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A N I N T E R N A T I O N A L J O U R N A L

Commercial Gouache as a Dye for Termites in Laboratory Assays

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ABSTRACT

Studies were performed to evaluate the suitability of a commercial gouache as an external marker for termite workers in lab assays. Dyed and non-dyed termites presented similar survival for the first 300 minutes since the experiment has began, and differed after this time. Mortality did not differ for termites dyed in two different colours from the same brand of gouache. It was concluded that commercial gouache could be a suitable marker for Cornitermes cumulans (Isoptera: Termitidae) termites, provided following restrictions were applied (i) tested hypotheses did not rely on times to death greater than 300 min., or (ii) experiments included "sham-dyed" termites (i. e., a "control" group with "dyed" individuals, rather than the tradicional control group composed by non-dyed individuals).

Key words: biological dyes, behaviour, lab assays, Isoptera

INTRODUCTION

Studying termites behaviour is often based on lab assays, not only to guarantee better control of experimental procedures, but also because the cryptic habits of these insects impair more accurate field observations. In such assays, it is usual to mark individuals using a variety of dyes such as Nile Blue, Sudan Red 7B, Sudan Black B, fuchsin, etc (Su et al., 1991), to allow more specific observations to be made. Termites to be dyed are forced feeding on filter paper previously stained by dye or forced to drink a water solution of such chemicals (Evans, 2000). Despite being largely used, this technique is far from being ideal. Firstly, after ingestion, some dyes may promote high levels of termite mortality (e.g. Sudan Blue 35, Grace and Abdallay, 1990). Secondly, this technique demands time, because it relies on termites ingesting tinged filter paper. Thirdly, when behavioural assays are to be registered by video cameras, internal dyes not always provide enough contrast, impairing recordings. This problem is particularly true for termites, whose behavioural assays need to be carried out under very faint luminosity. Finally, specifically for the case of Brazil, such dyes are not always readily available to researchers because, being imported, stocks are subjected to economical constraints or (prosaic) delivery delays.

A valid alternative to circumvent dye toxicity and the morosity of the marking technique, would be to mark individuals externally, as it is usual for studies on butterflies (Azerefegne et al., 2001).

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High contrast, can be achieved by the use of an opaque pigment. And to circumvent the troubles associated to importation, it would be highly desirable to use dyes readily available in the internal market.

This work aimed to test the suitability of a cheap, readily available, and topically appliable dye to mark termites in lab assays. *Cornitermes cumulans* (Kollar) (Isoptera, Termitidae) are suitable termites for this test, because they are large bodied, abundant and present in nearly all over the Brazilian territory, being considered pest in several environments. *Cornitermes spp.* are Neotropical termite species occurring in several habitats, including forests, "cerrados" (Brazilian savannas) and man-modified habitats, such as pastures or even gardens within cities, where they feed on living and dead grass and herbs (Cancello, 1989). Several species of this genus (among them *C. cumulans*) build large epigeous nests which are simultaneously inhabited by inquilines, such as other termite genera, ants, beetles, birds, snakes, etc (Redford, 1984).

We tested two distinct null hypotheses: (i) time spent till death would not differ between termites marked externally and those not marked; and (ii) for the same brand of dye, different opaque pigments do not promote differential mortality on termites.

MATERIALS AND METHODS

C. cumulans workers (third instar and beyond) used in this experiment were collected in Viçosa, State of Minas Gerais, in southeastern Brazil. The experiment aimed to verify the effects of an artificial dye (commercial gouache) on survival of termite workers kept in Petri dishes in the lab. Workers of *C. cumulans* were taken to the lab and confined in groups of four individuals per Petri dish (diameter = 9.0 cm). Before being confined, termites had their tergites painted with water soluble commercial gouache ("Têmpera Guache", from Acrilex ™ "Tintas Especiais", São Bernardo do Campo, São Paulo, Brazil). Special care was taken so that the gouache did not ooze to the insects' pleuron, which could block their spiracles. Two colours were used: black and white. Each colour was applied to all four individuals kept in a group, and five groups were tested per colour. An additional set of five groups not receiving any dye was used as a control.

Termites were reared in dark conditions, in a constant temperature chamber (25°C ± 0.5) and no food or water was provided. Petri dishes were exposed to light only during the counting of survivors (no more than 5 min.). Observations started 8h after incubating the termites, and proceeded periodically, until all individuals were dead. Data were submited to Survival Regression Analysis, based on a Weibull model, using package SurvReg from R Project to Statistical Computing, freely available at http://termix.ufv.br/CRAN. For more details see also Ihaka and Gentleman (1996).

RESULTS

Survival patterns of dyed termites was indistinguishable from that of non-dyed termites for ca. 300 minutes since the begining of the experiment (Fig. 1). After that threshold, mortality rate was accelerated for dyed termites when compared with non-dyed ones, but such mortality showed similar patterns for termites dyed in two different colours of the same brand of gouache. Survival curves presented a shape parameter, α = 4.8909, implying that mortality tended to be more expressive after an initial time-lag had elapsed (Fig. 1).

On average, termites dyed in black survived 883.6 min.; those dyed in white survived 858.5 min., while termites not receiving any dye survived 1521.8 min. Mean time to death of non-dyed termites differed significantly from mean time to death of dyed termites (χ^2 = 101.24, df = 2, n = 60, p = 1.037×10^{-22}). In addition, mean times to death for black-dyed and white-dyed termites did not differ statistically (χ^2 = 0.20, df = 1, n = 60, p = 0.639).

DISCUSSION

Termites marked with commercial gouache died faster than termites not marked. Reasons for that could be physical blockage of gas exchange, leading to death by suffocation, or dye toxicity, leading to death by poisoning. Gas exchange in insects is known to happen mainly via spiracles (Chapman, 1991) and, in some termites, it is suspected to also occur through the cuticle

(Shelton and Appel, 2001). Termite mortality observed here, therefore, could not easily be attributable to suffocation. Firstly, the dye was avoided to ooze the pleuron, when marking termites, thereby ruling out suffocation by blockage of spiracles. Secondly, in an unnoticed blockage of any spiracle, respiration could still be performed through the cuticle (Shelton and Appel, 2001). In fact, mortality of tinted termites occured more intensely after some 300 min. had elapsed (Fig. 1), which seemed too long for a termite to tolerate a suffocation.

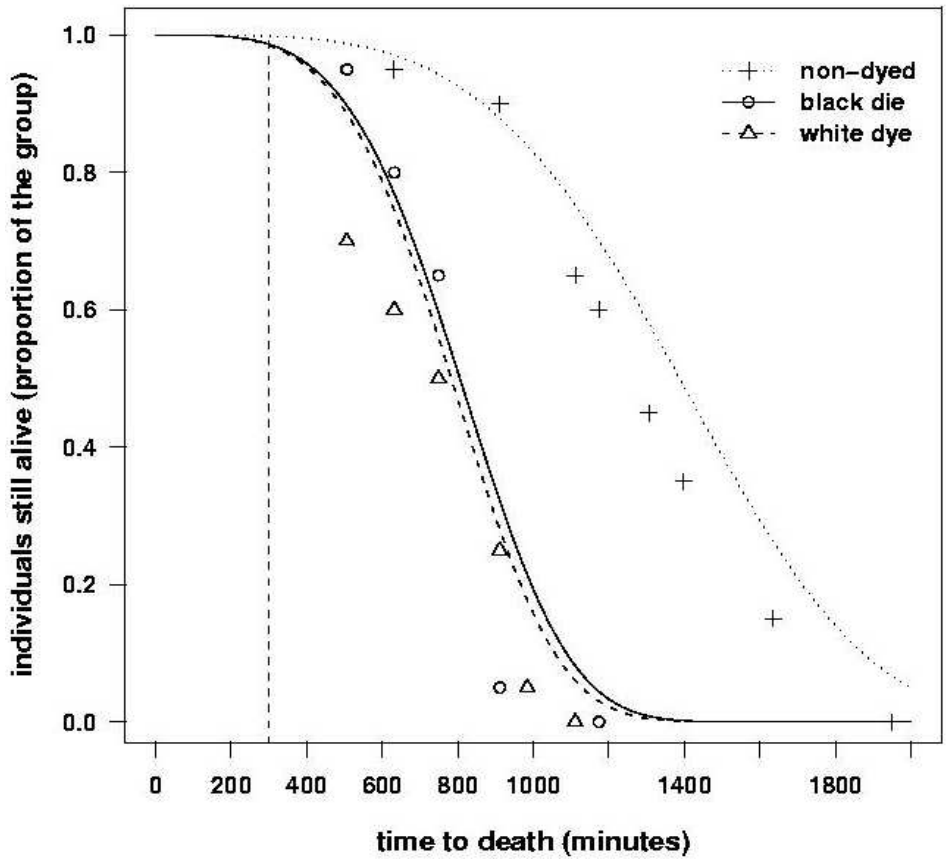

Figure 1 - Survival curves for starving termite workers coloured, with commercial gouache, in black ("black dye") and in white ("white dye"), and termite workers which did not receive any dye ("non-dyed"). Note that curves merge at times to death below 300 min.

It seemed more likely, therefore, that tinted termites were intoxicated by the dye. This was based on two complementary evidences: (i) ingestion of dyes could cause termite mortality (Grace and Abdallay, 1990); and (ii) *C. cumulans* could face death when submited to topic application of chemicals (DeSouza et al., 2001).

Whether dyes used here intoxicated termites due to ingestion or after percolation through the cuticle, could not be discriminated. What seemed more significant, however, was the fact that white and black dyes had very similar effects (no statistical difference, Fig. 1). This showed that either the pigments were equally toxic, or dye toxicity was from gouache components other than the pigments themselves.

In view of these results, one should not conclude that this commercial gouache was inappropriate to label termites. Rather, it can be used for dye marking termite workers in behavioural studies, as long as experimental procedures were conveniently adjusted. Firstly, experiments should

include “sham-dyed” termites to control for variance due to dye toxicity. “Sham” techniques are very useful in experiments where it is unavoidable to cause some kind of injury/stress on tested organisms. Such techniques consist of inflict in control insects, injuries that are similar to the injury suffered by the treatment under study (Proux et al., 1993, Sullivan et al., 2000). Specifically for the case related here, since both dyes had similar effect on termites, it could suffice to dye the control-group in one colour, and the treatment-group is dyed in other colour.

It is also true that experiments using this dye should not investigate hypotheses that explicitly rely on too long times for death (e.g. > 300 min. or so). Below these values, mortality due to dye toxicity was negligible and statistically indistinguishable from natural mortality of non-dyed termites (Fig. 1), therefore would most likely not affect the behaviour under study.

Finally, for studies involving lab grouped termites, experimental units should use the same density, i.e., the same number of termites per unit area. This is important because mortality of lab confined termites is strongly related to the density of the experimental group (Miramontes and DeSouza, 1996), and this is important even for termites poisoned by chemicals (DeSouza et al., 2001). Thus, this commercial gouache may be a viable alternative for marking termites where other dye markers are not available.

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RESUMO

Estudos foram conduzidos para avaliar a adequabilidade de um corante barato, prontamente disponível e aplicável topicamente, como marcador para operários de *Cornitermes cumulans* (Insecta: Isoptera) em ensaios de laboratório. Os padrões de sobrevivência dos cupins marcados não se distinguem daqueles apresentados pelos cupins sem marcação, cerca de 300 minutos desde o início do experimento. Após esse limiar, a mortalidade dos cupins marcados é acelerada quando comparada com cupins não marcados. Tal mortalidade, entretanto, não difere entre cupins marcados com duas cores diferentes da mesma marca comercial de guache. Concluiu-se que o guache comercial é adequado como marcador de operários de *C. cumulans*, desde que (i) experimentos não investiguem hipóteses explicitamente dependentes de tempos de sobrevivência maiores do que 300 min., ou então que (ii) experimentos incluam um grupo “controle” composto de cupins corados com um pigmento diferente da cor usada no grupo “tratamento”.

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