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SPECIAL ISSUE: FEMALE MATING FAILURES

Mating performance of *Glossina palpalis gambiensis* strains from Burkina Faso, Mali, and Senegal

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Abstract

The mating performance of *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae) mass-reared in Burkina Faso (BKF strain) was compared with that of target populations originating from the Bamako peri-urban area of the Niger River Basin, Mali (MLI strain) and the Niayes area, Senegal (SEN strain). The tests were carried out using a field cage either set up outdoors in Burkina Faso or inside the laboratory in Austria. The target population strains (MLI and SEN) were a few generations from the wild whereas the laboratory-reared flies (BKF) were adapted to laboratory rearing over many generations. The laboratory-reared BKF strain significantly out-competed the MLI strain in the mating tests, but showed close to equal competitiveness with the SEN strain. At least one-third of possible matings occurred during each observation period. The females from the two target populations readily mated with males from the BKF strain. The selected mating parameters and behaviour in the cage showed that there was mating compatibility between the strains and this absence of obvious mating barriers indicates the potential of using BKF strain males in programmes that have a sterile insect technique (SIT) component targeting the two *G. p. gambiensis* populations of Mali and Senegal.

Introduction

In West Africa, tsetse flies of the *palpalis* group (e.g., *Glossina palpalis* Robineau-Desvoidy ssp. and *Glossina tachinoides* Westwood) are the most important cyclical vectors of the debilitating disease African animal trypanosomosis (AAT) or nagana in livestock and human African trypanosomosis (HAT) or sleeping sickness in humans (Leak, 1998; van den Bossche et al., 2010). The presence of these flies is a major hindrance to the development of more sustainable and effective livestock systems and the flies are rightly considered a root cause of poverty and hunger (Feldmann et al., 2005). In the moist savannah of West Africa, the habitat of riverine tsetse flies is restricted for most of the year to the riparian forests that border the rivers and their tributaries (Leak, 1998). There are four control tactics which are currently considered acceptable for the integrated management of these disease vectors, i.e., insecticide-impregnated targets/traps (Green, 1994), the live-bait technique (dip, spray, or pour on application of residual insecticides on livestock) (Thompson et al., 1991), the sequential aerosol technique (SAT) (Kgori et al., 2006), and the sterile insect technique (SIT) (Politzar & Cuisance, 1984; Oladunmade et al., 1990; Vreysen et al., 2000). Integrating these techniques following area-wide integrated pest management (AW-IPM) principles – the control effort is directed against the entire pest population in a circumscribed area – in most cases leads to more sustainable control (Vreysen et al., 2007).

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The SIT requires the production of the target insect in large numbers in specialized rearing factories, sterilization of the male flies usually with ionizing radiation, and sequential release of these sterile flies over the target zone to outnumber and outcompete the wild male population. This robust technology has been successfully used in the past decades to contain, suppress, or eradicate several economically important insect pests of crops, livestock, or humans (Steiner et al., 1965; Hendrichs et al., 2002; Dyck et al., 2005). The technique was, likewise, used effectively to manage tsetse populations as was demonstrated in Burkina Faso (Politzar & Cuisance, 1984), Nigeria (Oladunmade et al., 1990), and Tanzania (Williamson et al., 1983; Vreysen et al., 2000). The application of the technology in an AW-IPM approach is complex and there are various technical and administrative prerequisites that need to be in place to be successful (Vreysen et al., 2000, 2007). The biological quality of the sterile flies that are released is arguably the most important technical aspect for the successful application of the SIT (Krafsur & Hightower, 1979; Vreysen, 2005).

Most of the pest insect species that have been controlled using the release of sterile insects have a high reproduction rate, which facilitates the rearing of the millions of individuals required. Rearing of tsetse flies, and especially colonizing a new population, is time consuming, labour intensive, and challenging. Using flies from an existing laboratory-adapted colony to develop new colonies or using flies from a regional mass-rearing facility for release in another country would be more cost effective approaches. Although pooling of regional resources will significantly reduce the financial resources required to implement a control programme, the mating compatibility and competitiveness between the released sterile flies and the target wild strain is of prime importance in such a regional or international approach.

Following the establishment of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), several governments in West Africa, including those of Mali, Burkina Faso, and Senegal, embarked on feasibility studies to assess whether the SIT could or should be integrated in their national tsetse control efforts. Entomological and veterinary baseline data were collected in the three countries and population genetics studies were conducted to better understand the dynamics of the target species *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae) (Bouyer et al., 2010; Solano et al., 2010; Koné et al., 2011). The Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide (CIRDES) in Burkina Faso is the only place in West Africa where an in vitro colony of *G. p. gambiensis* is being maintained. The colony was used in the 1980s for the eradication of a *G. p. gambiensis* population from 3 500 km^2 of agro-pastoral land in Sidéradougou, Burkina Faso. Mating studies were therefore needed to assess whether mating barriers existed between the CIRDES strain as the potential release strain and the *G. p. gambiensis* target populations from Mali and Senegal.

This article presents the results of an assessment of the mating performance of the *G. p. gambiensis* strain maintained at CIRDES (Burkina Faso origin) with those of the two target populations in Mali and Senegal. The studies were carried out in Bobo-Dioulasso (Burkina Faso) and Seibersdorf (Austria) using walk-in field cages to approximate as closely as possible the actual field conditions during sterile insect releases.

Materials and methods

Fly strains

The field cage experiments at CIRDES were carried out with strains of *G. p. gambiensis* that were maintained on an in vitro silicon membrane feeding system (Feldmann, 1994). The Burkina Faso (BKF) strain was initially colonized in 1972 at Maisons-Alfort, France, using material collected in Guinguette, Burkina Faso. The strain was transferred to CIRDES, Burkina Faso, in 1975 with the last addition of 'local' wild material, collected in Mare aux Hippopotames in 1981 (Figure 1). This translates to nearly 200 generations in laboratory rearing. The Mali (MLI) strain originated from the Niger River Basin and was sampled in the area between Bamako and Koulikouro (Figure 1) and was colonized in December 2001 and as of May 2002 maintained on an in vitro silicon membrane feeding system. The MLI strain had thus been cultured only for about five generations before the test. All flies used for the tests were fed on bovine and porcine blood according to standard rearing procedures at CIRDES (Bauer et al., 1984). Virgin flies were used for the mating observations, males 7 days post emergence and females 3 days post emergence.

The field cage experiments at the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria, were carried out with the BKF strain established from material derived from the CIRDES colony and with a newly established strain from Senegal (SEN). The SEN strain was thus up to five generations in laboratory rearing. All strains were maintained on an in vitro silicon membrane feeding system. The flies were fed bovine blood that was collected in large volumes from an abattoir, frozen at −20 °C, and irradiated with 1 000 Gy in a commercial irradiator. Aliquots of the blood were thawed and used as required. The flies were fed three times a week, pupae were

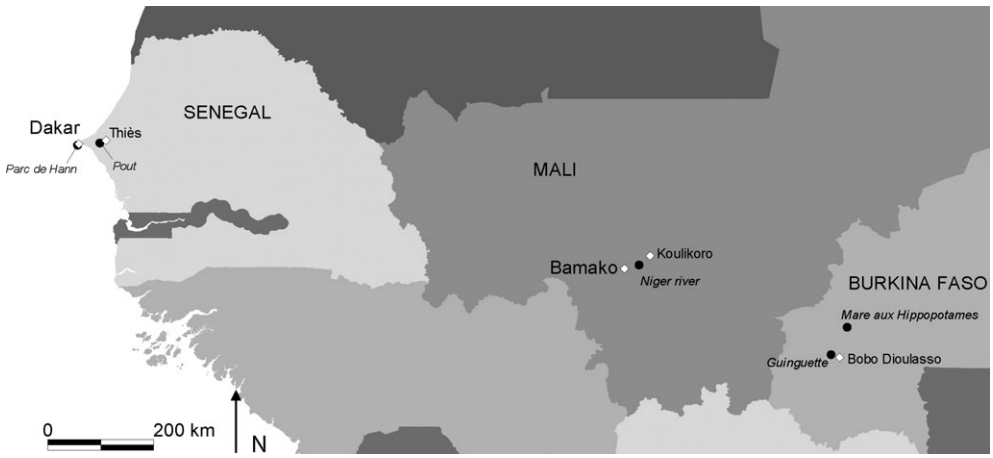

Figure 1 The locations of source materials for the *Glossina palpalis gambiensis* strains.

incubated at 24 °C for 4 weeks, and adults emerged at 24 °C, 75% r.h., and L12:D12. Virgin flies were used for the mating observations, males 6–8 days post emergence and females 2–3 days post emergence. Mature flies readily engage in mating activity, however, due to the small source SEN colony size it was not possible to get adequate numbers of virgin flies from a contiguous 24-h period for the daily experiments and thus flies emerging over a 72-h period were pooled.

The colony of the BKF strain was established at the IPCL from 7 830 pupae received in four shipments from CIRDES and put in culture in early 2009. The average weight of the pupae shipped from CIRDES was 20.4 mg and emergence was 91%, with 50.8% females.

A new SEN colony was established at the IPCL in 2009–2010 with flies originating from the Niayes in Senegal (Figure 1). Collections of wild female flies were initiated in September 2009 in Pout and Parc de Hann (Dakar). The flies were sampled with unbaited Vavoua traps and transported to an insectary in Dakar where they were transferred to standard holding cages. The flies were initially fed on goats, but as of March 2010, the feeding was shifted to an in vitro membrane feeding system. In this new protocol, the flies were fed on a goat for the first two blood meals, but thereafter they were fed in vitro on blood that was freshly collected from the jugular vein of two cows. The pupae that were produced in the insectary in Dakar were transported from Senegal to Austria in Petri dishes that were placed in insulated transport boxes (AcuTemp®; Dayton, OH, USA) that contained phase change material (ClimSel C24®; Climator, Skövde, Sweden) to maintain a near constant temperature. The shipment periods were <50 h each. The temperature varied between 18 and 22 °C, and humidity fluctuated between 74 and 83% r.h. The shipments were initiated on 18 October 2009 and a further 12 shipments were carried out.

The field cage and its environment

CIRDES, Bobo Dioulasso. A single cylindrical, netting cage, 2.9 m in diameter and 2.0 m high supported by external guy lines (Mutika et al., 2001) containing a *Pterocarpus erinaceus* Poir. (Fabaceae) pruned to 2 m high at the centre of the cage was set up outdoors. The cage was in complete shade at the start of the tests [09:00 hours Greenwich Mean Time (GMT)] but most of the cage was under direct sunlight at the end (12:05 hours GMT). There was a gentle breeze in the cage but the site was protected from strong winds by trees a few metres away (*Azadirachta indica* A.Juss., *Thevetia nereifolia* Juss., *Delonix regia* Bojer ex Hook., and *Cassia siamea* Lam.). Temperature and relative humidity were measured at the centre of the cage and light intensity averaged from three levels.

IPCL, Seibersdorf. A similar field cage as described above, but with an internal polyvinyl chloride (PVC) tubing frame, was used with a potted citrus tree at the centre. The cage was set up in the laboratory under 58-W fluorescent lights (eight tubes) providing luminescence up to 550 lux in a room with ceramic tiles on the floor and walls, where temperature was controlled between 24–25 °C, and 60–65% r.h.

Field cage test protocol

CIRDES, Bobo-Dioulasso. The mating performance of *G. p. gambiensis* males was assessed using BKF and MLI strain males competing for mating opportunities with MLI strain females. All the flies that were used in the tests were

treated in a similar manner. Each day, only males of one strain were marked on the thorax using a dot of water-based paint. Flies were marked the day before observations. Marking was alternated between the strains in successive replicates. All flies were immobilized at about 4 °C during marking and the strain that was not marked was also subjected to the same immobilizing temperature. Thirty virgin males of each strain competed for mating opportunities with 30 virgin females from the MLI strain, thus 90 flies were used each day. The flies were not fed on the day of the test but had been fed the day before. Females were released from a Geigy cage at 09:00 hours at the centre of the cage followed by males 5 min later. Fly activity was observed for 3 h and mating pairs were collected into individual vials as they formed and the time was recorded. The observer was in the field cage for the entire period of the experiment. When the pairs separated, the time was recorded. The latency time was then calculated as the period in minutes between time when males were released and formation of each mating pair. Mating duration was calculated as the length of copulation in minutes, which is from formation of mating pair to separation. Flies that did not mate were collected at the end of the observations each day and discarded. Dissections for assessment of spermathecal fill were carried out in the afternoon. The tests were conducted daily for eight consecutive days in October 2002.

IPCL, Seibersdorf. The mating compatibility of *G. p. gambiensis* males was assessed using BKF and SEN strains competing for mating opportunities with SEN strain females. A similar marking, holding, and release protocol was followed for the nine tests carried out at Seibersdorf in 2010 but with only one test a week. Females that mated with males from the SEN strain were returned to the colony.

Mating indices and data analysis

The propensity of mating (PM) was defined as the overall proportion of released females that mated. It represents the overall mating activity of the flies under the given environmental conditions and is used to assess suitability of the conditions and the flies for the test (Cayol et al., 1999; Mutika et al., 2001). The relative mating index (RMI) is a measure of mating competitiveness and is defined as the number of pairs of one strain as a proportion of the total number of matings; in case of equal competition between the strains, each strain would have an RMI value of 0.5 (Cayol et al., 1999; Mutika et al., 2001). The relative mating performance (RMP) is a measure of mating compatibility and is defined as the difference between the numbers of matings of the BKF strain and the other strain as a proportion of the total number of matings. Values can range from −1 to +1, where positive values indicate preferential mating by the BKF strain (satyrism) and negative values preferential mating of the SEN or MLI strain (reproductive isolation).

When dissection was carried out, the spermathecal value was obtained by assessing the content of the two spermathecae and adding the values. Each spermatheca was classed as empty (0), quarter full (0.25), half full (0.5), three quarters full (0.75), and full (1) (Nash, 1955). Dissections and the subjective spermathecal content assessments were carried out in saline solution using a Carl Zeiss stereomicroscope at 50× magnification.

Data for latency time, mating duration, and spermathecal value were tested for homoscedacity followed by analysis of variance or Kruskal–Wallis analysis as appropriate (Minitab, 2000). Replicated tests of goodness of fit (heterogeneity G-test) were carried out on the number of males that mated from each strain (summarized as the relative mating index) (Sokal & Rohlf, 1995).

Results

CIRDES, Bobo-Dioulasso

Temperature ranged from 26–41 °C and relative humidity from 33–73% during the observation periods. Atmospheric pressure remained constant during each day's observation period but varied between 959 and 964 hPa with the day of the test (atmospheric pressure readings supplied by ASECNA, Bobo-Dioulasso). Light intensity varied between 1 270 and >20 000 lux. When flies were released they generally flew to, and landed on, the walls of the cage and the edge of the cage where the wall meets the roof. A few flies landed on the ground, roof of the cage, on the tree, and buzzed around the observers. Mating pairs were recorded 2 min after release of males until the end of the 3-h observation period with the majority forming during the 1st h.

The MLI male flies formed mating couples slightly, but not significantly, earlier than the BKF males ($F_{1,152} = 2.90$, $P>0.05$). Insemination occurred in about 90% of the pairs collected with 59% being fully inseminated. The insemination rate was not significantly different between strains ($H = 3.76$, d.f. = 1, $P>0.05$) (Table 1). The average rank latency time was 70 and 82 min for the MLI and BKF strains respectively. The duration of copulation was not significantly different ($F_{1,152} = 1.59$, $P>0.05$). The mean (± SD) mating propensity was 0.64 ± 0.21 with a relative mating performance of 0.26 in favour of the BKF males. The relative mating index was 0.64 ± 0.11 for BKF and 0.36 ± 0.11 for MLI. A G-test on the number of mating pairs (RMI values) indicated that there were significantly

Table 1 Mean (± SD) mating parameters for *Glossina palpalis gambiensis* when male flies originally from Burkina Faso (BKF) competed in a field cage with male flies from Senegal (SEN) for mating opportunities with SEN females, or with male flies from Mali (MLI) for mating opportunities with MLI females

Competition	Strain	n	Pre-mating time (min ± SD)	Mating duration (min ± SD)	Mean spermathecal value[1]
BKF vs. SEN	BKF	88	42.73 ± 49.19	56.22 ± 18.33	1.73 ± 0.47
	SEN	84	43.62 ± 47.41	56.93 ± 22.44	Not dissected[2]
BKF vs. MLI	BKF	97	77.71 ± 56.53	49.64 ± 16.92	1.63 ± 0.64
	MLI	57	62.44 ± 48.68	46.28 ± 14.23	1.42 ± 0.77

[1]Spermathecal value is the combined content of the two spermathecae, classified as empty (0), quarter full (0.25), half full (0.5), three quarters full (0.75), and full (1).
[2]SEN female flies were not dissected because they were required for colony build-up.

more matings between BKF males and MLI females than between MLI males and MLI females (G = 10.510, d.f. = 1, P = 0.0012).

About 56% of mating pairs were seen on the cage walls, 16% from the edge of the cage where the wall meets the roof, 10% in flight, and the remainder on the ground, tree, and roof of the cage (Table 2). This overall trend in distribution of the location in the cage where mating pairs were first observed was similar for both strains.

IPCL, Seibersdorf

Establishment of a SEN colony. In the insectary in Dakar, feeding response of the female flies sampled in Pout was in general good (>85%) and daily mortality fluctuated between 3.8 and 10.2%, excluding the period 13–27 June 2010, when daily mortality was 22.8%. A total of 2 185 *G. p. gambiensis* pupae were shipped from the insectary in Dakar, to the IPCL, in Seibersdorf, Austria. Upon receipt, pupal weight varied between 17.03 and 21.01 mg with a mean (± SD) weight of 18.4 ± 1.75 mg. The average pupal weight remained stable and the proportion of small pupae declined with time. A total of 1 920 pupae originating from females collected in Pout were incubated. Emergence rate of the batches of the pupae was at least 80% with an average (± SD) of 88.9 ± 7.75%, of which 49.3% were females and 50.7% were males. The mean daily mortality (1.3 ± 0.58%) of the female flies was slightly higher during the initial months of colony establishment. Female fecundity was low in the first 10 weeks of 2010 (on average 0.4 ± 0.58 pupae per female per 10 days) but improved to 0.50 ± 0.05 pupae per female per 10 days by the 38th week of 2010.

Field cage studies. Following release, most of the females landed on the PVC tubes supporting the roof of the cage,

Table 2 Average distribution of locations at which mating pairs were collected in the field cage, after competition between male flies originally from Burkina Faso (BKF) vs. males from Mali (MLI) for mating opportunities with MLI females, or vs. males from Senegal (SEN) for mating opportunities with SEN females. For the BKF vs. MLI male-male competition experiments, the supporting frame was outside the cage, whereas the frame was inside the cage for the BKF vs. SEN experiments

	% mating pairs for each strain			
	BKF vs. MLI		BKF vs. SEN	
Location	BKF (n = 97)	MLI (n = 57)	BKF (n = 88)	SEN (n = 84)
Cage wall top	25.8	35.1	10.2	7.1
Cage wall midway up	10.3	14.0	1.1	1.2
Cage wall bottom	27.8	21.1	4.8	3.6
Cage wall-roof edge	14.4	17.5	19.3	14.3
Tree	2.1	0	1.1	0
Ground	3.1	3.5	1.1	2.4
In flight	10.3	7.0	5.7	7.1
Roof	6.2	1.8	4.6	11.9
Cage frame	Not applicable	Not applicable	52.3	52.4
Total	100	100	100	100

the top portions of the sides of the cage, and very few landed on the citrus tree at the centre of the cage. When males were released they also flew to and landed mainly on the polyvinyl tubes supporting the roof and some immediately attempted to mate. Half the mating pairs formed within the first 30 min. Unreceptive females either flew away or initiated a series of movements to prevent the male from engaging genitalia. A female could remain in one landing position for more than 2 h. Only one of the 61 SEN females that mated with BKF males and were dissected was not inseminated. Latency time ($F_{1,170} = 0.01$, $P>0.05$) and mating duration ($F_{1,170} = 0.05$, $P>0.05$) were not significantly different for these strains (Table 1). The mean (± SD) mating propensity was 0.6 ± 0.13 with a relative mating performance of 0.02 in favour of the BKF males. The mean relative mating index for BKF was 0.51 ± 0.21 and SEN 0.49 ± 0.21. There was no significant difference in the number of mating pairs between BKF males and SEN females vs. SEN males and SEN females ($G = 0.093$, d.f. = 1, $P>0.05$).

About 52% of mating pairs were seen on the cage frame, 12% on the cage walls, 17% on the edge of the cage where the wall meets the roof, and the remainder in flight, on the ground, tree, and roof of the cage (Table 2). This overall trend in distribution of the location in the cage where mating pairs were first observed was similar for both strains.

Discussion

Operational success in AW-IPM programmes that include an SIT component depends on the biological quality of the sterile insects that are being released over the target area (Calkins & Parker, 2005; Vreysen et al., 2007). Assessing the sexual competitiveness of the produced and released sterile insects is an important component of the quality assurance of the sterile flies (Vreysen, 2005; Vreysen et al., 2007). In addition, in those cases where sterile males are released that originate from a different geographical area, mating compatibility between the released and the target strain becomes of paramount importance (FAO/IAEA/USDA, 2003). Project managers need to have the assurance that mating barriers between the released and target strain are absent, before a decision is made which strain to colonize and considerable funds are invested to establish the strain and mass-rear the flies.

Both the governments of Mali and Senegal have expressed an interest to incorporate the SIT in their arsenal of potential control tactics against the tsetse fly *G. p. gambiensis*. The colony that has been maintained at the CIRDES since the 1980s (Bauer et al., 1984) was the obvious choice to provide seed material for establishing a new colony in Mali or to provide sterile males for release in the Niayes of Senegal. However, before deciding on any future control strategy, it was deemed necessary to conduct mating studies in field cages to assess the presence or absence of mating barriers and to assess the mating performance of the males of the different strains. This became especially relevant for Senegal in view of recent population genetics data. Whereas significant gene flow was detected between the *G. p. gambiensis* populations of the Niger River Basin in Mali and those residing in the Mouhoun River Basin in Burkina Faso (Koné et al., 2011), there was no gene flow detected between the *G. p. gambiensis* population in the Niayes and those of the nearest population at Missira in the main tsetse belt in eastern Senegal (Solano et al., 2010). Although these findings were crucial from an operational and strategic point of view and encouraged the Government of Senegal to opt for an eradication strategy using an AW-IPM approach, the study also revealed genetic distances between the two populations that were equivalent to those observed between subspecies i.e., between *G. p. gambiensis* and *G. p. palpalis* (Solano et al., 2010).

The data from the mating studies indicate the absence of mating barriers between the BKF strain and the MLI and SEN strains. The BKF male flies successfully inseminated the female flies from the other target populations. Flight and mating activity in the cage were similar for all strains. These results imply that the BKF flies, despite being in culture for more than 30 years, have not developed pre-mating barriers with the *G. p. gambiensis* populations of the Niayes or the Niger River Basin. The colony of the BKF strain could, therefore, be a source of sterile males for the programme in Senegal. The results of the BKF-SEN studies likewise confirmed that genetic isolation does not necessarily imply mating isolation. Earlier studies using smaller laboratory cages with the allopatric sub-species *G. p. gambiensis* and *G. p. palpalis* and *G. p. gambiensis* and *Glossina fuscipes fuscipes* Austen likewise revealed readiness of the female flies to accept matings of the other subspecies (Gooding, 1988; Vreysen & van der Vloedt, 1990; Vreysen, 1995). In addition, introducing *Glossina morsitans* Westwood pupae into an isolated area inhabited by a low density population of *Glossina swynnertoni* Austen resulted in a significant reduction in the *G. swynnertoni* population according to theoretical expectations (Vanderplank, 1947).

These mating studies are an important component of the feasibility studies carried out in Mali and Senegal to assess whether tsetse-free zones could be created using AW-IPM approaches with an SIT component. These studies are important as they provide an opportunity to assess the mating behaviour and performance of reared and wild insects under controlled conditions. Direct observation of

tsetse mating behaviour in field cages has been done previously under controlled conditions (Mutika et al., 2001; Abila et al., 2003) but the open field conditions used at CIRDES were a first attempt to observe mating behaviour in outdoor field cages. Dean et al. (1969) used a similar approach but they worked with bigger cages, 26.7 and 752.5 m^2, in which competitiveness and compatibility were assessed indirectly by making inferences from production of larvae. The flight activity that was noted in both field cage setups was similar in that there was infrequent movement from resting positions and that the flies spent most of their time grooming themselves as noted for *Glossina pallidipes* Austen (Mutika et al., 2001). The high temperature experienced towards the end of the observation periods in the cage set up in the open in Burkina Faso may have influenced flight activity. There seems to be a complex mix of environmental conditions that are necessary for mating activity to take place and this may include temperature and light intensity above a certain threshold, and adequate humidity (Okiwelu, 1982). The behaviour of females that were not ready to mate was similar to that described for *Glossina austeni* Newstead and *G. m. morsitans* (Huyton & Langley, 1982), and *G. pallidipes* (Mutika et al., 2001). These types of field cage study have likewise been used to assess the effect of various abiotic or biotic parameters on the mating behaviour and competitiveness of tsetse flies, e.g., the effect of age on *G. f. fuscipes* and *G. p. palpalis* (Abila et al., 2003), of low temperatures on *G. pallidipes* (Mutika et al., 2002), and of low temperatures in combination with irradiation on *G. p. gambiensis* (GN Mutika, unpubl.).

In the last decade, the importance of using field cages to study the mating behaviour of insect populations from different geographic origins in the context of the SIT has been exemplified for several species of fruit flies, e.g., the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Cayol et al., 2002), and the South American fruit fly, *Anastrepha fraterculus* Wiedemann (Allinghi et al., 2007). These studies had far-reaching implications for the application of the SIT: no mating barriers were found between populations of the Mediterranean fruit fly from different continents and hemispheres. As a result a genetic sexing strain that was developed using an Egyptian strain of *C. capitata* (Franz et al., 1994) is now being used in most mass-rearing facilities in the world that use the SIT against this pest (Hendrichs et al., 2007). Conversely, distinct mating barriers were found between populations of *A. fraterculus* originating from Argentina, Peru, and Brazil implying that AW-IPM programmes with an SIT component can only be implemented using strains for rearing and release that originate from the same geographic region. These mating studies have now been expanded to other insect groups such as Lepidoptera, and a recent study found also no mating barriers between the codling moth, *Cydia pomonella* L., populations from different continents and hemispheres (Taret et al., 2010).

The results of field cage studies are important, as they approximate the natural conditions more closely and they allow the insects to behave more naturally than they would in small laboratory cages (where mating is basically forced upon them). The data from field cages, as carried out here, relate more to intrinsic biological features that determine isolation and competitiveness. In the wild, the released sterile flies are, however, competing with wild insects and there are several other important aspects that contribute to their sexual competitiveness which might not necessarily be revealed in a field cage, i.e., survival, mobility, dispersal, and aggregation patterns (Vreysen et al., 2011). These field cage studies therefore need to be complemented by an assessment of the performance of the sterile insects in the field and to assess their mating success by investigating mating frequencies of wild and sterile insects, e.g., rate of induced sterility (Vreysen, 2005). These pilot trails are planned for Senegal to assess how well the released flies of the BKF strain will perform under the very harsh ecological conditions of the Niayes (Bouyer et al., 2010).

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