# A Laboratory Maintenance Regime for a Fungus-Growing Termite *Macrotermes gilvus* (Blattodea: Termitidae)

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**ABSTRACT** The optimum maintenance conditions of the fungus-growing termite, *Macrotermes gilvus* (Hagen) (Blattodea: Termitidae), in the laboratory were studied. Termites were kept on a matrix of moist sand and with fungus comb as food. The survival of groups of termites was measured when maintained at different population densities by changing group size and container volume. Larger groups (≥0.6 g) were more vigorous and had significant higher survival rates than smaller groups (≤0.3 g). The population density for optimal survival of *M. gilvus* is 0.0025 g per container volume (ml) or 0.0169 g per matrix volume ($cm^3$), i.e., 1.2 g of termites kept in a 480-ml container filled with 71 $cm^3$ of sand. In termite groups of smaller size (i.e., 0.3 g) or groups maintained in smaller container (i.e., 100 ml) the fungus comb was overgrown with *Xylaria* spp., and subsequently all termites died within the study period. The insufficient number of workers for regulating the growth of unwanted fungi other than *Termitomyces* spp. in the fungus comb is the most likely reason. Unlike some other mound-building termite species, *M. gilvus* showed satisfactory survival when maintained in non-nutritious matrix (i.e., sand). There was no significant difference in the survival rate between different colonies of *M. gilvus* ($n = 5$), with survival in the range of 78.5–84.4% after 4 wk. Advances in the maintenance of *Macrotermes* will enable researchers to study with more biological relevance many aspects of the biology, behavior, and management of this species.

**KEY WORDS** laboratory maintenance, termite survival, *Macrotermes gilvus*, population density, matrix

Laboratory bioassays with subterranean termites usually involve collecting termite individuals from their nest or feeding sites and subsequently maintaining them in containers for an extended period of time. The housing containers are normally supplied with a food source and a matrix suitable for holding moisture and allowing termites to construct tunnels through it. However, termite individuals separated in this way from their colony often survive only for short periods. Numerous factors such as colony origin (Su and La Fage 1984), population density and container volume (Lenz and Williams 1980), type of rearing medium (Haverty 1979), caste composition (Watson et al. 1978), and temperature (Howick et al. 1975) are known to influence the survival rate of termites. If laboratory bioassays with subterranean termites are carried out without the consideration of above-mentioned factors, there will be a high possibility that survival rates of termites in untreated control groups may be as low as in treated termite groups (Lenz 2009). Survival rates in untreated control groups should reach acceptable levels to ensure the reliability of experimental results.

The fungus-growing termites of the subfamily Macrotermitinae are broadly distributed throughout Africa, South, and Southeast Asia (Eggleton 2000). One of the important mound-building species *Macrotermes gilvus* (Hagen) (Blattodea: Termitidae) in Southeast Asia is commonly found in gardens and along the perimeters of structures and buildings. In recent years, *M. gilvus* has gained notoriety as secondary pest in houses that were previously baited for lower termites such as *Coptotermes* spp. (Lee 2002, Lee et al. 2007). Acda (2004) reported that *M. gilvus* causes severe damages to wooden structures and is known as one of the most destructive termite species in the Philippines. Because of its economic importance, the need for evaluating the efficacy of termite management systems and susceptibility of materials to *M. gilvus* under laboratory condition has become more urgent.

Although laboratory maintenance conditions for other termite species (e.g., *Coptotermes*, *Reticulitermes*, and *Nasutitermes*) have been well-investigated, maintenance of *Macrotermes* spp. in the laboratory has been less successful to date. Unlike the lower termites, macrotermitines rely for their survival on a symbiotic fungus, *Termitomyces* spp. (Rouland-Lefevre 2000). Maintaining fungus-growing termites (i.e., *Macrotermes*, *Microtermes*, and *Odontotermes*) is much more challenging than other termite species, which do not culture fungus gardens, as Becker (1969) has already stated. As a consequence, for example, during insecticide testing, *M. gilvus* had to be exposed to treatments

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for shorter periods compared with other termite species because of significant natural mortality under current laboratory bioassay conditions (Acda 2007). Recently, Li et al. (2015) successfully maintained colonies of *Odontotermes formosanus* (Shiraki) up to 2.5 yr using the laboratory artificial rearing system. Clearly, there is a need to establish the optimum maintenance condition to achieve longer survival of *M. gilvus* groups.

A successful bioassay study depends on how closely the laboratory routine matches the conditions the termites experience in their natural subterranean habitat. In this study, we tested and discussed the range of factors that may influence termite survival. We determined how group size and container volume affect survival of *M. gilvus*. The percentage of termites surviving was established at the end of the experimental period. The presence of fungi other than *Termitomyces* spp. was monitored throughout the experiments. We also compared survival on sand with and without the incorporation of carton material from *M. gilvus* mound. Lastly, we tested whether there are significant variations in termite survival between different colonies.

## Materials and Methods

**Termite Sampling.** Mounds of *M. gilvus* were sampled in a residential area (5° 32′ N, 100° 29′ E) near Universiti Sains Malaysia, Minden campus in Penang Island, Malaysia. *M. gilvus* mounds were excavated by digging a circular trench around the perimeter of the mound. Sideways pressure was applied to remove the outer mound casing cautiously to minimize injury to termites. Fungus combs that housed the termites were collected into holding containers, thereby minimizing injury to termites during transport back to the laboratory.

In the laboratory, termites were separated from fungus combs by gently tapping the fungus combs over a tray. Termite individuals were carefully picked up with a piece of moistened filter paper. Termites were sorted into different castes (i.e., major workers, minor workers, major soldiers, and minor soldiers). Only healthy, freely moving termites were selected for testing.

**Experimental Assembly to Investigate Effects of Group Size and Container Volume.** The effect of group size and container volume on termite survival was evaluated with *M. gilvus* from a single colony (height, 54 cm; diameter, 110 cm). Termite groups of four sizes, ranging from 0.3 g (~55 termites) to 2.4 g (~440 termites), were housed in containers of three different volumes (ml) with certain volume of sand ($cm^3$) to form 1-cm-thick layer for each container size (i.e., 100 ml, 24 $cm^3$; 480 ml, 71 $cm^3$; 3,400 ml, 299 $cm^3$), as illustrated in Fig. 1. Termite population density was expressed as the relation of group size to container volume (g/ml) and relation of group size to sand volume (g/$cm^3$), respectively. Apart from container volume, we also had included matrix (sand) volume as a factor in determining population density because this was where termites located and foraged. Containers with a round base were used in this study because termite performance was greater in containers with a circular base compared with those with a rectangular base (Lenz and Williams 1980, Lenz et al. 1993). The lids of the plastic containers were perforated with numerous 1-mm-diameter holes for ventilation. Caste composition in each group size was based on the natural caste ratio of *M. gilvus* (Lee et al. 2012) with 10% soldiers and minor: major workers in a ratio of 1.4: 1 (Table 1). Six pseudo-replicates were set up for each combination of group size and container volume.

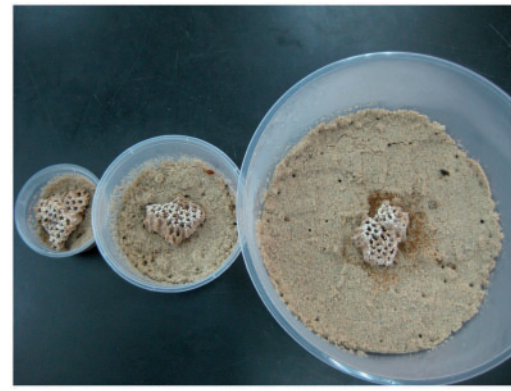

**Fig. 1.** Experimental assembly used to evaluate survival rates of groups of *M. gilvus* when kept under different combinations of group size and container volume (i.e., 100, 480, and 3,400 ml from left to right).

Each experimental unit was filled with sieved (mesh 40) sterile sand to form a 1-cm-thick layer and then moistened with 20% water w/w. Termites are known to adjust their food consumption rates according to the relative amount of food available to them (Lenz et al. 2009). Termite groups were provided with fungus combs as food source and the amount added was proportional to the size of termite groups. 0.3, 0.6, 1.2, and 2.4 g group of termites were supplied with 3, 6, 9, and 12 g of fungus combs, respectively. Test arenas were held in total darkness in plastic storage containers (63 by 47 by 39 cm) under constant environmental conditions of 26 ± 1°C and 90% relative humidity. A dish with water was placed into the plastic storage containers to maintain a humid environment for experimental groups. All groups were monitored daily to see whether termites carried out routine work and for any abnormal appearance in fungus comb (e.g., presence of white cottony mycelium on surface of combs). The experimental units were dismantled after 28 d and surviving termites counted. The survival rate was calculated by dividing the number of surviving termites by the initial group size.

**Experimental Assembly to Investigate Effects of Incorporating Nest Carton Material in Sand.** In this study, we assessed the suitability of a sand-carton material mixture (9:1 wt/wt) compared with sand alone for laboratory maintenance of *M. gilvus* from a single colony (height, 39 cm; diameter, 73 cm). Carton material was collected from the inner part, i.e., nursery zone, of termite mounds. The sand–carton material

**Table 1. Group size, container, and matrix volume, and the resulting population density used in the experiment**

| Group size (g) | Caste composition[a] | Container volume (ml) | Matrix (sand) volume ($cm^3$) | Population density of termites (g/ml)[b] | Population density of termites ($g/cm^3$)[c] |
|---|---|---|---|---|---|
| 0.3 (~55 termites) | 21 Mw, 29 mw, 1 Ms, 4 ms | 100 | 24 | 0.003 | 0.0126 |
| | | 480 | 71 | 0.000625 | 0.00423 |
| | | 3,400 | 299 | 0.0000882 | 0.001 |
| 0.6 (~110 termites) | 42 Mw, 58 mw, 2 Ms, 8 ms | 100 | 24 | 0.006 | 0.0252 |
| | | 480 | 71 | 0.00125 | 0.00847 |
| | | 3,400 | 299 | 0.00018 | 0.00201 |
| 1.2 (~220 termites) | 84 Mw, 116 mw, 4 Ms, 16 ms | 100 | 24 | 0.012 | 0.0505 |
| | | 480 | 71 | 0.0025 | 0.0169 |
| | | 3,400 | 299 | 0.00035 | 0.00402 |
| 2.4 (~440 termites) | 168 Mw, 232 mw, 8 Ms, 32 ms | 100 | 24 | 0.024 | 0.1011 |
| | | 480 | 71 | 0.005 | 0.0339 |
| | | 3,400 | 299 | 0.00071 | 0.008 |

[a] Mw, major worker; mw, minor worker; Ms, major soldier; ms, minor soldier.
[b] Calculation of population density is based on the container volume.
[c] Calculation of population density is based on the matrix volume.

mixture was prepared by adding 12 g of coarse carton (particle size 5–10 mm) into 108 g of sand and then mixing it thoroughly inside the 480-ml container. Twenty-four millilitres of water were added giving a matrix with a water content of 20%, and 1.2 g of termites (84 major workers, 116 minor workers, 4 major soldiers, and 16 minor soldiers) were then introduced into the container, while 9 g of fungus comb was provided as food source. The above-mentioned procedures were repeated when testing the suitability of sand alone in maintaining *M. gilvus* (120 g sand, 24 ml water). A total of six replicates were set up for each of the two matrices. The experimental groups were maintained under the same conditions to those in the study of the effects of population density. The experiment was again terminated after 28 d and the number of surviving termites was recorded.

**Experimental Assembly to Investigate Effects of Colony Origin.** In this study, we assessed whether significant variations in survival rates exist among different colonies of *M. gilvus*. Termites were collected from five *M. gilvus* mounds (height, 30–55 cm; diameter, 65–110 cm) on the Minden campus. The experimental details were similar to those described earlier, using sand alone as the matrix. The combination of 1.2 g of termites in 480-ml containers was used in this study based on the high survival rates it provided. A total of six replicates were set up for each of the colonies. The experiment was terminated after 28 d. The number of surviving termites was counted.

**Data Analysis.** To normalize the data, termite survival rates (%) were subjected to arcsine square root transformation. Two-way analysis of variance (ANOVA) with group size and container volume as main effects was performed to analyse the influence of these factors on percentage survivals of termites, and the means were separated using Tukey's honestly significant difference (HSD) test. One-way ANOVA was used to analyse the differences in survival of the different termite castes. Pearson correlation test was used to analyze the relationships between population densities and survival rates.

Differences in percentage survival of termites in sand-carton material mixture and sand matrix were compared using Student's *t*-test. Survival rates among different colonies of *M. gilvus* were also compared using one-way ANOVA, and the means were separated using Tukey's HSD test. All the analyses were employed using SPSS, v.11.0 (SPSS Inc., Chicago, IL) at $\alpha = 0.05$.

## Results

**General Observations.** Termites tunnelled through the matrix within 1–3 d after being introduced into the plastic containers. Tunnels and plastering on the container walls were clearly visible through the side and bases of the containers. At the time of dismantling the experimental units, termite cadavers were found buried in the very bottom layer of the sand physically well-separated from the living termites.

A rapidly growing fungus identified as *Xylaria* sp. appeared within 3 d in some of the experimental units, especially in the 0.3 g termite groups. For example, in the 0.3 g termite groups, white cottony mycelium (Fig. 2a) spread on the surface of fungus combs within 3 d in 8 of the 18 replicates (Table 2). *Xylaria* sp. grew rapidly on fungus combs and eventually developed into upright stromata with blackish colour in the lower and whitish colour toward the upper part (Fig. 2b; Batra and Batra 1979). From appearance, the *Xylaria* sp. was presumed to be *Xylaria piperiformis* Berkeley based on the black stromatal surface layer and whitish interior (Fig. 2c; Rogers et al. 2005). Dead termite bodies and decaying cadavers were found entangled in the mass of *Xylaria* mycelium.

**Group Size and Container Volume on Termite Survival.** The percentage survival of termites was significantly affected by group size ($F_{3,36} = 16.07$; $P < 0.001$), container volume ($F_{2,36} = 33.70$; $P < 0.001$), and group size by container volume ($F_{6,36} = 6.741$; $P < 0.001$). For the 100-ml container, there were no significant differences in the mean survival among different group sizes of termites ($F_{3,12} = 1.459$; $P = 0.275$;


**Fig. 2.** Growth of *Xylaria* spp. on fungus combs. (a) White cottony mycelium on surface of combs. (b) Upright stromata. (c) Black stromatal surface layer and whitish interior.

**Table 2. Growth of *Xylaria* spp. in experimental units throughout the 28-d period**

| Group size (g) | Container volume (ml) | Number of experimental units with growth of *Xylaria* spp.[a] | | | | |
|---|---|---|---|---|---|---|
| | | 3 d | 1 wk | 2 wk | 3 wk | 4 wk |
| 0.3 | 100 | 2 | 4 | 4 | 5 | 5 |
| | 480 | 2 | 4 | 5 | 5 | 5 |
| | 3,400 | 4 | 6 | 6 | 6 | 6 |
| 0.6 | 100 | — | 3 | 4 | 4 | 4 |
| | 480 | — | — | 2 | 2 | 2 |
| | 3,400 | — | — | 1 | 1 | 1 |
| 1.2 | 100 | 1 | 5 | 6 | 6 | 6 |
| | 480 | — | 1 | 1 | 1 | 1 |
| | 3,400 | 2 | 2 | 2 | 2 | 2 |
| 2.4 | 100 | — | 2 | 6 | 6 | 6 |
| | 480 | — | — | 1 | 1 | 2 |
| | 3,400 | — | — | 1 | 1 | 1 |

[a] For each combination of group size and container volume six replicates.
— No growth.

Fig. 3). None of the 1.2 and 2.4 g of termites had survived in the 100-ml container by the end of the experiment. However, for both of the 480-ml and 3,400-ml containers, the mean survival in 0.6, 1.2, and 2.4 g of termites was significantly greater than that in the 0.3 g groups (480 ml: $F_{3,12} = 8.713$, $P = 0.002$; 3400 ml: $F_{3,12} = 377.260$, $P < 0.001$).

There were no significant differences in the mean survival of the 0.3 g groups among different container sizes ($F_{2,9} = 0.5$; $P = 0.622$; Fig. 3). In general, the mean survival of the 0.3 g group was very low; 13.2 and 13.6% were recorded for 100 and 480 ml, respectively. None of the 0.3 g of termites survived in the 3,400-ml container by the end of experimental period. For the 0.6 g group, the mean survival was significantly different among various container sizes. The mean survival in the 100-ml container was 22.5%, but it increased to 55.9 and 61.1% in the 480- ml and 3,400-ml containers, respectively. The mean survival in the 3,400-ml container was significantly higher than that in the 100-ml container ($F_{2,9} = 5.415$; $P = 0.029$). This trend of increased percentage survival in larger containers was also found among the 2.4 g group. None of the 2.4 g termites survived in the 100-ml container by the end of the experimental period. In contrast, 50.2 and 65.0% of termites survived in the 480-ml and 3,400-ml containers, respectively. The mean survival in the 480-ml and 3,400-ml containers was significantly higher than in the 100-ml container ($F_{2,9} = 92.365$; $P < 0.001$). For the 1.2 g group, the mean survival was also significantly different among various container sizes. The 1.2 g group of termites experienced 100% mortality in the 100-ml container. The mean survival in 480 ml was 69.5%, which was significantly higher than that of the 3,400-ml container, with 57.3% survival ($F_{2,9} = 346.615$; $P < 0.001$).

The mean survival of termites differed significantly among the different termite castes ($F_{3,124} = 21.69$; $P < 0.001$). In general, major soldiers experienced the highest mortality (76.2 ± 5.5%), followed by minor soldiers (50.4 ± 4.3%), minor workers (49.4 ± 2.6%), and major workers (32.8 ± 2.1%).

**Population Density on Termite Survival.** When the relation of group size to container volume or sand volume is expressed as population density (g/ml or g/cm$^3$), it reveals that *M. gilvus* showed satisfactory survival rates (> 55%) at densities within the range of 0.00018–0.005 g/ml or 0.00201–0.0169 g/cm$^3$, respectively. No significant correlation between termite survival and population density was detected (g/ml: $r = -0.503$, $P = 0.096$; g/cm$^3$: $r = 0.008$, $P = 0.981$).

**Type of Maintenance Substrates and Colony Origin on Termite Survival.** On average, the survival rate of termites in sand (72.5 ± 1.6%) was significantly higher than that in the sand–carton material mixture (18.5 ± 1.3%; $t = 23.434$; df = 8; $P < 0.05$). There were no significant differences in the mean survival of termites among different colonies ($F_{4,25} = 0.942$; $P = 0.456$), with survival ranging from 78.5 to 84.4%.

## Discussion

This study gives us an insight into the influence of some key factors on the survival rates of *M. gilvus* under laboratory conditions. Batra and Batra (1979) stated that the difficulty in maintaining laboratory cultures of Macrotermitinae is primarily caused by rapid growth of *Xylaria* on fungus combs once removed from the nest. In our study, 89% of the smallest group size of termites (i.e., the 0.3 g group) and 88% of termite groups maintained in the smallest container (i.e., 100 ml) were swamped by abundant growth of *Xylaria* spp. by the end of the experimental period of 28 d (Table 2). We also found that no experimental groups survived the full experimental period once *Xylaria*

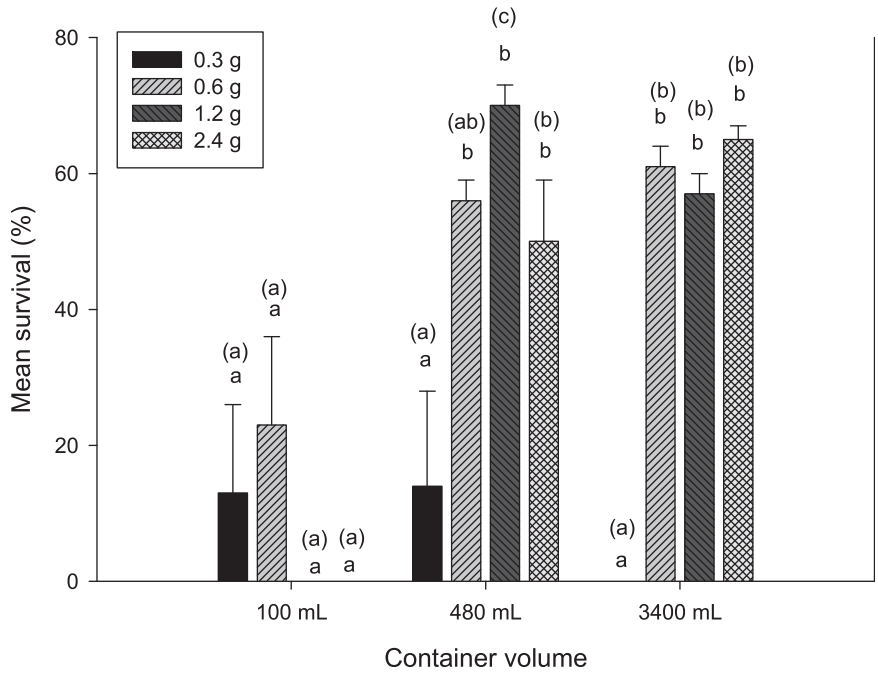


**Fig. 3.** Mean survival of four group sizes of *M. gilvus* maintained under different container volumes. Letters above each bar are assigned for comparison of group size within each container volume and letters in parentheses are used for comparison of container volumes within each group size. Bars labelled with the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD test). A vertical line above each bar represents SE.

began to grow on fungus combs and had formed stromata, this included even a few groups of the largest size which succumbed to *Xylaria* and died. However, all groups, i.e., only those of larger size, that managed to suppress the growth and spread of *Xylaria* and thus continued to have full access to the fungus combs had high survival rates. Nevertheless, we do not exclude the possibility that the unequal proportions of group size and fungus comb amount used in this assay may also affect the termite survival.

Population density that is conducive for maintenance differs greatly between termite species. Most of the previous studies expressed density of termites in relation of group size to container volume. Lenz et al. (1987) found that *Heterotermes indicola* (Wasmann) survived better at a surprisingly low population density of only 0.0023 g/ml. On the other hand, for *Coptotermes acinaciformis* (Froggatt), survival was highest at a population density of 0.016 g/cm$^3$ (Lenz and Williams 1980). Our results indicated that *M. gilvus* was able to tolerate a wide range of population densities, ranging from 0.00018 to 0.005 g termites per ml of container volume or 0.00201–0.0169 g termites per cm$^3$ of sand volume. The survival rates of termites under this range of population densities was >55%, except for the groups which consisted of only 0.3 g of termites (Fig. 4). For example, the 0.3 g termite groups housed in 480-ml container with resulting population density of 0.00423 g/cm$^3$ or 0.000625 g/ml registered only 13.6% survival rate. This demonstrated that 110 termite individuals (~0.6 g) appear to be the minimum number for maintaining a vigorous group in each of these containers. DeSouza and Miramontes (2004) stated that termites showed highest survival rates at certain population densities. In our study, *M. gilvus* showed highest survival at a population density of 0.0025 g/ml or 0.0169 g/cm$^3$ (1.2 g of termites in the medium-sized container of 480 ml with a sand volume of 71 cm$^3$).

In general, the small 0.3 g groups showed very poor survival. Two possible explanations may account for this. First, the number of workers present in the 0.3 g group was insufficient to perform their routine activities (e.g., tunnelling, construction, and food processing) properly, which subsequently imposed considerable stress on the termite groups. In other words, termite individuals in the 0.3 g group need to perform their basic tasks more frequently compared with that of larger group of termites (also see Jones 1990). Thus, this small group of termites experienced high mortality because of higher stress levels and energy expenditure. Previous studies indicated that larger groups of termites are much more robust and have lower mortality rates than smaller groups of termites (Becker 1969, Lenz and Williams 1980, Lenz et al. 1984, Jones 1990, Lenz 2009). Second, the number of workers present may too low to prevent the growth of *Xylaria* spp. and other microbes in the arena and on the fungus comb. Termite workers are known to play an important role in regulating the growth of fungi other than *Termitomyces* by mechanical removal of mycelium and also by inhibition through salivary secretions (Wood and Thomas 1989). We observed that

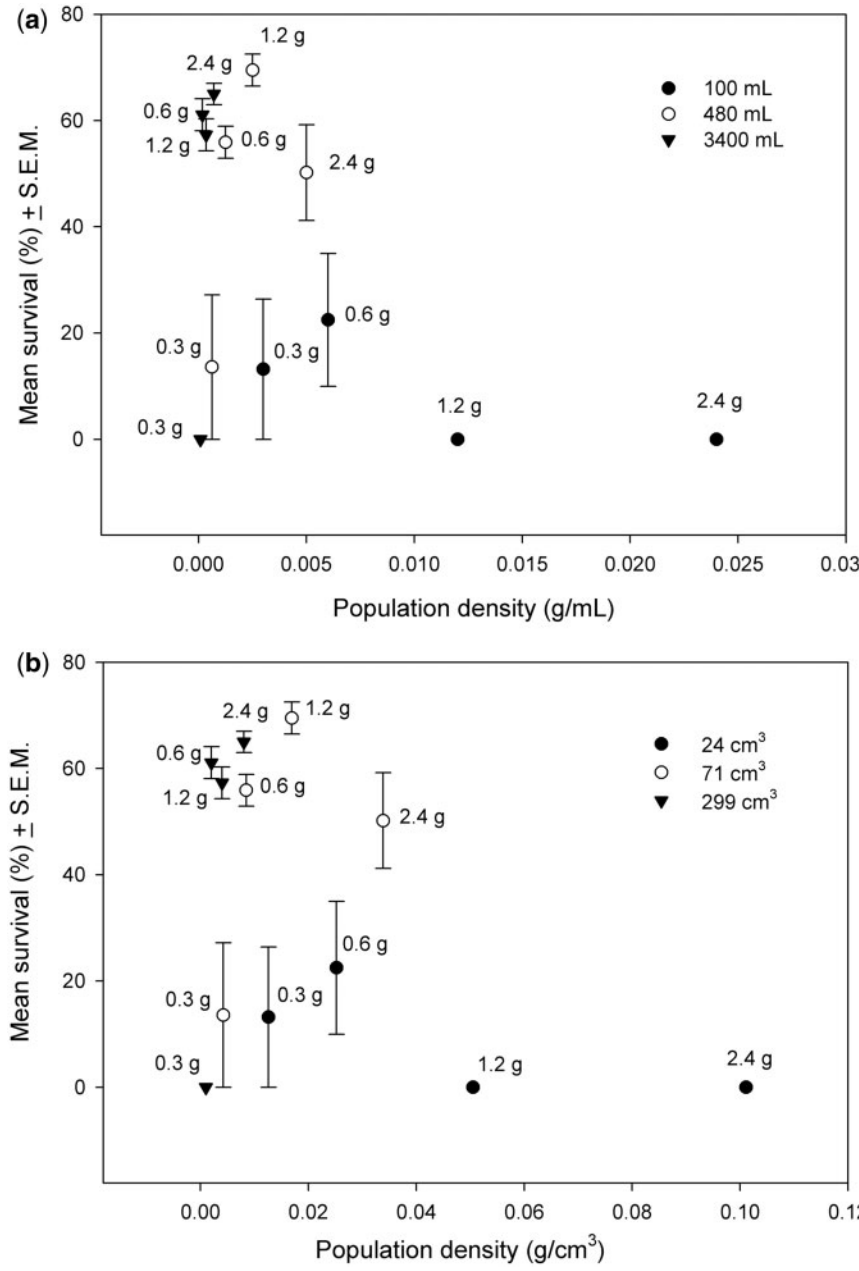


**Fig. 4.** Mean percentage of survival of termites at different population densities based on the relation of group size to container volume (a) or matrix volume (b).

dead and decaying termite bodies were found entangled in the mass of *Xylaria* mycelium growing rapidly on combs.

The volume or type of containers used must provide sufficient area and ventilation for termite groups to perform their routine activities in favorable conditions. In our study, *M. gilvus* showed very poor survival when maintained in the 100-ml container. This was possibly due to overcrowding in limited space and depth of the matrix imposing stress on the termite groups.

Mound-building termite species such as *C. acinaciformis* poorly survived when maintained in nonnutritious matrix (e.g., sand; Lenz et al. 1987). This form of *C. acinaciformis* has a significantly higher survival rate when maintained in their own carton materials compared with nest materials of other termite species. Unlike previous studies, the test of different substrates showed that *M. gilvus* survived much better on sand than a sand–carton material mixture. *M. gilvus* showed good survival rate even when maintained in

non-nutritious matrix may because of the provision of fungus combs in our experimental setup. Fungus comb is the major food source for the termites and fulfilled the nutritional requirements of termite groups throughout the experimental period (Hinze et al. 2002). The poor performance of termite groups when maintained in the sand–carton material mixture demonstrated the possibility that nest carton of *M. gilvus* is of only limited nutritious value. Unlike *M. gilvus* carton, nest cartons of two mound-building species, *C. acinaciformis* and *Globitermes sulphureus* (Haviland), contain considerable amount of nutrients, which enable longer-term survival of termite groups (Lee and Wood 1971a,b; M. L. personal communication). The poor survival of termite groups in the sand–carton material mixture may also be owing to the present of *Xylaria* spp. in carton material, which may increase the stress level of the termite groups.

Lenz (1985) showed that different *C. acinaciformis* colonies exhibited similar response to variation in population densities but there was a significant variation in termite vigor (i.e., survival and wood consumption) between colonies. In contrast, there was no marked variation in the mean survival between colonies for *M. gilvus* in our study. Variability in survival between colonies become distinct when exposed to adverse conditions, e.g., when termite groups are supplied with an unfavorable food or maintained in overcrowded density (Lenz 1985, Lenz and Dai 1985). However, we cannot exclude the possibility that the survival rates may differ if colonies of *M. gilvus* were sampled from widely separated geographical region or if the groups were exposed to more adverse conditions. Previous studies have demonstrated that a minimum of three source colonies should be tested in laboratory bioassays (Haverty 1979, Su and La Fage 1984). Interpretation of results obtained from five different colonies in our studies proved that our experimental settings did provide an optimal environment for *M. gilvus*. Survival of *M. gilvus* was satisfactory, with survival rates higher than 78% at the end of the 28-d period.

In summary, termite survival varied between different combinations of group size with container volume. Smaller groups of termites ($\leq$0.3 g) are less vigorous and have lower survival rates than larger groups of termites. Also, termites survived poorly when kept in smaller containers (i.e., 100 ml). Optimal population density for survival of *M. gilvus* is 0.0025 g/ml or 0.0169 g/cm$^3$. Successful maintenance of *M. gilvus* for an extended period is much dependent on whether the termite groups are able to suppress the growth of fungi, such as *Xylaria* spp., that coexist with *Termitomyces* spp. on the fungus comb. Unlike some of the mound-building termite species, non-nutritious matrix appears to be a suitable medium for maintaining groups of *M. gilvus*, for as long as fungus comb is provided as a food source. Different colonies responded uniformly to the experimental conditions without significant variation in the survival rate.

The present study resulted in significant advances in the maintenance of *M. gilvus* that will enable future studies into various aspects of the biology of *M. gilvus* and the response of this species to the effects of termite management systems and the resistance of material to attack by this species. For example, Lee et al. (2014) suggested that neurotoxins should be tested as bait toxicants against *M. gilvus* as actives with the potential to affect all stages and castes of this macrotermitid. With the new laboratory maintenance system, it will be possible to select those active bait ingredients in laboratory screening tests, which show best promise for field evaluation. This approach will allow for a more economic and effective way of developing management systems against *M. gilvus* and other Macrotermitidae.

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