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The effect of age on the mating competitiveness of male *Glossina fuscipes fuscipes* and *G. palpalis palpalis*

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Abstract

The effect of age on male *Glossina fuscipes fuscipes*, Newstead, and *Glossina palpalis palpalis,* Austin (Diptera: Glossinidae) competiveness were investigated with a view to estimate optimal age for sterile male release. Sterile insect technique involves the mass production, sterilization and sequential release of males of the target species to out compete the wild male population. Mating between released sterile males and wild females produce inviable progeny and the population is reduced over several generations to unsustainable levels. It is vital that the released male are of high quality and are sexually competitive. Age is one parameter affecting the sexual competiveness of the male tsetse fly. The optimal release age was estimated by assessing sexual competitiveness of flies of different age categories, 1, 5, 8 and 13-days after adult eclosion. A walk-in field-cage was used in order to approximate as closely as possible the actual field scenario during sterile insect release programes. It was shown that 8 and 13-day old males mated significantly more frequently, i.e. were more competitive, in the presence of equal numbers of 1 and 5-day old males. The age of male tsetse flies significantly affected competitiveness in both species studied. The ability of *G. f. fuscipes* to inseminate was not age dependent, and insemination occurred in all females that mated regardless of male age. In *G. p. palpalis*, however, 1-day old males were least able to inseminate. Mating duration was not significantly affected by age in both species. Eight to thirteen day old males of the test species are here recommended as the optimal sterile male release age.

Keywords: optimal mating age, male competitiveness, sterile insect technique, *Glossina fuscipes fuscipes*, *Glossina palpalis palpalis*

Résumé

L'effet de l'âge sur la compétitivité des mâles de *Glossina fuscipes fuscipes* et de *Glossina palpalis palpalis* a été étudié en vue de déterminer l'âge optimal pour le lâcher de mâles stériles. La technique de l'insecte stérile (TIS) consiste en une production de masse, en la stérilisation et au lâchage de mâles de l'espèce cible afin qu'ils competissent avec la population de mâles sauvages. L'accouplement entre mâles stériles lâches et femelles sauvages ne produit pas de progéniture, ce qui conduit au bout de plusieurs générations à une réduction de la population à un niveau non sur vivable. Il est primordial que les mâles lâches soient de bonne qualité et sexuellement compétitifs. L'âge est l'un des paramètres affectant la compétitivité des mouches tsetse mâles. Il était donc nécessaire d'estimer l'âge optimal pour lâcher des mâles en comparant la compétitivité de différentes catégories d´âges (1, 5, 8 et 13 jours après leur émergence). La méthode dite du « field-cage » a été utilisée afin d'étudier le comportement des mâles TIS dans les conditions aussi proches que possible de la réalité. Il a été démontré que les mâles de 8 et 13 jours s'accouplent plus fréquemment que les mâles de 1 a 5 jours. Pour les deux espèces étudiées, l'âge affecte significativement la compétitivité des tsetse mâles. La capacité des mâles de *G. f. fuscipes* à inséminer n'est pas fonction de l'âge ; toutes les femelles accouplées sont inséminées. Chez *G. p. palpalis* cependant, les mâles de 1 jours sont les moins inséminées. La durée de l'accouplement n'est pas significativement affectée par l'âge dans les deux espèces. Les mâles de 8 et 13 jours des deux espèces testées sont les plus recommandés pour le lâcher des mâles stériles.

Mots clé: âge optimale d'accouplement ; compétitivité des mâles ; technique de l'insecte stérile ; *Glossina fuscipes fuscipes ; Glossina palpalis palpalis*

Introduction

Mating behavior in tsetse flies (*Glossina*) has been the focus of recent research (Mutika *et. al*., 2001; Olet *et. al*., 2002; Carlson and Schlein, 1991) because of its implications in the efficiency of the sterile male insect technique as a control strategy (Knipling, 1955; 1959; 1963). Tsetse cause significant economic loss by transmitting both human and animal African trypanosomosis; a disease caused by protozoan blood parasites of the genus *Trypanosoma*. It is estimated that annual losses caused by animal trypanosomosis in agricultural production in Sub-Saharan Africa are in the order of UK £ 3 billion and more than 100 human lives a day (Budd, 1999).

Vector control has been one option in the control of both human and animal forms of the disease. Tsetse control methods have evolved from game and bush clearing at the beginning of the 20th century to insecticide spraying in the 1950s (Allsopp, 2001), and more recently traps, targets (Brightwell *et. al*., 1997) and live bait technologies have been used to control tsetse (Thomson and Wilson 1991). These methods have been used successfully with dramatic reductions in tsetse population sizes (Leak, 1999). However, in the absence of sustained control efforts and protection of cleared areas, tsetse fly populations have recovered from residual pockets and/ or re-invaded from neighbouring territories (Brightwell *et. al*., 1997).

The area-wide-approach to tsetse control aims at control of entire populations and to effectively prevent re-invasion or recovery from residual pockets. The sterile insect technique is one way in which this can be achieved (Knipling, 1963). The sterile insect technique relies on the release of mass reared, sexually sterilized males and is environmentally benign. Mating between released sterile males and wild females results in embryonic arrest followed by expulsion of the dead embryo (Van der Vloedt, *et. al*., 1978). Successful area-wide use of sterile insect programs have been implemented against insect pests and disease vectors such as the Mediterranean fruit fly, *Ceratitis capitata* (Hendriches *et. al*., 1995), the New World screw-worm, *Cochliomyia hominivorax* (Lindquist *et. al*., 1992), and the mosquito, *Culex quinquefasciatus*, the vector of bancroftian filariasis (Patterson *et. al*., 1977; Curtis and Andreasen, 2000). More recently, Vreysen *et. al*. (2000), have demonstrated the application of the use of sterile insect techniques in the area-wide eradication of *Glossina austeni* and elimination of animal trypanosomosis transmission on Ungunja Island, Zanzibar (Dyck *et. al*., 2000). This case, in addition to earlier successful attempts in Burkina Faso (Politzar and Cuisance, 1984) and Nigeria (Oladunmade, *et. al*., 1990), have inspired a continental strategy to progressively reduce isolated tsetse populations to unsustainable numbers. This undertaking will require mass production of sterile males for sustained sequential release until significant population reductions are achieved (Feldmann and Hendrichs, 2001).

The labor and attendant costs of producing sufficient numbers of sterile male tsetse flies for release inevitably requires that the mating success and competitiveness of the released flies are optimal (Mutika, *et. al*. 2001; Alphey and Andreason, 2002). Olet, *et. al*. (2002) investigated sexual receptivity and age in *G. pallidipes* and showed that older males copulated more frequently than younger ones. Releasing sterile males of an optimal age would improve efficiency of the sterile males to out compete the wild males. This will contribute towards the efficiency and reduce costs of a release program. It is therefore vital to investigate the most succesful age for copulation and sperm transfer in male tsetse flies, with a view to establish the optimal age for sterile male release for each species. Earlier investigations on the age and sexual competitiveness of tsetse have been done in fly holding cages that confined the flies to unnaturally small and restricted space. Here we use a walk-in fieldcage (Calkins and Webb, 1983; Mutika, 2001) to investigate the effect of age on the mating competitiveness of the test species; *Glossina fuscipes fuscipes* Austen, and *Glossina palpalis palpalis* Austen. Information on optimal sterile male release age, duration of copulation, insemination rates, and ambient environmental conditions of the experiments are reported.

Materials and Methods

The tsetse flies

The *G. f. fuscipes* colony used in this study were first colonized in 1986, while the *G. p. palpalis* dated from 1981 and have been in culture for 64 and 88 generations respectively. Both colonies were maintained *in vitro* on silicone membrane with bovine blood diet, at the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf, Austria. The flies were kept under standard colony conditions of pure bovine blood diet. The flies were offered a blood meal everyday except on Sundays. Holding temperature was maintained at 24 ± 1 °C, and 70 to 80% relative humidity (Feldmann, 1994a,b). The males were chilled at 4 °C and color marked using Rowny® acrylic paint one day before the experiments. All female flies used in experiments were 8 days old which is when female receptivity to males was determined to be maximal (Olet et al., 2002). Males are capable of inseminating at emergence because of their well-developed accessory glands (Forster, 1976). In both colonies of the test species that were used in this study, the males were able to mount and inseminate successfully on the first day after eclosion, in the absence of older males.

The field-cage

The cage used was the same as that described by Calkins and Webb (1983). The cage netting forms a cylinder of 2.9 m diameter and 2.0 m height. The entrance is through a front vertical zipper. Two small citrus trees located in the centre of the cage may not duplicate but imitate field like situation. The cage was located in a glasshouse with temperature controls (Mutika *et. al*., 2001). The experiments were done once a week for four consecutive weeks.

Environmental conditions

During the experiments, temperature, humidity and atmospheric pressure were recorded every 30 minutes using a digital Meteoscope II (Augenoptikversand, Sindelfingen, Germany). Light intensity was measured from three locations within the cage; top, foliage level and floor using Lightmeter TES-1334 (TES Electrical Electronic Coorporation, Taipei, Taiwan). The observer was present in the field-cage throughout the full duration (2 and 3 hours for *G. f. fuscipes* and *G. p. palpalis* respectively) of the experiments and movements were kept minimal. The experiments were repeated four

times for each of the test species. Each repeat is referred to as a run.

Mating Competitiveness

Mating propensity was defined as the proportion of females that mated, and are indicative of the tendency of the flies to mate. The relative mating index was defined as the number of mating pairs accounted for by the age category as a proportion of the total number of mating pairs. This measure represents the competitiveness of each age category tested relative to the other categories (Cayol, *et. al*, 1999).

Virgin male and female *G. f. fuscipes* and *G. p. palpalis* were collected from the colony on the first day of eclosion from pupae. To ensure that female and male flies were not mated, they were removed as they emerged from pupae. The experiments began at 10.00h and terminated at 13.00h local time to cover the morning peak activity hours of flies in the field. In each test species a total of 30 females were released in the field cages and observed for the presence of non-fliers. Any non-flier was removed and replaced.

Different age categories chosen following the mating regime system reported in Abila *et. al*. (in press). Equal numbers (n=15) of male flies, 1, 5, 8 and 13 days after eclosion, were marked with different colors on the thorax and introduced in the cage resulting in a total of 60 males in each test species. As in the case of the females, any non-flier was removed and replaced. For each mating pair that formed, the age of the male fly was identified by the age-specific color code.

Mating duration

The flies were observed carefully for any signs of male pursuit of females. Once genitalia were engaged and the pair was in copula, the pair was collected into individual tubes. Age of the male, and the starting and separation time of each successful mating, were recorded to the minute. The duration was then calculated as the difference in minutes between ending and starting times. Once copulation ended, the male was removed and the female was kept in the fly holding room overnight to allow sperm migration to the spermathecae.

Insemination

Dissections were done in physiological saline solution under binocular microscope, to determine insemination rate and spermathecal fill (Pollock, 1982). The spermathecae were removed, and mounted on a microscope slide with a cover slip. Spermathecal fill was estimated by microscopic viewing each spermatheca under 100 x power. The spermathecal fill was scored as; empty (0), quarter full (0.25), half-full (0.50), three-quarter-full (0.75) and full (1.0). The amount of sperm transferred was then computed as the mean spermathecal fill values of both spermathecae (Nash, 1955).

Data Analysis

Data were subjected to Arcsine transformation before analysis of variance and for significant variations; the homogeneity of means was tested by the Least Square Difference (LSD) procedure.

Results

Environmental conditions

Light intensity was not controlled and varied naturally. The values recorded range from 596 lux within the foliage of the citrus trees and the maximum was 11380 lux at the roof of the cage. The average light intensity was computed to have been 3944 ± 432 lux. Temperature varied from 20.0 to 28.5 °C. The average was 24.4 ± 0.4. Relative humidity ranged from 40% to 75%, with an average of 53.7 ± 1.8 %. The average atmospheric pressure during the experiments was 997 ± 1.9 mb. Figure 1 summarises the temperature and relative humidity observed.

Mating Competiveness

When released from the holding cages, the flies dispersed randomly throughout the field-cage. Many settled within the foliage provided by the citrus trees in the field cage. Flies preferred to rest on the lower side of the leaves and branches. Some flies rested on the roof and walls of the cage.

The overall mating propensity of the experiment was 0.59 ± 0.05 in *G. f. fuscipes* and 0.69 ± 0.18 in *G. p. palpalis* (see Table 1).

There was no definite pattern of pairing behavior observed in relation to time of the experiments (Figure 2). Runs 1 and 2 had similar patterns of activity except that the peak activity times varied between the 2nd and 3rd hours, respectively. The majority of pairs were formed within the first 60 minutes of the experiments. There were long periods of relative immobility followed by a sudden surge in activity and mating strikes, genital engagement and copulation.

During the experiments, some males were observed trying to engage genitalia with already copulating females, but were unsuccessfull in dislodging the earlier male during the occasions that this behavior was observed. In *G. f. fuscipes*, most (38.6%, N=70) of the mating pairs were formed within the folliage provided by the citrus plants in the cage. Another 32.9% were formed on the walls of the cage and 15.7% on the wall edges, the rest forming on the roof.

Analysis of variance revealed a highly significant difference in the relative mating indices influenced by age of male in both test species; ($P<<0.001$, $F = 21.44$, $df = 3$) in *G. f. fuscipes* and ($P<<0.001$, $F = 65.04$, $df = 3$) in *G. p. palpalis*. LSD tests reveal two groups in *G. f. fuscipes* in which the means were not significantly different. The 8 and 13-day old were not significantly different, but were superior to the 5 and 1-day old male. In *G. p. palpalis*, all the four age categories differed significantly from one another. The 13-day old males mated most frequently followed by the 8, 5, and 1-day old males in decreasing order of competitiveness. The runs did not significantly affect the variation ($P\sim1.0$, $F\sim 0$, $df = 3$) in both species (Table 2 and Figure 3 a and b).

Table 1: Sumary of mating results in the field cage

Run	No. of Females	No. of Males	Sex ratio	*G. f. fuscipes* Pairs formed Total	Mean ± SE	MP	*G. p. palpalis* Pairs formed Total	Mean ± SE	MP
1	30	60	1:2	17	4.25 ± 2.14	0.57	23	5.75 ± 2.43	0.77
2	30	60	1:2	17	4.25 ± 2.21	0.57	13	3.25 ± 1.32	0.43
3	30	60	1:2	22	5.50 ± 1.85	0.73	23	5.75 ± 2.56	0.77
4	30	60	1:2	14	3.50 ± 2.02	0.47	24	6.00 ± 2.58	0.8
Overall	120	240	1:2	70	4.38 ± 0.41	0.58	83	5.19 ± 0.65	0.69

MP - mating propensity, the proportion of females that mated

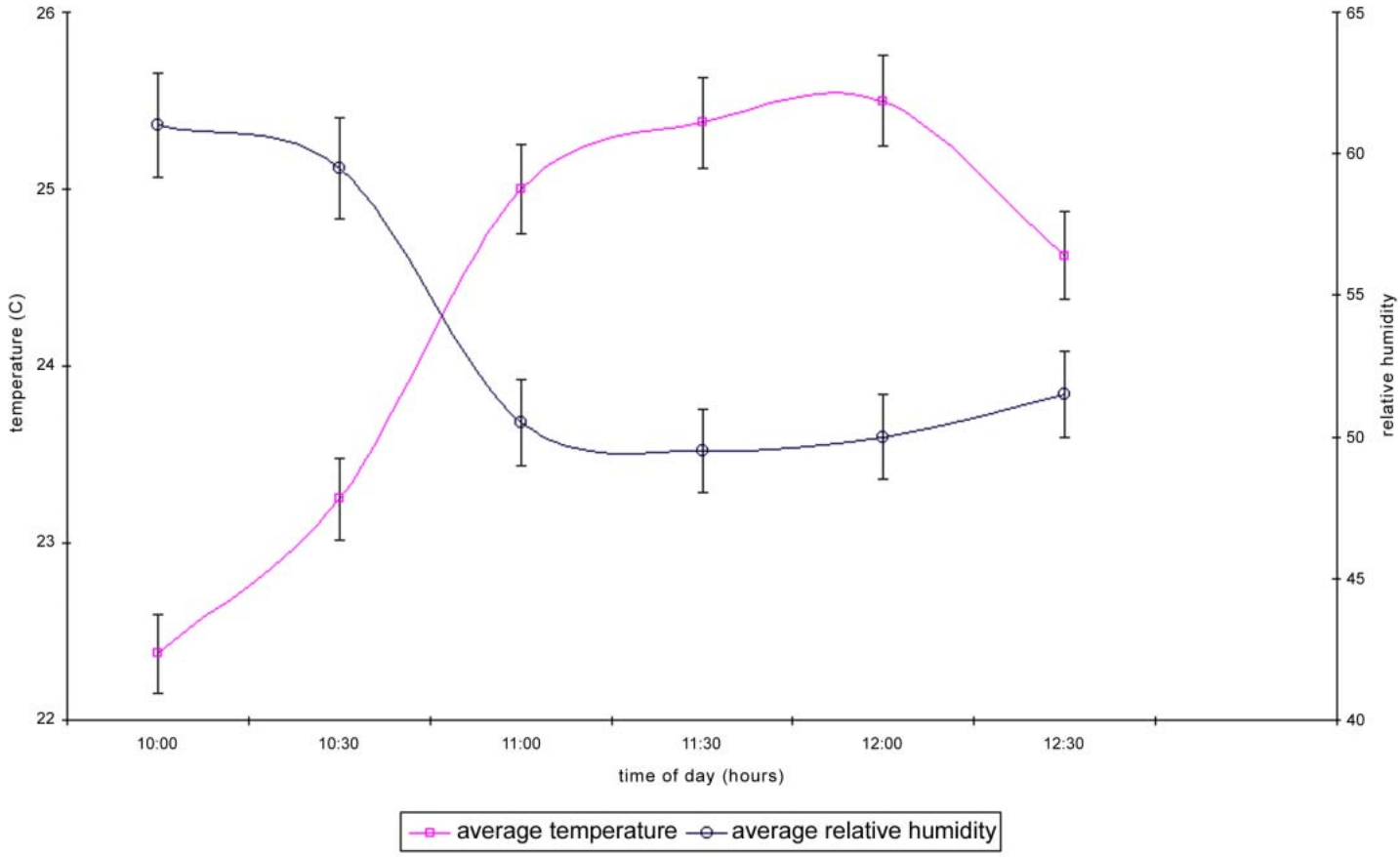


Figure 1: Mean temperature and humidity recorded during the experiments

Mating duration

After genital engagement copulation ensued, which had a variable duration. In both test species, no significant differences in mating duration were seen (Table 2).

Insemination

All the *G. f. fuscipes* females that mated (N=70) were inseminated with varying spermathecal indices. The variation in insemination indices in *G. f. fuscipes* were not significanly affected by the age of males (P = 0.0649, F = 2.530, df=3). Nevertheless, the mean spermathecal fill value declined with age of the male (Table 2). In *G. p. palpalis* age significantly affected the ability of males to inseminate (P<<0.001, F=6.74, df = 3). The 1 and 5-day old male *G. p. palpalis* were less able to inseminate compared to the 8 and 13 day old males. Only 6.64% (n = 8, N = 83) were not inseminated, showing an insemination rate of 93.36% among females that copulated.

Discussion

The results of this study confirm that age influences the mating success of male *G. f. fuscipes* and *G. p. palpalis*. Young males (1 and 5-day old) are less competitive in comparision to the eight and thirteen day old categories. Nevertheless, in contrast to the findings of Mellanby (1936), that *G. palpalis* (this has been subsequently refered to as *G. f. fuscipes* as reviewed by Newstead (1911)) males are unable to inseminate before 5 days, in our experiments, one day old flies (n=3) were able to score an overall mean spermithecal value of 0.75 ± 0.00. Among the tested age categories, the optimal age for release of *G. f. fuscipes* males was shown to be at least 8 days in this study. It is here recommended that sterile male *G. f. fuscipes* releases should be composed of flies that are 8 to 13-day old. In *G. p. palpalis*, however, the 13-day old males were shown to mate siginificantly more than the next best age (8-day old). The appropriate sterile male release age for *G. p. palpalis* is therefore 13-days old flies.

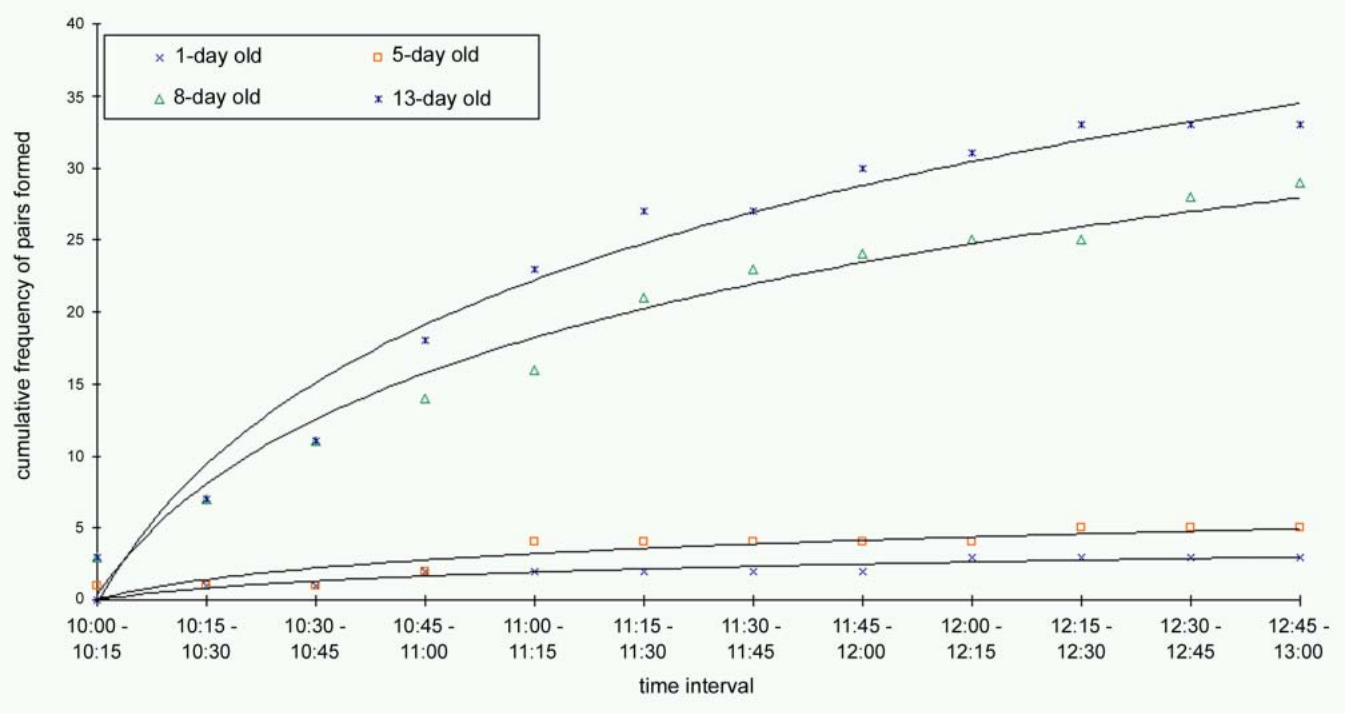
It would be worthwile to release the 8-day old sterile *G. p. palpalis* males if their longevity in the field can maintain adequate numbers upto the 13th day after emergence. A recent study addresing the effects of gamma radiation on the longevity of male *G. pallidipes* (Opiyo *et. al.* 2001) reports that longevity spaned a period of 60 days in the laboratory. Earlier studies however indicate that irradiation reduced longevity in *G. p. gambiense* (Politzar *et. al.*,

Table 2: Effect of age on the competitiveness of male *G. f. fuscipes* and *G. p. palpalis*

Age (days)	Total number of pairs formed		De-transformed Relative mating index (± SE)		Mating duration ± SE (minutes)		Mean Spermathecal Value (± SE)	
	G.f.f.	*G.p.p.*	*G.f.f.*	*G.p.p.*	*G.f.f.*	*G.p.p.*	*G.f.f.*	*G.p.p.*
1	3	3	0.04±.01a	0.04±.02a	41.67±17.7a	41.67±37.67a	0.75±.00a	0.17±.17a
5	5	14	0.06±.06a	0.17±.02b	56.60±10.01a	83.93±9.30a	0.65±.13a	0.51±.11a
8	29	22	0.42±.06b	0.26±.04c	40.24±3.59a	78.64±9.50a	0.68±.05a	0.73±.06b
13	33	44	0.48±.04b	0.53±.01d	35.97±3.19a	86.89±4.64a	0.74±.05a	0.80±.04b

Any two values in the same column followed by the same letter are not significantly different (P<=0.05)
The relative mating index is the number of mating pairs as a proportion of the total number of mating pairs.

A



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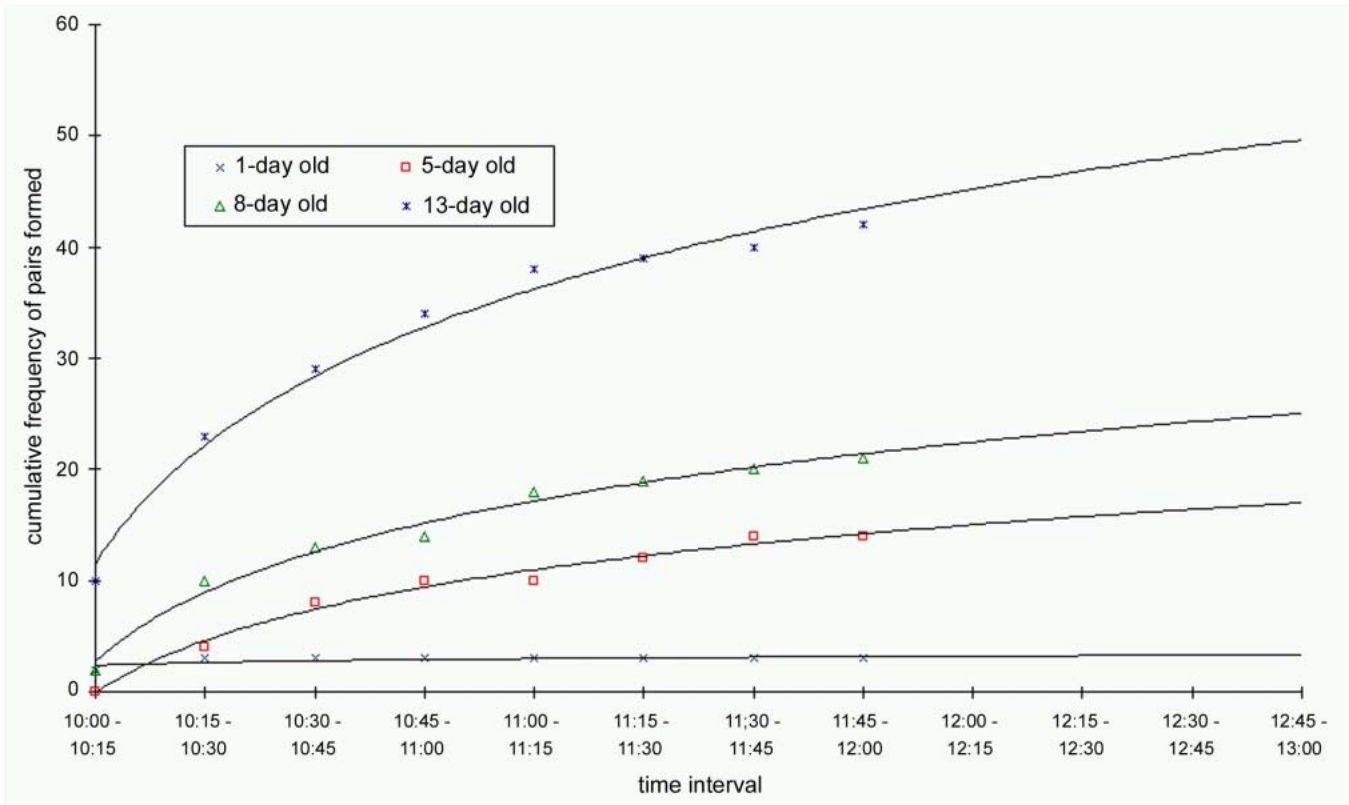


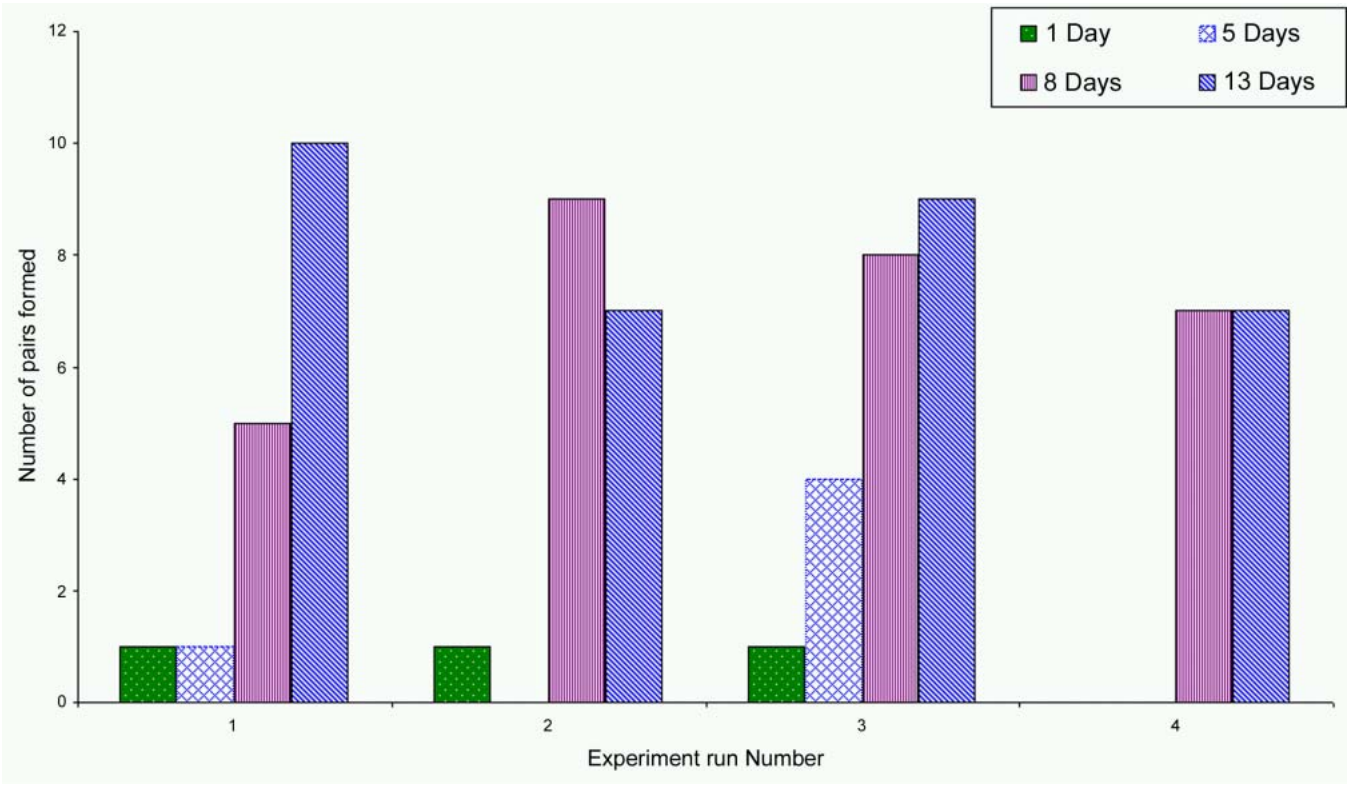
Figure 2 a: Cumulative frequency of pair formation with time in *G. f. fuscipes*. **2b:** Cumulative frequency of pair formation with time in *G. p. palpalis*

1979), *G. tachinoides*, *G. f. fuscipes*, and *G. brevipalpis* (Vreysen *et. al*., 1996).

The sterile male release ages recommended here are consistent with those obtained in a similar study using fly holding cages with *G. pallidipes* that estimated the optimal release age at seven days (Olet *et. al.*, 2002). However, it is possible to explain the descrepancy as a result of the systematic age categorisation (1, 5, 8, and 13 in this study while Olet *et. al*., 2000 used; 3, 5, 7, 9, 11, 13, and 15 day old males) and/ or real differences resulting from using different species.

Regardless of the development of physiological sexual maturity, changes in the competitiveness with age may have a more fundamental influence on mating competitiveness and success across several tsetse species (Wall and Langley, 1993). In *G. p. palpalis*, 3

A



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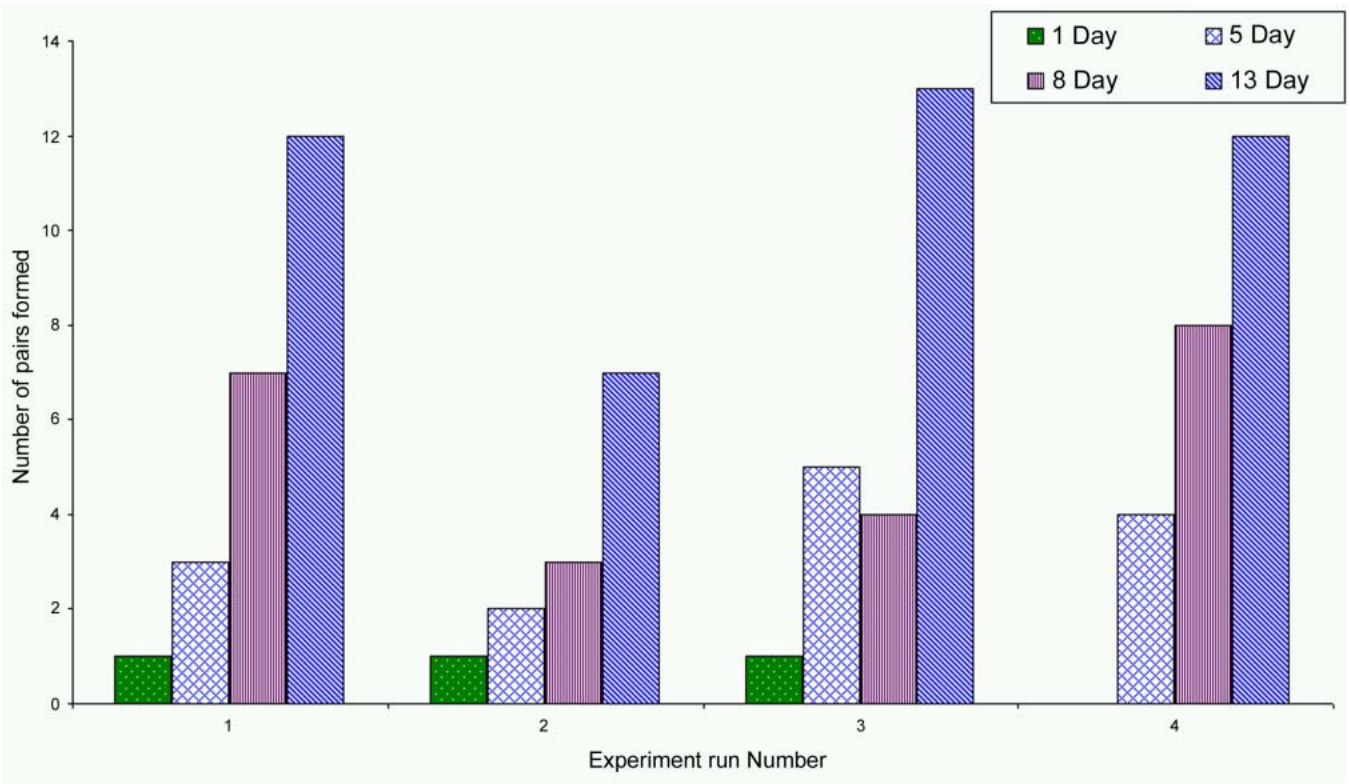

Figure 3a: Number of pairs formed by age category against run in *G. f. fuscipes*. **3b:** Number of pairs formed by age category against run in *G. p. palpalis*

- 6 day old males were observed to account for only 18% of copulations when in competition with equal numbers of older males (Nash, 1955). Ten-day-old *G. m. morsitans* Westwood males inseminated significantly more females (and to a greater degree) than 5-day old males (Southon and Cockings, 1963). In a study of the effects of age on responses of *G. m. morsitans* and *G. pallidipes* males to decoy females in the laboratory, it was shown that there was a significant increase in activity levels with age, which resulted in older males mating more frequently (Wall, 1988).

The age of male flies did not significantly affect mating duration in either test species. Mating duration in both test species were in the order of 40 minutes. It has been suggested before that long copulatory periods exceeding 30 minutes are caused by unsuccessful attempts at engaging genitalia at the on-set of copulation, but no attempt was made to relate this to the age of the male fly (Mutika *et. al*., 2001).

This study has revealed that the age of male *G. f. fuscipes* and *G. p. palpalis* influences sexual competitiveness, but not mating duration and insemination. These results should provide a picture of actual field conditions although only laboratory maintained strains of the test species were used. These conditions approximate actual sterile insect release programs, but may fall short of the actual field scenario, where colonized and wild males compete for fertile females. Using the walk-in field cage, however, provides the nearest possible field-like conditions for this type of investigation. Any future attempts to address these concerns in a single experiment will go a long way in improving our understanding of the reproductive behavior and factors affecting the competitiveness of released sterile males.

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