species involved.

In tsetse, relatively few studies have focused on factors affecting female choice of larviposition site (Parker, 1956; Nash et al., 1976; Rowcliffe and Finlayson, 1981; Leonard and Saini, 1993; Muzari and Hargrove, 2005; Basson and Terblanche, 2010). Although it is described that the pupae of *G. brevipalpis* can be found in the soil under a low overhanging canopy of shrubs and bushes as well as soil

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between the buttressed roots of certain large trees (Du Toit, 1954), little is known about the ecological factors that influence larviposition site selection. Factors which have been found to influence site selection in tsetse females include shade, color and soil texture (Parker, 1956; Rowcliffe and Finlayson, 1981). In addition to these environmental factors it has been shown in *G. morsitans morsitans* and *G. m. centralis* that a pheromone produced during pupation may attract gravid females to a larval site (Nash et al., 1976; Leonard and Saini, 1993; Saini et al., 1996). The identification and manipulation of attraction by such an 'aggregation factor' would allow for the development of larviposition traps, specifically targeting gravid females and thus removing both adult flies and their larvae from the targeted area.

To characterize some of the factors that may influence larviposition site selection by *G. brevipalpis* females, the effect of pH, salinity and the presence of either heterospecific (*G. austeni*) or conspecific tsetse pupae on site selection was determined under laboratory conditions in the current study. Salinity and pH have been found to influence site selection in Diptera such as blowflies (Calliphoridae) and mosquitoes (Culicidae) and may also play a role for tsetse (Emmens and Murray, 1983; Bentley and Day, 1989). Another possibility is that gravid females may be attracted to specific sites by a pheromone released during pupariation. Pheromone mediated aggregation, as described in *G. morsitans,* has not been previously tested for in *G. brevipalpis* (Nash et al., 1976; Saini et al., 1996). This study therefore aimed to determine if pH, salinity and presence of tsetse pupae can influence *G. brevipalpis* females when selecting sites for larviposition.

2. Materials and methods

2.1. Experimental insects

All experiments were conducted on colonised *G. brevipalpis* females maintained under standard conditions (23–24 °C, 75–80% RH and a 12D:12N photoperiod) at the Agricultural Research Council–Onderstepoort Veterinary Institute (ARC-OVI) in Pretoria, South Africa. Colony flies are fed on bovine blood using an artificial membrane system according to The Food and Agriculture Organisation of the United Nations (2006). *G. austeni* pupae used in the pupal aggregation trials were obtained from colonised flies at ARC-OVI maintained under identical conditions to *G. brevipalpis*. These experimental insects were from long-term colonised populations.

2.2. Trial Cages

Cages used for larviposition site selection trials consisted of 30 cm × 26 cm × 30 cm metal frames covered with white polyester netting. In addition to a sleeve, one side of the cage attached along three edges by Velcro® to allow access to the interior. The base of the cage was divided into equal quarters by a plastic container (individual sections 14.7 cm × 12.3 cm and 5.0 cm deep) allowing for the simultaneous evaluation of four treatments in one cage.

2.3. Substrate preparation

Sand commercially used in children's sand boxes was washed, autoclaved and oven dried at 100 °C for 24 h before being used as a substrate base for the selection sites. pH levels were set at pH of 5, 7 or 9. A citrate/phosphate buffer (pH 5); a phosphate buffer (pH 7) and a sodium hydroxide/glycine buffer (pH 9) were used to sustain substrate pH at the specified values in the sand (Choudhuri, 1958; Emmens and Murray, 1983; Van Straalen and Verhoef, 1997). Salinity was set at 0 g/L, 1.3 g/L and 4 g/L. Saline solutions at concentrations of 1.3 g/L and 4 g/L were produced with the addition of sodium chloride (NaCl) to distilled water. Salinity and pH values were monitored using a HI 9828 multi-parameter meter (HANNA, USA) and a pH 220-S soil pH meter (Lutron, Taiwan) respectively.

During pupal aggregation trials, substrate was prepared by placing 20 freshly deposited larvae of either *G. brevipalpis* or *G. austeni* on dry sand.

2.4. Larviposition site selection

All larvipositioning site trials were conducted under the same standardised conditions (23–24 °C, 75–80% RH and 12D:12N photoperiod) and in the rooms where the colonies are maintained. To compensate for potential minor fluctuation in environmental conditions within the room, experimental cages were set up with trays that contained four selection sites in a randomised 4 × 4 Latin square design. For each trial type, salinity, pH and aggregation, twelve replicates were conducted. To simulate the larvipositioning conditions in the colony one site was left empty during each trial. Each other site received 200 g of prepared sand into which 20 mL of the appropriate solution had been mixed to modify the pH (5, 7 or 9) or salinity (0 g/L, 1.3 g/L and 4 g/L) as per the trial type.

In pupal aggregation trials two sites received larvae, with one receiving 20 *G. brevipalpis* larvae and the other 20 *G. austeni* larvae. Larvae were allowed to pupate naturally with the result that pupation occurred either on the surface or after burrowing into the substrate, resulting in a similar mixture of buried and exposed pupae in each site. One of the remaining two containers was kept empty and the other held only dry sand with no pupae.

Cages were stacked into towers of four cages according to trial type, and the position of these was switched from week to week. Forty gravid females, undergoing their first cycle of parturition, were introduced for site selection and remained in the cages for five days. To minimise disruption experimental flies were only fed on the third day of the five-day long behavioral trials. Larvae were allowed to pupate without any disturbance in each site.

Trays were changed daily and replenished with freshly prepared substrate. In the pupal aggregation trials, trays were changed on the third day with only the sand control and empty control sites being cleaned. The two sites containing pupae were left undisturbed. All pupae deposited in the sand and empty sites were collected.

2.5. Data analysis

Analysis of variance (ANOVA) was used to differentiate between substrate treatment effects at the 5% level. Treatment means were separated using Fisher's protected *t*-test least significant difference (LSD) at the 5% level of significance (Snedecor and Cochran, 1980). Data was analysed using the statistical programs GenStat1 (Payne et al., 2007) and SPSS.

3. Results and discussion

In pH trials, 480 females produced 305 larvae (63.5% deposition) over five days (Table 1). The total number of larvae deposited at each treatment ranged from 70 (23%) at pH 5 to 90 (29.5%) at pH 9 (Table 1). The mean number of larvae deposited in each of the treatments did not differ significantly ($P > 0.05$) between treatments or from that of the control. Similarly, there was no significant difference in the numbers of larvae deposited on each of the five days separately, including day 3 on which the highest mean number of pupae ($\bar{x} = 3.75$, Table 1) was deposited in the pH 9 treatment (Kruskal–Wallis *H* test, χ^2 (3) = 6.86, $P > 0.05$).

During the salinity trials, 480 females produced 323 larvae (67.3% deposition) over five days. The number of larvae deposited over each treatment ranged from 65 (20.1%) with only distilled water added to the substrate to 103 (31.9%) at 4 g/L salinity. Similar