Tolerance of Low Temperature and Sterilizing Irradiation in Males of Glossina pallidipes (Diptera: Glossinidae)

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ABSTRACT. Investigations into the possibility of using the chilled adult release system are continuing as an alternative method to the release of sterile tsetse flies, Glossina pallidipes Austen (Diptera: Glossinidae) in cardboard boxes. Exposing tsetse flies to 4°C for 6 h caused negligible mortality. A combination of chilling and irradiation resulted in reduced quantities of seminal contents being transferred to females. Mortality of flies after bulk irradiation was lower when a thermos flask was used than expanded polystyrene. Mortality after removal from cold storage increased with age. Flies that did not have a blood meal for 3 d prior to exposure to cold had a lower overnight survival than flies that were deprived of a blood meal for 1 or 2 d. Exposure of adult male tsetse flies to low temperature should be for as short a duration as is practical, so that the fitness of the released sterile flies is not unduly compromised. It is also necessary to ensure that losses are minimized during bulk irradiation of adult flies. It would be desirable to have minimal losses after the combined effects of irradiation, cold, and transportation, such that a sufficient number of sterile male flies will still be available to successfully compete for mating opportunities with wild females.

Key Words: glossinidae, chilled adult release system, SIT, tsetse, trypanosomiasis

Some species of tsetse flies, Glossina spp., Theobald (Diptera: Glossinidae), are vectors of the causal agents of trypanosomiasis commonly known as sleeping sickness (human African trypanosomosis) or nagana (animal African trypanosomosis). Considerable resources have been invested in research work to develop means to combat these diseases with control efforts directed at both the vector and parasite. The current approach, spear headed by the Pan African Tsetse and Trypanosomiasis Eradication Campaign, includes application of the sterile insect technique (SIT) as a component of area-wide pest management where it is desirable (Kayabo 2010), and several field programs were executed in the past using the SIT (Dean et al. 1969, Dame and Schmidt 1970, Curtis 1980, Williamson et al. 1983b, Offori 1992, Vreysen et al. 2000, Feldmann 2004).

SIT programs for fruit flies have developed the chilled adult release system (Mangan 1996), which is considered more desirable as no extra material is strewn in the environment, reducing pollution, cost, and the volume needed to hold the flies during releases. Work on chilling of adults of blood-feeding insects has shown that there are high levels of mortality under certain conditions (Elwaer and Elowni 1991, Jones and Kunz 1998). However, brief exposure to low temperature may be included during processing, thus sacrificing a few of the insects to bring about the desired process efficiency. Chilling has been used successfully in the transportation of large numbers of adult horn flies, Haematobia irritans L., and it was recognized that depletion of oxygen and accumulation of carbon dioxide were critical factors in survival of flies (Miller et al. 1977, Jones and Kunz 1998).

For the New World Screwworm, Cochliomyia hominivorax Coquerel, cold storage has been used for irradiated adults and pupae for transportation from rearing plants to distant distribution centers (Hightower and Garcia 1972, Elwaer and Elowni 1991, Lindquist et al. 1993), similarly for H. irritans (Miller et al. 1977). The added advantage of the procedure is the ability to extend the storage of insects when releases cannot be undertaken due to unfavorable weather conditions or other logistical problems. It is important to determine cold or heat tolerance and the critical time and temperature regimes for radiation-sterilized insects (Bursell 1960, Chirico et al. 1994).

During the last major application of SIT for tsetse eradication in Zanzibar (Vreysen et al. 2000), the sterile males were loaded in biodegradable cardboard boxes for aerial release. It would be worthwhile if the chilled adult release system could be developed for tsetse flies to reduce material, labor, and flight costs. However, the feasibility of the entire process has not been explored for tsetse flies.

Normal development in tsetse flies is thermodynamically controlled (Phelps and Burrows 1969a, b; Zdarek and Denlinger 1995) and prolonged exposure to low temperature for any of the life stages is potentially lethal (Bursell 1960, Sinclair 1999, Somme 1999). Various approaches to improve handling procedures have been investigated, for instance, anoxia has been found to improve cold tolerance in house flies (Coulson and Bale 1991). Even though it has been demonstrated that irradiation in a nitrogen atmosphere is beneficial in terms of quality of flies (Williamson et al. 1983a), the procedure still remains to be widely adopted in field programs. The work that is reported in this article provides a quality assessment of the effect of chilling and gamma irradiation sterilization on Glossina pallidipes Austen, taking survival, mating behavior, insemination ability, and sperm transfer as the parameters of interest. The work builds on previous experiments (Mutika et al. 2002, Mutika and Parker 2006) to determine response of tsetse flies to several procedural events in the production of sterile male flies in an attempt to simulate the major temperature events following the proposed release method.

Materials and Methods

Fly Strains. All experiments were carried out with a strain of G. pallidipes that is maintained on silicon membranes at Seibersdorf, Austria, at 24 ± 0.5°C and 75 ± 5% relative humidity (RH). The colony was derived from field pupae collected in Uganda in 1975 and first colonized in Amsterdam, The Netherlands. The flies are fed three times a week, pupae are incubated at 24°C for 4 wk, and adults emerge at
26.5°C directly into production cages at required proportions, avoiding chilling to separate the sexes (Opiyo et al. 2000). The colony is fed commercially procured bovine blood collected from an abattoir, frozen at −20°C, and irradiated with 1 kGy in a commercial 220 PBq 60Co wet storage panoramic shuffle irradiator. Aliquots of the blood are thawed and used as required. Reference to pupae in this article includes the pharate adult stage.

The Field Cage and Its Environment. Field cage experiments were conducted in a cylindrical netting cage (Calkins and Webb 1983), 2.9 m in diameter and 2.0 m in height, set up in a glasshouse under natural light (Cayol et al. 1999, Mutika et al. 2001). To monitor environmental conditions, temperature and humidity were measured using a Luft Opus I thermo hygrometer (G. Luft Mess- und Regeltechnik GmbH, Fellbach, Germany), and light intensity was measured at 30-min intervals using a TES-1334 light meter (MTP Instruments Inc., Quebec, Canada), starting at 1000 hours and ending at 1200 hours. The temperature (mean ± SE) ranged from 20 ± 0.8°C up to 25 ± 1°C and RH (mean ± SE) from 72 ± 4% down to 61 ± 5% from the beginning to the end of observations. Light intensity (mean ± SE) varied from 2,030 ± 350 Lux to a peak of 3,500 ± 609 Lux.

Mating Activity. Batches of pupae were transferred to 26.5°C for emergence. The total number of pupae in each batch varied but was sufficient to ensure a minimum of 120 males emerged each day. Six days following eclosion, four groups of males were selected. Two colors of acrylic paint were used to mark each of two groups of flies with a dot sufficient to ensure a minimum of 120 males emerged each day. Six days following eclosion males in each treatment were individually labeled and observed to record the time when the mating occurred. The time when each successful mating pair was seen was taken as the period from the end of the release of the males to the time a successful engagement of genitalia occurred. The time when each successful mating pair was seen was recorded and the pair immediately collected into a tube. The tubes were individually labeled and observed to record the time when the mating pairs separated. Duration of mating was then calculated as the difference between pre-mating and end time in minutes. The total number of pairs collected each day the test was run was expressed as a proportion of the total possible pairs to give the mating propensity (Mutika et al. 2001). Dissection of females that mated was carried out in saline solution under a stereo microscope at 50× magnification to estimate the quantity of seminal fluid in the spermathecae (Nash 1955).

Chilling. Chilling at Low Density. Male G. pallidipes of different ages, held in standard 110-mm diameter by 50-mm deep cages, were starved for 1, 2, or 3 d and chilled at 4°C for 6 or 24 h at 40, 50, or 70% RH then returned to normal colony holding conditions. Most flies were 7–8–d old with additional ages observed to provide comparative data. Chilling at High Density in Bulk. Most of the chilling was conducted in one of two programmable temperature and humidity controlled chambers, Weiss Technik 1600SP (Weiss Umwelttechnik GmbH, Reiskirchen, Germany) and Jumo ZPR-2000 (LEEC Limited, Nottingham, UK), enabling gradual reduction and increase of temperature and humidity at the start and end of the chilling period, respectively. Additional work was conducted in an LMS Cooled incubator (LMS Ltd, Kent, UK) at 4°C, and the humidity was not controlled but remained around 40%.

Adult G. pallidipes males were chilled at 4°C prior to irradiation in the cobalt-60 gamma source. For irradiation, the flies were placed in small rearing cages or in an expanded polystyrene (Styropor) cylindrical container or in a wide-mouth vacuum flask. The latter two containers fitted snugly into the irradiation chamber. The expanded polystyrene container was used twice but then discontinued due to high mortality, whereas the thermos flask, which held ~3,000 flies, was used five times. The flies were irradiated with a dose of 120 Gy at ~20 Gy/min at room temperature. The flies irradiated in the large containers were apportioned into holding cages and placed in an incubation chamber together with cages of the control group for up to 6 h with temperature stable at 4°C and RH fluctuating around 50%. The flies were then returned to normal colony holding conditions together with an irradiated control group that was not chilled and survival without feeding was observed.

Cold Acclimation. An experiment was also set up to assess cold acclimation. Flies were subjected to 6 h chilling at 4°C, 70% RH, 1 h after 30 or 60 min conditioning at the same temperature. This was intended to determine whether there was any rapid chill hardening in tsetse flies (Chen et al. 1991).

Effect of Humidity. A subsidiary experiment was conducted to further investigate the effect of humidity when the previous experiments indicated a strong effect. Flies were irradiated and chilled in a high (72% RH) or low (~40% RH) humidity environment to assess survival. Pupae were irradiated for 27 d at 23–24°C then transferred to 26.5°C for eclosion. Six days following eclosion males in each treatment were split into three groups. One group was irradiated in air with 120 Gy sterilizing dose, one with 180 Gy in nitrogen, and the third group was not irradiated. Each of the three groups was further split into two subgroups. Half was exposed to 4°C for 6 h at high humidity with the other half at low humidity and returned to the holding room overnight. In total, 25 cages with 30 flies each (except two with 24 and 14) were observed. The flies were not fed again. Survival was first recorded on the morning after return to normal colony conditions and every working day thereafter. Mortality and general flight activity (no attempt to score) in individual cages positioned at random in the chamber were checked every morning until all flies died.

Statistical Analysis and Mating Indices. Homoscedasticity of the mating parameters data was confirmed using Levene’s test, and approximate normality was confirmed using the graphical summary subcommand in Minitab (2000). An analysis of variances was carried out on pre-mating period, duration of mating, and mean spermathecal value followed by separation of means using Tukey’s comparisons at the 5% level. The mating propensity and relative mating index were calculated (Mutika et al. 2001). Proportions of males that mated in field cage tests
were analyzed using the replicated tests of goodness of fit (G-statistic) (Sokal and Rohlf 1995). Survival of hunger stressed male flies was analyzed using the binary logistic regression function in Minitab with total number of flies that were alive each day taken as success and total number of flies that died each day as failure.

**Results**

**Mating in Field Cage.** The average numbers (± SE) of experimental flies were 72.5 ± 3.1 in control, 44.3 ± 3.2 irradiated in air, and 52.5 ± 2.2 irradiated in nitrogen for each of the 11 replicates in the field cage mating tests. Mean (± SE) overnight mortality prior to the field cage test was 0.2 ± 0.5% for control flies, 3.2 ± 1.6% for males irradiated in air, and 12.9 ± 3.3% irradiated in nitrogen saturated atmosphere. Nonflyers were 2.4 ± 0.6, 3.2 ± 1.1, and 5.1 ± 2.7%, respectively.

**Mating Propensity.** The mating propensity was 0.4 ± 0.05 for the competition between unirradiated males and males irradiated in air and 0.28 ± 0.06 for the competition between unirradiated males and males irradiated in nitrogen saturated atmosphere. There were two replicates where mortality recorded after irradiation in nitrogen followed by chilling was very high (26 and 38%) and none of the males secured a mating during the subsequent field cage test. There were a total of 76 males that were irradiated in air and 56 males that were not irradiated that succeeded in mating out of the total of 330 males of each treatment in the 11 replicates, giving relative mating indices (± SE) of 0.54 ± 0.07 and 0.46 ± 0.07, respectively.

**Mating Proportion.** The proportions of males that mated in each of the 11 replicates were significantly different in 1st (G = 4.44, df = 1, P < 0.05) and 10th replicates (G = 6.20, df = 1, P < 0.05) giving an overall (heterogeneity G) significantly higher proportion of males irradiated in air over the unirradiated (G = 19.02; df = 10; P < 0.05). However, the males irradiated in an atmosphere saturated with nitrogen and subsequently stored at low temperature were significantly less successful (total G) in securing mating opportunities compared with nonirradiated males (G = 28.31; df = 11; P < 0.01) with significant differences on third (G = 4.19; df = 1; P < 0.05), fifth (G = 8.32; df = 1; P < 0.01), and seventh replicates (G = 3.86; df = 1; P < 0.05) giving a significant pooled G (G = 15.81; df = 1; P < 0.01). There were 28 males irradiated in nitrogen saturated atmosphere and 66 control males out of a possible 330 that successfully mated giving a relative mating index of 0.76 ± 0.05 for males that were not irradiated and 0.24 ± 0.05 for males that were irradiated in nitrogen saturated environment.

**Mating Parameters.** The treatment of the males did not significantly affect the prematting period (F = 0.50; df = 2, 223; P > 0.05), average copulation duration (F = 0.15; df = 2, 216; P > 0.05), or mean spermathecal values (F = 2.23; df = 2, 223; P > 0.05). Even though a few spermathecae were full, the majority of flies failed to transfer sperm that completely filled the spermathecae (Table 1). There were 19 out of 226 males that failed to transfer seminal fluid, but of those, 3 had a copulation duration insufficient to allow sperm transfer. The flies that failed to transfer sperm were 12 control, 4 irradiated in nitrogen, and 3 irradiated in air.

**Survival After Chilling.** Through direct observation as the temperature in the chamber was lowered, it was noted that flies could still move/twitch their legs and drag themselves around down to 6°C, whereas they were completely immobile below this temperature.

**Effect of Fly Age on Survival.** Survival of unirradiated flies older than 9 d was significantly reduced (F = 289.83; df = 1,438; P < 0.01) the day after removal from 6 h low-temperature storage (Table 2).

**Effect of Length of Starvation on Survival.** The starved flies could still feed through a silicone membrane like other colony flies. Starvation for 1 to 2 d prior to exposure to low temperature did not significantly affect survival, but there was a marked increase in overnight mortality on the first day after removal from low-temperature storage if the flies were starved for 3 d prior to chilling (Table 3).

**Effect of Length of Chilling on Survival.** Chilling for 6 h had a significant impact on survival in all the incubators except when the temperature was gradually lowered and raised (Z = −0.35; P > 0.05; odds ratio 0.99), whereas chilling for 24 h significantly reduced survival rates after removal from the chilling chamber (Z = −33.30; df = 1; P < 0.01; odds ratio 0.56) (Fig. 1). Similar trends when flies were starved were noted for irradiated flies (Figs. 1 and 2; Table 4) except that survival was similar for flies irrespective of age. The probability of dying rose with an increase in the number of days post removal from low-temperature storage (Z = −176.05; P < 0.01; odds ratio 0.50).

**Effect of Irradiation on Survival.** Irradiated males that were not chilled survived better than the control males that were neither chilled nor irradiated (Z = 10.45; P < 0.01; odds ratio 1.58). Chilled but not irradiated males (Z = −1.52; P > 0.05; odds ratio 0.95) and chilled and irradiated males (Z = 1.51; P > 0.05; odds ratio 1.04) did not differ significantly in survival from the control over the 17 d.

**Effect of Irradiation Container on Survival.** Chilling using a styrene container resulted in low survival rates by the following morning after removal from the chilling chamber. The overall length of survival was also much lower for flies chilled in the expanded styrene container compared with nonirradiated (Z = 10.45; P < 0.01; odds ratio 1.58) and chilled but not irradiated males (Z = −1.52; P > 0.05; odds ratio 0.95) and chilled and irradiated males (Z = 1.51; P > 0.05; odds ratio 1.04) did not differ significantly in survival from the control over the 17 d.

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**Table 1.** Mean mating parameters (± SE) when male _G. pallidipes_ unirradiated or irradiated in air or nitrogen atmosphere competed for mating opportunities with 7-d-old females in a field cage

| Treatment          | n  | Premating time (min)a | Mating duration (min) | Spermathecal fill |
|--------------------|----|-----------------------|-----------------------|------------------|
| Control            | 122| 66.05 ± 3.17a         | 28.43 ± 0.87a         |                  |
| Irradiated in air  | 76 | 68.03 ± 3.77a         | 27.86 ± 1.31a         |                  |
| Irradiated in N2   | 28 | 60.54 ± 5.70a         | 29.00 ± 1.40a         |                  |

The males were stored at 4°C for 6 h the day prior to the field cage test. aValues in the same column followed by the same superscript letter do not differ significantly at the 0.05 level (Tukey’s test).

**Table 2.** Influence of age on survival of chilled unirradiated, unfed male _G. pallidipes_ 1 d after removal from 6 h low-temperature storage

| Age (d) at start of chilling | Number of cages | Mean number of flies in each cage ± SE | Mean overnight survival % ± SE |
|-----------------------------|-----------------|----------------------------------------|-------------------------------|
| 4                           | 1               | 130                                    | 100                           |
| 5                           | 11              | 30 ± 0.3                               | 98.8 ± 0.7a                   |
| 6                           | 70              | 30 ± 0.4                               | 98.6 ± 0.4a                   |
| 7                           | 136             | 32 ± 1.2                               | 97.6 ± 0.3a                   |
| 8                           | 134             | 33 ± 1.4                               | 96.3 ± 0.4a                   |
| 9                           | 27              | 30 ± 0.4                               | 93.0 ± 1.8a                   |
| 12                          | 26              | 31 ± 0.2                               | 77.0 ± 3.0b                   |
| 13                          | 25              | 30 ± 0.2                               | 73.3 ± 3.7b                   |
| 14                          | 10              | 30 ± 0.5                               | 66.2 ± 7.6b                   |

aValues followed by the same letter do not differ significantly at the 0.05 level (Tukey’s test).

**Table 3.** Influence of length of starvation before chilling on survival of unirradiated, unfed male _G. pallidipes_ 1 d after removal from 6 h low-temperature storage

| Days of starvation | Number of cages | Number of flies per cage ± SE | Mean overnight survival % ± SE |
|--------------------|-----------------|------------------------------|-------------------------------|
| 1                  | 174             | 35 ± 1.5                     | 94.4 ± 0.8a                   |
| 2                  | 197             | 30 ± 0.2                     | 96.8 ± 0.4a                   |
| 3                  | 69              | 30 ± 0.3                     | 83.7 ± 2.2b                   |

aValues followed by the same letter do not differ significantly at the 0.05 level (Tukey’s test).
polystyrene container. Out of 3,168 flies irradiated and chilled in an expanded polystyrene container, 60 ± 8% survived the first 18 h post irradiation and chilling. Survival after irradiation and chilling in a thermost flask was relatively high (84 ± 2% out of 11,000 flies). It was possible to maintain the flies in an inactive state when irradiating in a thermost flask, whereas the flies were active in the expanded polystyrene container by the time irradiation was completed. Generally, the survivors were active in the holding cages up to 7 d after removal from the chilling chamber without any feeding; thereafter, movement became sluggish and eventually ceased.

**Rapid Chill Hardening.** Briefly exposing the flies to low temperature prior to a longer storage period (rapid chill hardening) led to a lower survival rate (Table 5). A longer interval between the initial brief exposure to low temperature and the 6 h exposure led to a slightly lower mortality effect. The survival of flies was significantly affected by treatment ($Z = -18.43; P < 0.01$) and number of days post exposure to low temperature ($Z = -81; P < 0.01$).

**Effect of Humidity on Survival.** When flies were irradiated in air followed by storage at low temperature at either low or high humidity, around 50% survived 6–8 d post chilling without a blood meal with no significant effect of humidity. Flight activity in the cage was minimal after 1 wk. The majority of the flies died within 14 d after removal from the low-temperature chamber (Fig. 3) with very few surviving longer but incapable of flight activity. Neither the position of the cage in the chilling chamber nor the specific cage affected mortality.

**Fig. 1.** The survival of unirradiated, unfed male *G. pallidipes* exposed to cold and varying humidity in different types of chambers (LMS cooled incubator, Jumo ZPR-2000 and Weiss Technik): 1—control, in 23°C, 75% RH; 2—ZPR2000, ramp, 4°C for 6 h, 70% RH; 3—ZPR2000, no ramp, 4°C for 6 h, 70% RH; 4—Weiss, ramp, 4°C for 6 h, 50% RH; 5—LMS, no ramp, 4°C for 6 h, 40% RH; 6—Weiss, ramp, 4°C for 24 h, 40% RH.

**Fig. 2.** The survival of irradiated, unfed male *G. pallidipes* stored at low temperature and constant humidity for 6 h: 1—control chilled; 2—irradiated chilled; 3—control, not chilled; 4—irradiated not chilled.

**Fig. 3.** Mortality of starved *G. pallidipes* after irradiation with 120 Gy and storage at 4°C for 6 h in high or low humidity (1—unirradiated control; 2—irradiated and not chilled control; 3—irradiated, chilled and high humidity; 4—unirradiated, chilled and high humidity; 5—unirradiated, chilled, and low humidity; 6—irradiated, chilled, and low humidity; 7—unirradiated, not chilled, and low humidity).

### Table 4. Influence of length of starvation before chilling on survival of irradiated, unfed male *G. pallidipes* 1 d after removal from low-temperature storage

| Days of starvation before chilling | Number of cages | Number of flies per cage ± SE | Mean overnight survival % ± SE |
|-----------------------------------|-----------------|------------------------------|-------------------------------|
| Overnight survival                |                |                              |                               |
| 1                                 | 15 5 3 3       | 6 2.2 98.7                  | 6 0.4a                       |
| 2                                 | 141 37         | 6 2.1 99.5                  | 6 0.1a                       |

*Values followed by the same letter do not differ significantly at the 0.05 level (Tukey’s test).

### Table 5. Influence of preexposure to low temperature on survival of male *G. pallidipes* 1 d after removal from 6 h storage at low temperature

| Treatment                          | n    | Mean per cage ± SE | Number of cages | Mean overnight survival % ± SE |
|------------------------------------|------|--------------------|-----------------|-------------------------------|
| Control                            | 259  | 32 ± 1             | 8               | 100 ± 0a                      |
| 6 h continuous chill               | 280  | 31 ± 1             | 9               | 93.0 ± 2.9b                   |
| 30 min + 6 h chill                 | 281  | 31 ± 0             | 9               | 85.6 ± 3.0b                   |
| 60 min + 6 h chill                 | 257  | 32 ± 1             | 8               | 91.2 ± 2.7b                   |

*Values followed by the same letter do not differ significantly at the 0.05 level (Tukey’s test).

§Initial storage at low temperature for 30 or 60 min, then returned to normal colony conditions for 60 min followed by 6 h storage at low temperature.
Discussion

The survival and mating ability of *G. pallidipes* that were immobilized at low temperature was investigated in this study with the ultimate aim being to include chilling during the dispersal of sterile male flies as a component of area-wide pest management. This work on chilling showed that it is possible to subject *G. pallidipes* to immobilizing low temperature under certain conditions without adversely affecting the overall physiological quality of males. The combination of irradiation and chilling in a low-humidity environment caused high levels of first day mortality with equally high variation among samples when *G. pallidipes* were irradiated in bulk especially with the use of an expanded poly-styrene container. There is a possibility that the foaming agent, fire retardant, or glue used in the construction of the poly-styrene container was toxic to the flies and the use of this container was discontinued. Prolonged exposure to low temperature has a negative effect on the physiology of tsetse flies; as such it would be necessary to keep the flies at low temperature for the shortest possible time and to also improve the irradiation environment to limit physiological damage. Irradiated and chilled males participated in mating activities, albeit at lower levels compared with unirradiated males that were not chilled.

In an area-wide pest management program that includes SIT, the large numbers of males that are released would ensure a numerical advantage for the sterile males. There may be other factors in the handling procedures that require further refinement before large-scale adoption of chilled adult release for tsetse flies. Sterilization of insects in an atmosphere with reduced oxygen has been shown to have added benefits to the efficiency of the SIT with limited genetic and somatic damage (Langley et al. 1974, Vreysen et al. 1996). However, males irradiated in nitrogen require a higher dose to achieve the same level of sterility as males irradiated in air.

In the majority of batches that were observed, the mean overnight survival rate after 6–24 h chilling at 4°C was sufficiently high to permit a reasonable number of males to be available for successful mating opportunities when competing with control males. There is a possibility that the low humidity level experienced by some batches in the incubator (below 35%) could have lowered the water balance in *G. pallidipes* (Bursell 1959), a fact that was noted to be important in *Glossina morsitans morsitans* Westwood (Williamson et al. 1983a), but a few observations at higher humidity produced similar levels of high first day mortality. The incomplete filling of spermathecae by males that are irradiated and chilled requires further investigation to determine whether this handicap can be compensated for by other quality factors, such that the integrity of the SIT is not jeopardized. It should also be borne in mind that female tsetse flies may require only a single mating experience and that even a limited quantity of sperm may be sufficient for their lifetime (Dame and Schmidt 1970).

Any possible effect of the release mechanism on the chilled flies remains to be investigated. Tolerance to chilling is also ecologically important because it is recognized that the occurrence of the *Glossina* species extends to altitudes where temperature can occasionally drop below 10°C for short periods. Experimental work with *G. pallidipes* has shown high variability in survival response when exposed to low-temperature (Terblanche et al. 2008). Survival of male flies after chilling indicated a similarity with work on other blood feeding insects that has also shown that neither cold acclimation nor dehydoration could improve cold tolerance in female bed bugs, *Cimex lectularius L.* (Benoit et al. 2009). Cold acclimation and continuous cold storage had similar survival rates in our work.

The numbers of sterile males that are required in a release program are large, thus it is necessary to irradiate the flies in bulk. Due consideration should be given to the selection of appropriate materials for the construction of containers that are used during bulk irradiation to avoid toxicity. Physical damage to the insects needs to be minimized by, among other conditions, maintaining flies in an immobile state throughout irradiation until final release.

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