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PLANAR ARENAS FOR USE IN LABORATORY BIOASSAY STUDIES OF SUBTERRANEAN TERMITES (RHINOTERMITIDAE)

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Abstract

Reliance on use of the Petri dish for bioassay studies has resulted in the majority of subterranean termite research being conducted in a manner that has not taken into account one of the most important feature of subterranean termites ecology: the soil in which they live. This artificial environment for bioassays, favored for its ease of use, may have resulted in accumulation of data about termite physiology, toxicology, pathology and behavior with limited biological relevancy. Caution should be taken when drawing conclusion from such experiments, because recent studies that made use of planar arenas (or 'two-dimensional arenas'), which provided soil-foraging conditions for termites and visibility for observation, have produced findings that are either contradictory to or more complete than corresponding Petri dish studies. The result of this study showed that groups of termites kept in planar arenas had better vigor and survivorship after 60 d than groups kept in Petri dishes. We also describe a list of technical advantages that the use of planar arenas can provide over the use of Petri dishes, and suggest that entomologists planning future laboratory studies on subterranean termites should consider using a similar protocol that involves planar arenas.

Key Words: Bioassay, arena, Petri dish, soil condition, subterranean termite

Resumen

Al depender en el uso de platos Petri para realizar estudios de bioensayo ha resultado en que la mayoría de las investigaciones de termitas subterráneas no toman en cuenta la característica más importante de la ecología de las termitas: el suelo en que viven. Este ambiente artificial para los bioensayos, favorecido por su facilidad de uso, puede tener como resultado la acumulación de datos sobre la fisiología, toxicología, patología y comportamiento de las termitas que son de relevancia biológica limitada. Se debe tener precaución en sacar conclusiones de estos experimentos, ya que estudios recientes que se hicieron utilizando arenas planas (o de arenas cerca de dos dimensiones), que proveen condiciones para que las termitas forajen en el suelo y la visibilidad para observarlas, han producido resultados que son contradictorios o más completos que los estudios correspondientes utilizando platos Petri. El resultado de este estudio mostro que los grupos de termitas mantenidos en "arenas planas" tuvieron mas vigor y supervivencia a los 60 días que los grupos mantenidos en platos Petri. También se incluye una lista de las ventajas técnicas que el uso de "arenas planas" pueden proveer sobre el uso de platos Petri, y sugieren que los entomólogos que planean estudios de laboratorio sobre las termitas subterráneas en el futuro deben considerar el uso de un protocolo similar que involucra a las "arenas planas".

Subterranean termites (Rhinotermitidae) inhabit extensive tunnel networks that they excavate through soil in search of resources. These cryptic nests with long underground galleries render subterranean termites difficult to detect, observe and control (King & Spink 1969; Su & Scheffrahn 1988). Due to their pest status, the genera *Coptotermes* and *Reticulitermes* have been the subject of numerous laboratory studies that focused on various aspects of their biology including, behavior, physiology, toxicology, and pathology. Although, by definition, any laboratory assay places the termites in an artificial environment, it is preferable that termites are provided with conditions that simulate as closely as possible their natural subterranean habitat; so that data can be interpreted with biological relevancy (Becker 1969; Lenz 1986). In natural conditions, the tunnel walls are lined with soil particles mixed with termite feces, which functions as a cement that consolidates the structure of the tunnel and prevents temperature and moisture extremes (Greaves 1962; Wood 1988). The microbial community associated with tunnel walls has been

shown to be altered by the termites themselves (Chouvenc et al. 2011a). Therefore, the tunnel system of subterranean termites should be considered as a modified environment, when compared with its surrounding soils, and is part of the extended phenotype of the termite colony (Dawkins 2004). This aspect should be carefully taken into account when interpreting data from laboratory assays about termite biology. According to Becker (1969), *"the experimental protocol must provide the necessary space and aeration and render possible the maintenance of suitable ecological and nutritional conditions. It must prevent the individuals from escaping, and should allow continuous observation without serious disturbance of the insects. . . . Soil-dwelling species need space for moving and gallery building as part of their natural behavior; and in long-term tests, Rhinotermitidae should be provided with soil to satisfy their building instinct."*

Despite Becker's recommendation, most of the current research on subterranean termites does not include an experimental protocol that takes into consideration of their complex biology. A survey of the 2009-2010 scientific literature where some type of bioassay on *Coptotermes* was performed revealed that out of 174 publications, 141 of them used Petri dishes or their equivalent for the bioassay (Chouvenc, personal observation). The use of the Petri dish appears to be favored because it is easy to setup and allows direct visual observation. The popularity of Petri dish assays has resulted in the majority of subterranean termite research being conducted using a type of bioassay that does not take into account one of the most important ecological features of subterranean termites: their soil-dwelling living condition. The inherent problems of using bioassays with limited biological relevancy were previously reviewed and discussed by Lenz (1986, 2009), who suggested that survival of termites in bioassays usually is unsatisfactory, even in the control groups, and that the value of such experiments was "meaningless." We also suggest that such experimental protocols can only provide limited information and that considerable caution should be exercised in drawing conclusions from them.

The use of jars containing soil is one option that provides a 'naturalistic' setting and is recommended by the American Wood Protection Association (2009), but the types of bioassays that can be performed in jars are limited by the inability to observe all termites in the units. A compromise between the non-soil but visible environment of Petri dishes, and the non-visible but soil environment of jars may be a planar arena, also called 'two-dimensional arena', consisting of a thin soil layer between two transparent plates. Bardunias & Su (2005) suggested that because natural termite tunnel networks are excavated within a limited soil horizon, a planar arena might allow for a
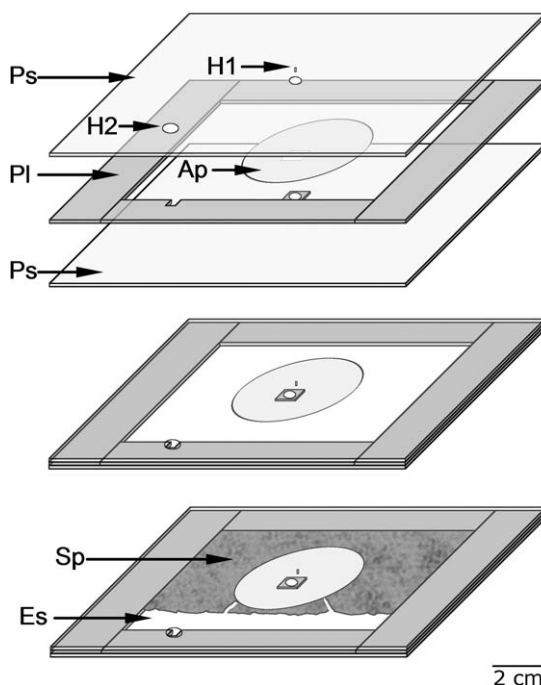

Fig. 1. Steps for the assembly of a planar arena. Ps = Plexiglas sheet, Pl = Plexiglas laminate, Hs = spacer and holes for the screw, H1 = 0.4 mm hole allowing introduction of liquids in the arena, H2 = 5 mm hole allowing the introduction of termites, Ap = absorbent pad (cellulose) with a hole for the spacer, Sp = sand particles, Es = empty space.

more natural tunnel expansion than a volume of soil that is deeper but with limited lateral distance. This type of arena originated with Luscher (1949), who proposed a "glass plate-termitary" consisting of two glass plates separated by spacers that create a space in between the plates, which are held together by clips, and where termites can be placed, and that can allow continuous observation of termite groups for extended periods. These arenas have only recently been used for laboratory bioassays on foraging behaviors of subterranean termites (Robson et al. 1995; Reinhard et al. 1997; Hedlund & Henderson 1999; Pitts-Singer & Forschler 2000; Campora & Grace 2001; Puche & Su 2001; Su & Puche 2003; Tucker et al. 2004; Su 2005a; Lee et al. 2007; Nobre et al. 2007; Whitman & Forschler 2007; Li & Su 2008, 2009; Bardunias & Su 2009, 2010).

Besides these few studies on some of the aspects of foraging behaviors in termites, only a small number of studies have used this type of experimental device to elucidate other aspects of termite biology. Su (2005b) used 'extended two-dimensional arenas' (providing a distance factor of 50 m) with laboratory groups of *Coptotermes for-*

mosanus Shiraki to study the effect of distant treatments on large groups (10,000) of foraging termites. This study showed that previous laboratory assays using small devices could only provide limited insight into the long term and long distance effects of toxicants on termite groups. Chouvenc et al. (2008) used large arenas (50 cm × 50 cm) with *Reticulitermes flavipes* (Kollar) to show that the survivorship of termites in groups of 960 individuals placed in soil and allowed to forage was not affected by the presence of a fungal pathogen, while small groups of termites kept in Petri dishes were susceptible to the fungus. Finally, Messenger & Su (2005) and Li et al. (2010) described the agonistic behavior in *Coptotermes* using planar arenas; and Li et al. (2010) compared the data obtained from Petri dish assays to data obtained from planar arenas filled with sand. The arena assay revealed important features of the agonistic behavior that were not previously observable from the Petri dish experiment alone. These few studies showed that the use of planar arenas allowed the monitoring of variables about toxicity, pathogenicity and behavior in environmental conditions with greater biological relevancy than other types of laboratory bioassays. Data from these studies led to different interpretations than previous work from their respective fields of research; and which suggests that a part of the results accumulated so far and the interpretations drawn from them may have a limited significance about subterranean termite biology.

Despite these recent studies showing the potential of the use of planar arenas, their use remains anecdotal in the subterranean termite literature. One aspect that these studies have not provided is the comparison of the survivorship between groups of termites in Petri dishes and planar arenas over extended periods of time. Such data would indicate if the vigor of groups of termites in planar arenas is improved over groups of termites kept in Petri dishes. The results of our current study, have induced us to advocate the use of planar arenas as an alternative to the types of bioassays currently used for laboratory studies of subterranean termites. We provide comparative survivorship between groups of termites kept in Petri dishes and planar arenas, and analyze the advantages and disadvantages of the use of such protocols.

MATERIALS AND METHODS

Collection of Termites

Termites were collected from three field colonies of *C. formosanus* in Fort Lauderdale, Florida using the method described by Su & Scheffrahn (1986), processed according to Tamashiro et al. (1973), and kept in groups of at least 1,000 for 10

to 15 d in containers stored at 28 °C. For each colony, 12 groups of 50 termites were prepared, using a caste ratio of 45 workers (undifferentiated larvae of at least the third instar) and 5 soldiers. The groups of termites were then placed in either 1 of the 2 environmental conditions: Petri dishes or planar arenas. Subsequently observations were made for 60 d.

Preparation of Petri Dishes

Sterile glass Petri dishes (50 mm diam) were prepared by placing a sterile absorbent pad (cellulose, 45 mm diam, 2 mm thick, Millipore Corp. Billerica, Massachusetts) in the center and adding 1 mL of sterile deionized water to the pad. A group of 50 termites was introduced in each Petri dish, which was wrapped with Parafilm to reduce desiccation. Six replicates were done for each termite colony, for a total of 18 replicates. Petri dishes were placed at 28 °C in the dark, and digital pictures were taken daily for a total period of 60 d. Small quantities of water were added to the absorbent pads when needed to maintain a humid environment in all Petri dishes. Dead termites were not removed.

Preparation of Planar Arenas

The planar arenas (Fig. 1) were each composed of 2 sheets of transparent Plexiglas™ (12 × 12 × 0.2 cm in thickness) separated from each other by Plexiglas laminates (2 cm wide and 0.2 cm thick) on the 4 sides, creating a 10 × 10 × 0.2 cm space inside the arena. A 0.4 mm diam hole was drilled close to the center of the top Plexiglas sheet, to enable the introduction of liquids with the help of a syringe, and a 5 mm-diameter hole was provided in one corner for the introduction of the termites into the arena chamber. An additional 4 mm-diameter hole was drilled at the center of both the top and bottom Plexiglas sheet and a 1 × 1 × 0.2 cm perforated spacer was glued on the bottom sheet. The spacer, used with a screw, prevents the sheets from warping in or out. Before assembly, all elements were washed with soap, immersed in bleach (3% sodium hypochlorate solution) for 2 h and rinsed 3 times with sterile DI water. A sterile absorbent pad (45 mm diam, with a 1 × 1 cm hole in the middle) was placed in each arena, centered with the central spacer, and the arena was filled with 18 g of wet sand (15 g of sterile dry sand, 150-500 µm sieves, and 3 mL sterile water), leaving a band of 10 × 2.5 cm empty on the opposite border, and exposing the absorbent pad to the empty space (Fig. 1). The arena pieces were kept together with eight 1-cm binder clips and the mounted arena was set up horizontally. The 4 sides of the arena were sealed with hot glue on the side, in order to prevent sand desiccation. One mL of sterile deionized water was injected via the

center hole onto the absorbent pad. A group of 50 termites was introduced into the arena with the help of a small funnel, and after all of these termites had entered the arena, the introduction hole was sealed with a thin transparent plastic cover. Six replicates were prepared for each termite colony, for a total of 18 replicates. Arenas were placed at 28 °C in the dark, and digital pictures were taken daily, for 60 d. Small quantities of water were added to the absorbent pad with a syringe through the center hole of the top Plexiglas sheet when needed, in order to maintain a humid environment in all arenas. Dead termites were not removed.

Survival Analysis

A Cox proportional-hazard regression analysis (using the program R-Project for statistical computing, version 2.4; http//cran.r-project.org/) was performed on all the individuals in order to estimate the effect of 2 variables on the termites' survivorship: the environmental condition, and the colony of origin. Through the analysis the Wald statistic was generated. The resulting hazard function defines the instantaneous rate of death at a particular time, while controlling the effects of other variables on survival. Pairwise comparisons of the death rates were adjusted by the Holm-Bonferroni method ($\alpha = 0.05$).

RESULTS

General Observations

Termites kept in planar arenas excavated sand particles and created a tunnel system within a couple of days (Fig. 2a). The empty space was filled with sand along with the extension of the tunnel system as described by Li & Su (2008). The cellulose absorbent pad was partially consumed but some of the cellulose matrix was not fed upon by the termites during the period of the study, instead the paper fragments were treated much like particles of soil and were deposited within the tunnel walls. Dead termites were mostly cannibalized, but in 3 cases, the cadaver was buried into the tunnel walls, which physically separated the cadaver from the rest of the termite group. On one of the buried cadavers, a saprophytic fungus identified as *Aspergillus nomius* Kurtzman, Horn & Hesseltine grew and sporulated within 48 h after the death of the termite (Fig. 3), but was not in contact with the rest of the termite group for the duration of the ex-
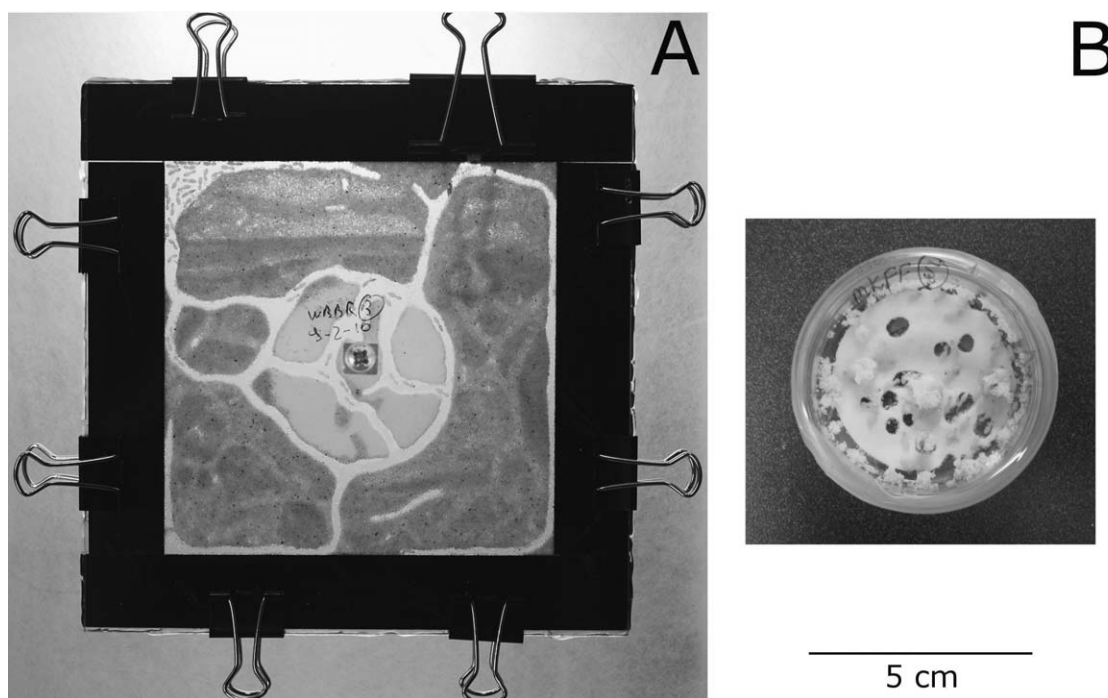

Fig. 2. Termite bioassays. A) Planar arena filled with sand; 50 termites, 30d after introduction, tunneled through the substrate and can feed on the absorbent pad made of cellulose. Observation of behavior and mortality is perfect throughout the duration of the experiment with minimal disturbance. B) Petri dishes with no sand; 50 termites can feed on the absorbent pad. Observation of behavior and mortality is difficult after 3d and major disturbance is necessary to accurately count and observe the termites.
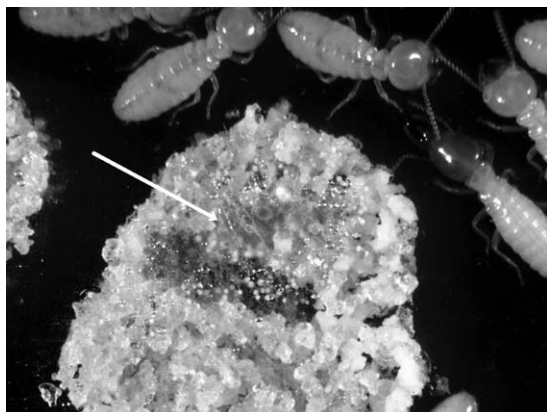
Fig. 3. Buried cadaver (arrow) of *Coptotermes formosanus*, with sporulating saprophytic fungus *Aspergillus nomius*, isolated from the rest of the termite group.
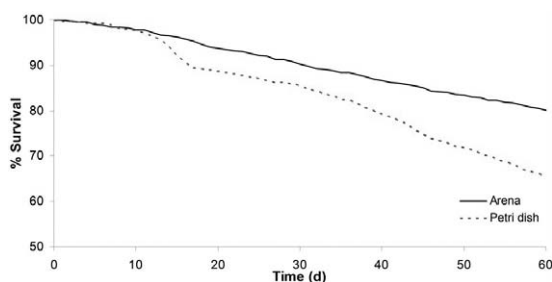

Fig. 4. Survival of *Coptotermes formosanus* in planar arenas and Petri dishes for 60 d. Groups of 50 termites in the arenas had higher survivorship than the groups in Petri dishes. Cox proportional regression (n= 1,800; Wald statistic = 46.7, df = 1, $P < 0.001$)

periment. After 30 d, some of the tunnel walls started to show the accumulation of termite fecal material on the lateral sand particles of the tunnel wall. At all times during the experiment, all live termites were visible and at 60 d all surviving termites appeared to be healthy.

Termites kept in the Petri dishes partially consumed the cellulose absorbent pad. A part of the chewed material was not fed upon, but deposited as "paper pellets" on the edges and the lid of the Petri dishes (Fig. 2b). As a result, visibility inside the Petri dishes was obscured. In order to accurately observe and count the number of live termites, the Petri dish had to be opened by removing the Parafilm seal to scrape off the deposited particles. Such disturbances were done multiple times (up to 3 times within 60 d) for some of the replicates. Dead termites were mainly cannibalized, but in the case of sudden accumulation of dead termites, the cadavers were piled at the edge. In some of the Petri dishes, a red stain appeared on the surface of the absorbent pad after 20 d. The red color was identified as the bacterium, *Serratia marcescens* Bizio, that colonized the humid absorbent pad. At 60 d in 2 of 18 Petri dishes all 50 termites were dead and most surviving termites in the other Petri dishes were slow and sluggish.

Termites Survivorship

After 60 d, termites kept in groups in the planar arenas had a better survival rate than the termites kept in groups in the Petri dishes (Fig. 4). The groups of termites in the Petri dishes had 1.89 × the hazard ratio of death of the groups kept in arenas ($n = 1,800$; Wald statistic = 46.7, df = 1, $P < 0.001$). The course of mortality in the arenas showed a uniformly gradual slope during the 60 d, with no sudden mortality peaks, as

not more than 2 termites died within a given day in a single arena. Conversely in the Petri dishes, a mortality peak was observed as up to 15 termites died within a day (15 d) in a single Petri dish.

Independent of the environmental assay, the overall survivorship of the termites differed significantly according to their colony of origin as the hazard ratios of death of two of the colonies were 1.98 × and 2.08 × that of the colony with the highest survivorship (Cox proportional-hazard regression, pairwise comparison among colonies, $P < 0.001$). The two colonies with the lowest survivorship had no difference in their hazard ratio of death (pairwise comparison, Wald statistic = 0.13, df = 1, $P = 0.72$).

DISCUSSION

The use of planar arenas for laboratory study of subterranean termites provides several advantages over Petri dishes (Table 1). Although it was thought that Petri dishes could allow good observation, our experiment showed that some groups of termite covered the inside of the dishes with fecal and paper pellets, which hindered visibility of the insides of the dishes. In comparison, the narrow gap between top and bottom sheets in the planar arenas did not elicit deposition of sand or fecal particles on the Plexiglas. Perhaps the lack of deposition resulted from a response to specific spacing similar to the "bee space," wherein honeybees do not deposit wax or propolis when presented with a limited distance between hive components (Langstroth 1853). If generally true, making use of "termite space" ensures that direct visual monitoring of termite survival, and detailed behaviors of the insects (Whitman & Forschler 2007; Chouvenc et al. 2008; Bardunias & Su 2009; Li & Su 2009; Li et al. 2010) can be conducted at all times without the need to disturb the arena in the manner that was needed for Petri dishes.

TABLE 1. COMPARATIVE CHARACTERISTICS OF PETRI DISHES AND PLANAR ARENAS FOR BIOASSAYS WITH SUBTERRANEAN TERMITES.

	Petri Dish	Planar arena
Environment	Open chamber	Tunnels, soil
Visibility	Medium: termite can deposit particles or move underneath the cellulose pad	high visibility at all time
Disturbance to monitor	Medium	Low
Microscopic photography	Difficult	Easy
Tunneling possibilities	Limited	Good
Distance of tunneling	Limited	Adjustable *
Food source	No shape limitation	All material that can be shaped 2 mm thickness
Termite activity after 60 d	Slow and sluggish	Normal
Survivorship after 60 d	65% survivorship	81% survivorship
Sudden peak of mortality in controls	Yes	No
Possibility to bury cadavers	Low	High
Number of termites	20-100 termites	50-1,000+ termites*
Unit preparation	Standard commercial Petri dish	Custom made from raw material
Time to setup for use and reuse	5-15 min	30-40 min

*Adjustable to the size of the arena (Li et al. 2010), or connect arenas with tubing to simulate long distances (Su 2005).

For microscopic photography, the limited depth of field is always problematic. Since a planar arena restricts termites to a narrower space than a Petri dish, termite behavior can be observed and recorded easily. Planar arenas consisted of smooth Plexiglas, which is also preferable for filming compared to the irregular surface of a glass Petri dish. In a planar arena, Li & Su (2009) were able to film the movement of each mouthpart through a microscope, and in this way they elucidated the function of each mouth appendage in sand displacement.

The planar arenas allowed termites to forage and establish a tunnel system that is characteristic of their biology. Although it was argued that the use of jars can provide more "realistic" tunneling behavior than planar arenas because of the introduction of a third dimension, it was shown that the tunneling patterns and geometry were similar in both assays (Bardunias & Su 2005). The depth of the three-dimensional jar setup is less important than the lateral distance and the length of the path traveled by termites (Bardunias & Su 2005). Therefore, the planar arena offers a good compromise between jars and Petri dishes, as it provides both good visibility and tunneling substrates.

Within both natural tunnels and passages excavated within planar arenas, termite movements are limited to specific paths, while in the open space of a Petri dish movement in any direction is unhindered. This has a significant implication for the laboratory bioassays with toxicants. The encounter patterns between individuals and the rates of contact within tunnels may differ from those in open space, which could lead to disparate rates and patterns of toxicant transmission. An advantage in using planar arenas is that it can simulate any remedial measure applied commercially where the toxicant is delivered via termite foraging tunnels. The effect of exposure to the tunnel wall environment and the ability of the toxicant to be moved through tunnels is thus a crucial component in colony elimination; and the results of a study performed in a planar arena with a soil component can predict the efficacy of a product in field trials (Su 2005b). Foraging distance is one of the critical aspects of termite biology that was commonly ignored in laboratory bioassays and remains a major characteristic to take into account when testing the effect of pesticides on field colonies (Su 2005b). Bioassays using Petri dishes can only provide information about direct toxicity, usually in a non-choice situation, while in the natural environment termite colonies can spread underground up to 100 m (King & Spink 1969). Individual arenas can provide longer distance of tunneling than jars or Petri dishes, depending on their size (Su & Puche 2003; Chouvenc et al. 2008; Bardunias and Su 2009; Li et al. 2010), and if connected with each other with

transparent tubing, it is possible to simulate termite tunnels of 50 m for large groups of termites (Su 2005b) and even longer distances (e.g., 135 m, Su, unpublished), still with the possibility to observe all individuals in the arenas at all times.

While Lenz (2009) stressed the importance of taking into account all characteristics of the termite biology that can influence the vigor of termites when using a laboratory assay, we assert here that the microbial community naturally associated with termites (Husseneder et al. 2009) and their tunnel system (Chouvenc et al. 2011a), also have been ignored in most bioassays. An extensive survey of the work accomplished in the field of termite biological control (Chouvenc et al. 2011b) showed that 90% of all laboratory bioassays that tested a pathogenic agent against groups of termites was done in Petri dishes or equivalent, which did not take into account the complex biology of subterranean termites nor the potential interaction between commonly associated microorganisms and the pathogen. In the current study, the colonization of the cellulose pad by *S. marcescens*, a known termite pathogen, which occurred in the Petri dishes but not in the arenas, suggests that the sand and the rich microbial community associated with the tunnel system (Chouvenc et al. 2011a) provides termites with a buffering environment against outbreaks of opportunistic parasitic or pathogenic organisms (Hughes et al. 2008; Cremer & Sixt 2009). In addition, the possibility for termites to bury cadavers in the soil particles of the planar arenas, allowed the group of termites to be physically separated from the cadaver, and prevented the spread of potentially harmful microorganisms (Chouvenc & Su 2010). The fact that no mortality peak was observed in the arenas but occurred several times in the Petri dishes supports the idea that a social insect nest represents a "homeostatic fortress" against adverse situations (Hughes et al. 2008). As mentioned previously, the termite tunnel system should be considered as part of the extended phenotype of the colony (Dawkins 2004), and should be taken into account when running all types of bioassays that use groups of subterranean termites.

Another limiting factor about the biological relevancy of a bioassay is the survival of termites throughout the duration of the experiments in challenged and unchallenged control groups (Lenz 2009). Our results demonstrated that termite groups kept in planar arenas had better survival and vigor (Arquette et al. 2006) than the groups kept in Petri dishes for 60 d. Petri dish bioassays probably imposed a stress on the groups of termites and did not facilitate satisfactory survival and vigor of the termites, while the planar arenas imposed a stress with lesser impact on termite survivorship and vigor. It is known that stressed organisms may display a compromised

immune system (Anderson & May 1981; Rolff & Siva-Jothy 2003), and it is possible that the termite mortality observed in the Petri dishes is partially due to such stress. However, the vigor of the termites also depends on factors operating prior to and during the bioassay (Lenz 1986, 2009). The colony origin, the age of the colony, and the time spent in groups in the laboratory prior to the experiment are factors that have to be taken into consideration (Becker 1969; Lenz 2009). The physical characteristics of the environment, such as relative humidity, access to water, temperature and food source that would allow optimal survival, must meet the requirements for each termite species (Becker 1978; Lenz et al. 1984; Lenz et al. 1987). Several studies demonstrated that the larger the size of the tested group, the better the vigor (Lenz & William 1980; Lenz et al. 1984; Lenz 1985); and, thus, results of experiment with small groups of termites should be interpreted cautiously.

Termites within the same colony often share physiological states, including nutrition, alarm, and stress level, in a manner that a similar group of non-social insects does not. There are unpredictable variations between colonies in coping with imposed stress, such as the exposure to unfavorable conditions (Carter et al. 1972; Lenz & Dai 1985; Rosengaus & Traniello 2001). Because of this, the response of termites from any single colony to a bioassay may not be representative of the wider population. Sampling from multiple colonies can mitigate, or at least uncover, unexpected variance between colonies for response to a bioassay (Carter et al. 1972; Lenz 1985; Su et al. 1985). Our results confirmed previous conclusions that a minimum of 3 colony sources should be used in making bioassays (Haverty 1979; Su & La Fage 1984).

Planar arenas can provide advantages for the study of subterranean termites compared to Petri dishes or jars, but there are some drawbacks that should be taken into consideration. Glass or plastic Petri dishes are readily available in different shapes and sizes from commercial providers for a relatively low price, and preparing or reusing an experimental unit for the bioassay is easy. In comparison, the planar arenas are usually custom-built, and the assembly of all individual pieces into the complete experimental unit requires some level of expertise. The fact that each individual arena has to be custom-made can be a limiting factor for laboratories that do not have the manpower or expertise to make such a device. In addition, the time to prepare a ready-to-use arena for the introduction of termites is longer than that of Petri dishes, mainly because of the meticulous care that must be exercised when introducing the sand particles, and when cleaning the unit before its reuse. Despite these requirements, we assert here that the additional effort to create and ser-

vice such device, which is indispensable for providing an appropriate environment for termites, and thus for obtaining high quality data with high biological relevancy is decisively a worthwhile. In addition, where some would consider the "custom made" aspect as a negative, we would argue that customization allows a wide range of possibilities in designing an experiment that is capable of providing the reliable data needed to answer a specific biological, toxicological or ecological question (Whitman & Forschler 2007; Bardunias & Su 2009, 2010).

To conclude, the use of planar arenas in the recent literature has significantly enhanced understanding of multiple aspects of subterranean termite biology, which could not have been achieved with other devices. We suggest that, except for some specific short-term studies, the use of Petri dishes should be restricted to preliminary or prescreening studies, and that authors should refrain from drawing conclusions about biological significance from these simplistic reductionist bioassays, that may yield severely distorted depictions of biological phenomena. The increased biological relevance of information obtained from assays conducted with planar areas greatly outweighs the additional effort and cost required to use them, and we strongly advise their use for the study of subterranean termites.

REFERENCES CITED

ANDERSON, R. M., AND MAY, R. M. 1981. The population dynamics of microparasites and their invertebrate hosts. Phil. Trans. R. Soc. B. 291: 451-524.

AMERICAN WOOD PROTECTION ASSOCIATION. 2009. Standard method for laboratory evaluation to determine resistance to subterranean termites. AWPA E1-09. 10 pp.

ARQUETTE, T. J., CHAMPAGNE, D. E., BROWN, M. R., AND FORSCHLER, B. T. 2006. Evaluation of novel and traditional measures for vigor of laboratory-cultured termites, *Reticulitermes flavipes* (Kollar). J. Insect Physiol. 52: 51-66.

BARDUNIAS, P. M., AND SU, N.-Y. 2005. Comparison of tunnel geometry of subterranean termites (Isoptera: Rhinotermitidae) in "two-dimensional" and "three-dimensional" arenas. Sociobiol. 45: 679-685.

BARDUNIAS, P. M., AND SU, N.-Y. 2009. Dead reckoning in the tunnel propagation of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 102: 158-165.

BARDUNIAS, P. M., AND SU, N.-Y. 2010. Change in tunnel heading in the Formosan subterranean termite

(Isoptera: Rhinotermitidae) in response to movement through opened space. Ann. Entomol. Soc. Am. 103: 449-454.

BECKER, G. 1969. Rearing of termites and testing methods used in the laboratory *In* K. Krishna and F. M. Weesner [eds.], Biology of termites. Vol. 1. Academic Press, New York. pp. 351-385.

BECKER, G. 1978. Temperatur-Optimum der Fraßaktivität verschiedener Termiten-Arten. Z. angew. Entomol. 86: 225-259.

CAMPORA, C. E., AND GRACE, J. K. 2001. Tunnel orientation and search pattern sequence of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 1193-1199.

CARTER, F. L., STINGER, C. A., AND SMYTHE, R. V. 1972. Survival of six colonies of *Reticulitermes flavipes* on unfavorable woods. Ann. Entomol. Soc. Am. 65: 984-985.

CHOUVENC, T., AND SU, N.-Y. 2010. Apparent synergy among defense mechanisms in subterranean termites (Rhinotermitidae) against epizootic events - The limits and potential for biological control. J. Econ. Entomol. 103: 1327-1337.

CHOUVENC, T., SU, N.-Y., AND ELLIOTT, M. L. 2008. Interaction between the subterranean termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) and the entomopathogenic fungus *Metarhizium anisopliae* in foraging arenas. J. Econ. Entomol. 101: 885-893.

CHOUVENC, T., SU, N.-Y., AND ELLIOTT, M. L. 2011a. Rich microbial community associated with the nest material of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Florida Entomol. 94: 115-116.

CHOUVENC, T., GRACE, J. K., AND SU, N.-Y. 2011b. Fifty years of attempted biological control of termites - Analysis of a failure. Biological Control (*In press*).

CREMER, S., AND SIXT, M. 2009. Analogies in the evolution of individual and social immunity. Phil. Trans. R. Soc. B 364: 129-142.

DAWKINS, R. 2004. Extended phenotype - but not too extended. A reply to Laland, Turner and Jablonka. Biol. Philosoph. 19: 377-396.

GREAVES, T. 1962. Studies of foraging galleries and the invasion of living trees by *Coptotermes acinaciformis* and *C. brunneus* (Isoptera). Aust. J. Zool. 10: 630-651.

HAVERTY, M. I. 1979. Soldier production and maintenance of soldier proportions in laboratory experimental groups of *Coptotermes formosanus* Shiraki. Insectes Soc. 26: 69-84.

HEDLUND, J., AND HENDERSON, G. 1999. Effect of available food size on search tunnel formation by the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 92: 610-616.

HUGHES, D. P., PIERCE, N. E., AND BOOMSMA, J. J. 2008. Social insect symbionts: evolution in homeostatic fortresses. Trends Ecol. Evol. 23: 672-677.

HUSSENEDER, C., BERESTECKY, J. M., AND GRACE, J. K. 2009. Changes in composition of culturable bacteria community in the gut of the Formosan subterranean termite depending on rearing conditions of the host. Ann. Entomol. Soc. Am. 102: 498-507.

KING, E. G., AND SPINK, W. T. 1969. Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus*, in Louisiana. Ann. Entomol. Soc. Am. 62: 536-542.

LANGSTROTH, L. L. 1853. Langstroth on the hive and the honeybee, a beekeeper's Manual. Northampton, Massachusetts: Hopkins, Bridgman.

LEE, S.-H., BARDUNIAS, P. M., AND SU, N.-Y. 2007. Optimal length distribution of termite tunnel branches for efficient food search and resource transportation. Biosyst. 90: 802-807.

LENZ, M. 1985. Variability of vigor between colonies of *Coptotermes acinaciformis* (Froggatt) (Isoptera: Rhinotermitidae) and its implications for laboratory experimentation. Bull. Entomol. Res. 75: 13-21.

LENZ, M. 1986. Principles behind the laboratory assessment of materials with subterranean termites - Recent perspectives and shifts of emphasis. Material and Organisms 21: 123-137.

LENZ, M. 2009. Laboratory bioassays with subterranean termites (Isoptera). The importance of termite biology. Sociobiol. 53: 573-595.

LENZ, M., AND DAI, Z.-R. 1985. On the validity of using susceptible timbers as indicators of termite vigor in laboratory studies on the resistance of materials to termites. Material and Organisms 20: 97-108.

LENZ, M., AND WILLIAMS, E. R. 1980. Influence of container, matrix volume and group size on survival and feeding activity in species of *Coptotermes* and *Nasutitermes* (Isoptera: Rhinotermitidae, Termitidae). Material and Organisms 15: 25-46.

LENZ, M., BARRETT, R. A., AND WILLIAMS, E. R. 1984. Implications for comparability of laboratory experiments revealed in studies on the effects of population density on vigor in *Coptotermes lacteus* (Froggatt) and *Nasutitermes exitiosus* (Hill) (Isoptera: Rhinotermitidae and Termitidae). Bull. Entomol. Res. 74: 477-485.

LENZ, M, AMBURGEY, T. L., DAI, Z.-R., KUHNE, H., MAULDIN, J. K., PRESTON, A. F., AND WESTCOTT, M. 1987. Interlaboratory studies on termite wood decay fungi associations. I. Determination of maintenance conditions for several species of termites (Isoptera: Mastotermitidae, Termopsidae, Rhinotermitidae, Termitidae). Sociobiology 13: 1-56.

LI, H.-F., AND SU, N.-Y. 2008. Sand displacement during tunnel excavation by the Formosan subterranean termite (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 101: 456-462.

LI, H.-F., AND SU, N.-Y. 2009. Buccal manipulation of sand particles during tunnel excavation of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 102: 333-338.

LI, H.-F., YANG, R.-L., AND SU, N.-Y. 2010. Interspecific competition and territory defense mechanisms of *Coptotermes formosanus* and *Coptotermes gestroi* (Isoptera: Rhinotermitidae). Environ. Entomol. 39: 1601-1607.

LUSCHER, M. 1949. Continuous observation of termites in laboratory cultures. Acta Trop. 6: 161-165.

MESSENGER, M. T., AND SU, N.-Y. 2005. Agonistic behavior between colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae) from Louis Armstrong Park, New Orleans, Louisiana. Sociobiol. 45: 331-345.

NOBRE, T., NUNES, L., AND BIGNELL, D. E. 2007. Tunnel geometry of the subterranean termite *Reticulitermes grassei* (Isoptera: Rhinotermitidae) in response to sand bulk density and the presence of food. Insect Sci. 14: 511-518.

PITTS-SINGER, T. L., AND FORSCHLER, B. T. 2000. Influence of guidelines and passageways on tunneling behavior of *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae). J. Insect Behav. 13: 273-290.

PUCHE, H., AND SU, N.-Y. 2001. Tunnel formation by *Reticulitermes flavipes* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in response to wood and sand. J. Econ. Entomol. 94: 1398-1404.

ROBSON, S. K., LESNIAK, M. G., KOTHANDOPANI, R. V., TRANIELLO, J. F. A., THORNE, B. L., AND FOURCASSIÉ, V. 1995. Non-random search geometry in subterranean termites. Naturwissenschaften 82: 526-528.

ROLFF, J., AND SIVA-JOTHY, M. T. 2003. Invertebrate ecological immunology. Science 301: 472-475.

ROSENGAUS, R. B., AND TRANIELLO, J. F. A. 2001. Disease susceptibility and the adaptive nature of colony demography in the dampwood termite *Zootermopsis angusticollis*. Behav. Ecol. Sociobiol. 50: 546-556.

SU, N.-Y. 2005a. Directional change in tunneling of subterranean termites (Isoptera: Rhinotermitidae) in response to decayed wood attractants. J. Econ. Entomol. 98: 471-475.

SU, N.-Y. 2005b. Response of the Formosan subterranean termites (Isoptera: Rhinotermitidae) to baits or nonrepellent termiticides in extended foraging arenas. J. Econ. Entomol. 98: 2143-2152.

SU, N.-Y., AND LA FAGE, J. P. 1984. Differences in survival and feeding activity among colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Z. Angew. Entomol. 97: 134-138.

SU, N.-Y., AND PUCHE, H. 2003. Tunneling activity of subterranean termites (Isoptera: Rhinotermitidae) in sand with moisture gradients. J. Econ. Entomol. 96: 88-93.

SU, N.-Y., AND SCHEFFRAHN, R. H. 1986. A method to access, trap, and monitor field populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the urban environment. Sociobiol. 12: 299-304.

SU, N.-Y., AND SCHEFFRAHN, R. H. 1988. Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiol. 14: 353-359.

SU, N.-Y., TAMASHIRO, M., AND HAVERTY, M. I. 1985. Effect of three insect growth regulators, feeding substrates, and colony origin on survival and presoldier production of the Formosan subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 78: 1259-1263.

TAMASHIRO, M., FUJII, J. K., AND LAI, P.-O. 1973. A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. Environ. Entomol. 4: 721-722.

TUCKER, C. L., KOEHLER, P. G., AND OI, F. M. 2004. Influence of soil compaction on tunnel network construction by the Eastern subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 97: 89-94.

WHITMAN, J. G., AND FORSCHLER, B. T. 2007. Observational notes on short-lived and infrequent behaviors displayed by Reticulitermes flavipes (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 100: 763-771

WOOD, T. G. 1988. Termites and the soil environment. Biol. Fertil. Soils 6: 228-236.