Effects of a Dye, Sudan Red 7B, on the Formosan Subterranean Termite, *Coptotermes formosanus* Shiraki (Isoptera:Rhinotermitidae)*

By NAN-YAO SU, JEFFERY P. LA FAGE and GLENN R. ESENTHER

Louisiana State University, Baton Rouge, Louisiana, U.S.A.
Forest Products Laboratory, Madison, Wisconsin, U.S.A.

Contents

1. Introduction
2. Materials and Methods
   2.1 Staining procedure and the immediate effects on termites - 2.2 The delayed effects and the durability of dyes - 2.3 Data analysis
3. Results and Discussion
   3.1 Immediate effects - 3.2 The delayed effects and the durability of dyes
4. summary
   References

1 Introduction

The Formosan subterranean termite, *Coptotermes fornosanus* Shiraki, is one of the most important structural pests. Control of this termite has been extremely erratic and unsatisfactory. In Louisiana many pest control operators have reported that chemical treatments for *C. formosanus*, carried out in the manner accepted for native subterranean termites (*Reticulitermes* spp.) often fail. This poor control was, in large measure, due to the very large size of *C. formosanus* colonies.

Studies of field colonies indicated that foragers may range more than 100 m in any direction and may be comprised of 10 million individuals (E. G. KING and W. T. SPINK, 1969; T. LI et al., 1976; P. Y. LAI, 1977). With colonies of this magnitude, it is difficult to disperse control agents throughout the entire colony. One proposed control strategy advises the use of poison

* This study was supported, in part, by a cooperative agreement between the Louisiana State University and the Forest Service, U.S. Department of Agriculture, Forest Products Laboratory, Madison, Wisconsin.

baits incorporating slow-acting toxicants to control the entire colony (G. R. ESENTHER and D. E. GRAY, 1968; G. R. ESENTHER and R. H. BEAL, 1974; R. L. BEARD, 1974; N.-Y. Su et al., 1982). For this technique to be successful, the total colony size must be known to estimate bait toxicant concentrations. Due to the size of the gallery system, direct counts of populations which are common practice for other species of termites, are not possible for *C. formosanus.* Several workers have attempted to estimate population size of subterranean termites using mark-recapture method (P. Y. LAI, 1977; C. BARONI-URBANI et al., 1978; G. R. ESENTHER, 1980). Although there are several methods for marking insects including mutilation, labeling, direct marking etc. (T. J. WALKER and S. A. WINERITER, 1981), most researchers prefer the use of dyes to directly mark subterranean termites because of the ease of marking a large number of individuals and the long durability (J. K. FUJII, 1975; P. Y. LAI, 1977; G. R. ESENTHER, 1980). However, information is lacking on the effects of the dyes on termites. This study was initiated to examine the effect of a dye, Sudan Red 7B (formerly Oil Soluble Red 7B, BASF Wyandotte Co., N.J.), on *C. formosanus*. Levels of the dye necessary to mark termites, its persistence in the termite and effect on termite survival were investigated.

2. Materials and Methods

2.1 Staining procedure and the immediate effects on termites

Termites were collected from cypress snags (*Taxodium distichum* [L.] Rich.) near Lake Charles, Louisiana, and held in a 246-1 container under laboratory conditions at 25 ± 2°C for two months prior to the test. Sudan Red 7B-impregnated absorbent pads (47 mm dia., Gelman Inst. Co., MI) prepared to deliver five dye concentrations in 2 % increments from 2 to 10 % (wt/wt) and a solvent- (acetone) control pad were placed into screw-top glass jars (50 mm dia., 65 mm height). Sixty ml of washed sand moistened with 12 ml of deionized water were added to cover the absorbent pad. Three hundred termites (240 larvae externally undifferentiated of at least the third instar and 60 soldiers) were introduced into each of the containers and held at 29 ± 1 °C. Twelve units were prepared for each concentration (total of 72 experimental units). Three units of each concentration were selected at random and disassembled every three days up to twelve days after the introduction and the termites counted and evaluated for the effects of the dye. The immediate effects of Sudan Red 7B on C. fornosanus were expressed as the Y_O mortality of workers and soldiers of each treatment when the unit was disassembled. Abbott's formula was used to correct for natural mortality (W. S. ABBOTT, 1925).

2.2 The delayed effects and the durability of dyes

After counting (above), 100 termites (80 workers and 20 soldiers) were selected at random from the survivors of disassembled units and transferred to screw-top glass jars (50 mm dia., 35 mm height) containing a block of slash pine (18 x 18 x 18 mm^3)

(*Pinus elliottii* Engelm. var. *elliottii*) covered by 30 ml of washed sand moistened with 6 ml of deionized water. These units were held at 29 ± 1°C for one month, after which time the termites were examined. The delayed effects of Sudan Red 7B on *C. formosanus* were measured by the % mortality of workers. The durability of the dye was determined by the Yo stained individuals for each treatment one month after being removed from the source of dye.

2.3 Data analysis

All data were transformed to square root of arc sine and subjected to analysis of variance using a completely randomized design. Means of three replications were tested for significance at the 5 % level, and separated by Duncan's multiple range test.

3. Results and Discussion

3.1 Immediate effects

Even at the lowest dye concentration (2%), workers which fed on the stained pads for a minimum period of three days showed red coloration visible through the cuticle. Except for a very small portion of individuals (less than 0.1 %) that obviously did not feed during that period possibly due to pre-molt fasting or other factors, all workers of all treatments acquired obvious marks. There were no distinctive marks on soldiers except for those individuals fed high dye concentrations over long exposure times. In these a slight red mark was visible at the tip of the abdomen. This result agreed with earlier observations in which we concluded that the dye was not transferred by trophallaxis (N.-Y. Su et al., unpublished). The results clearly indicated that even at a low concentration, workers could acquire the marking by feeding on the dyes even at a short period.

The immediate effects of the dye on survival are summarized in Table 1 and 2. Although soldiers did not acquire as distinct marking as workers, their mortalities were generally higher. Up to the dye concentration of 8 Yo, there is a general tendency toward higher mortality at higher concentrations and longer exposure times. The low mortality at the concentration of 10 Yo may have been due to the antifeedant effect of this high concentration resulting in the low consumption rate of dye. Worker mortality in all treatments immediately following exposure is relatively low, indicating the low acute toxicity of this dye on *C. formosanus* workers.

3.2 The delayed effects and the durability of dyes

The delayed effects of Sudan Red 7B on *C. formosanus* workers are summarized in Table 3. One month after being removed from the source

Table 1. Immediate effects of Sudan Red 7B on C. fornosanus workers expressed by % mortality.*

Concentration (%)	Exposure Time (days) 3	6	9	12
2	0.6a	1.2a	2.6 a	3.5 a
4	6.6b	6.5b	9.6b	14.0c
6	4.5 a	7.1 b	9.2b	10.3bc
a	2.9a	3.7 a	12.9c	13.5c
10	0a	0.2 a	11.7c	10.5bc

* Throughout the table means followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

Table 2. Immediate effects of Sudan Red 7B on *C. formosanus* soldiers expressed by % mortality. *

Concentration (%)	Exposure Time (days) 3	-6	9	12
2	7.0 a	16.5b	31.3c	38.3c
4	2.9a	51.8d	51.5d	73.2c
6	11.7b	39.6c	71.1e	58.0d
8	12.3 b	53.1d	55.6d	57.0d
10	12.3b	45.1d	62.5de	51.0d

* Throughout the table means followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

Table 3. Delayed effects of Sudan Red 7B on *C. formosanus* workers expressed by % mortality one month after transfer.*

Concentration (%)	Exposure Time (days) 3	6	9	12
2	2.4a	13.9a	13.5 a	56.4d
4	13.7a	24.1b	23.0b	41.7c
6	21.7ab	37.5c	28.8bc	37.0c
a	52.9d	55.2 d	50.0d	69.7e
10	5.9a	38.2c	52.5d	66.5e

* Throughout the table means followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

of the dye, workers of most of the treatments showed high mortality with a maximum of ca. 70 %, and more than 90 % of the workers from all treatments retain visible markings (Table 4). The delayed mortality due to marking technique, therefore, must be included in the estimate of the population, should such correction be necessary.

Table 4. The durability of Sudan Red 7 B in *C. formosanus* expressed by the % stained individuals one month after being removed from the source of dye.*

Concentration (%)	Exposure Time (days) 3	6	9	12
2	90.4 a	100c	100c	100c
4	98.7c	95.4 ab	100c	100c
6	93.7 a	98.0bc	100c	100c
8	98.4c	97.6bc	100c	100c
10	96.6ab	98.3c	100c	100c

* Throughout the table means followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

4. Summary

A dye, Sudan Red 7B, was selected as a candidate marking material for studying the population dynamics of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Its effect on survival and persistence in termites was investigated. Almost 100% of the workers that had been allowed to feed on absorbent pads containing 2% (wt/wt) dye for 3 to 9 days and 4% dye for 3 days, retained visible coloration one month after being removed from the source of dye. Termites from these treatments also exhibited the lowest mortality, ca. 10%. All termites from treatments with higher concentrations and/or longer exposure time retained the visible marking one month after the transfer, but, they exhibited higher mortality ranging from 20 to 70 %. Compared with workers, soldiers exposed to the dye generally exhibited higher mortality than workers and the coloration was less distinctive.

Zusammenfassung

Der Einfluß des Farbstoffs Sudan Bot 7B auf die Bodentermite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

Ein Farbstoff, Sudan Rot 7B, wurde als mögliches Markierungsmittel für Untersuchungen zur Populationsdynamik der Erdtermite *Coptotermes formosanus* Shiraki benutzt. Sein Einfluß auf die Überlebensrate und die Beständigkeit in Termiten wurde untersucht. Fast 100% der Arbeiter, die sich während 3 bis 9 Tagen von saugfähigen Papierscheiben mit 2 Gew.-% Farbstoff und während 3 Tagen mit 4 % Farbstoff ernähren konnten, behielten einen Monat, nachdem sie von der Farbquelle entfernt wurden, eine sichtbare Färbung. So behandelte Termiten zeigten die niedrigste Mortalitätsrate von ca. 10 %. Alle Termiten, die mit höheren Konzentrationen behandelt und/oder dem Farbstoff länger ausgesetzt waren, behielten die sichtbare Kennzeichnung einen Monat nach dem Umsetzen, aber ihre Mortalitätsrate war höher, sie schwankte zwischen 20 und 70%. Verglichen mit Arbeitern war die Sterblichkeit von Soldaten, die dem Farbstoff ausgesetzt waren, im allgemeinen höher, und die Färbung war weniger ausgeprägt.

Résumé

Effets d'un colorant, le Rouge Soudan 7B, sur le termite formosan souterrain, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

Un colorant, le Rouge Soudan 7B, a été choisi comme élément candidat marqueur pour étudier la dynamique de la population du termite formosan souterrain, *Coptotermes formosanus* Shiraki. On a étudié son effet sur la survivance et la persistance des termites. Près de 100 % des ouvriers qui ont été nourris sur des tampons absorbants contenant 2 % (pds/pds) de colorant pendant 3 à 9 jours et 4 Yo de colorant pendant 3 jours, ont retenu une coloration visible pendant un mois après suppression de la source de colorant. Les termites ainsi traités ont montré aussi le mortalité la plus faible, soit moins de 10 %. Tous les termites traités avec des concentrations plus fortes et/ou des temps d'exposition plus longs ont retenu le marquage visible pendant un mois après le traitement mais ils ont montré une mortalité plus forte allant de 20 à 70 % En comparaison avec les ouvriers, les soldats exposés au colorant ont montré en general une mortalité plus forte et leur coloration était moins distincte.

Resumen

Los efectos de colorante Sudan rojo 7B sobre la termita subterránea *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

Se procedió a seleccionar el colorante Sudán rojo 7B como posible sustancia marcadora para el estudio de la dinámica de población de la termita subterránea *Coptotermes formosanus* Shiraki. Fueron analizados sus efectos sobre la tasa de supervivencia, así como su permanencia en las termitas. Casi el 100 % de los obreros que habían podido alimentarse entre 3 y 9 días de papel absorbente con un 2 % en peso de colorante, o bien, durante 3 días de papel con un 4% en peso, conservaron una coloración visible a lo largo de un mes después de haber sido retirados de la fuente del colorante. Las termitas sometidas a este tratamiento demostraron también, con un 10%, la menor tasa de mortalidad. Todas las termitas sometidas a tratamientos con mayores concentraciones y/o una exposición más larga a1 colorante conservaron la coloración durante un mes después de retiradas, mas su tasa de mortalidad fue mayor, oscilando entre un 20 y un 70 %. En comparación con los obreros, los soldados sometidos al colorante sufrieron por lo general, una mortalidad mas elevada, a la vez que su coloración resultó menos marcada.

References

ABBOTT, W. S.: A method of computing the effectiveness of an insecticide. J. econ. Entomol. *18* (1925) 265 - 267.

BARONI-URBANI, C., JOSENS, G. and PEADIN, G. J.: Empirical data and demographic parameters. In: M. V. BRIAN (ed.): Production ecology of ants and termites. Cambridge, London, New York, Melbourne (Cambridge Univ. Press) 1978, 409 pp.

BEARD, R. L.: Termite biology and bait-block method of control. Bull. 748. The Connecticut Agric. exper. Stat., NH., 1974, 19 pp.

ESENTHER, G. R.: Estimating the size of subterranean termite colonies by a release-recapture technique. The Internat. Res. Group on Wood Pres. Doc. No. IRG/WP/112, 1980, 5 pp.

— and BEAL, R. H.: Attractant-mirex bait suppresses activity of *Reticulitermes* spp. J. econ. Entomol. *67* (1974) 85 - 88.

— and GRAY, D. E.: Subterranean termite studies in southern Ontario. Canad. Entomol. *100* (1968) 827 - 834.

FUJII, J. K.: Effects of an entomogenous nematode *Neoaplectana carpocapsae* Weiser, on the Formosan subterranean termite, *Coptotermus formosanus* Shiraki, with ecological and biological studies on *C. formosanus*. Ph.D. dissertation, Univ. of Hawaii, Honolulu, HI, 1975, 163 pp.

KING, E. G. and SPINK, W. T.: Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus*, in Louisiana. Ann. Entomol. Soc. Amer. *62* (1969) 537 - 542.

LAI, P. Y.: Biology and ecology of the Formosan subterranean termite, *Coptotermes formosanus*, and its susceptibility to the entomogenous fungi, *Beauovaria bassiana* and *Metarrhizium anisopliae*. Ph.D. dissertation, Univ. of Hawaii, Honolulu, HI, 1977, 140 pp.

LI, T., HE, K. H., GAO, D. X., and CHAO, Y.: A preliminary study on the foraging behaviour of the termite, *Coptotermes formosanus* (Shiraki) by labelling with iodine^{131}. Acta Entomol. Sinica. *19* (1978) 32 - 38.

SU, N.-Y., TAMASHIRO, M., YATES, J. R., and HAVERTY, M. I.: Effect of behaviour on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite. J. econ. Entomol. *75* (1982) 188 - 193.

WALKER, T. J., and WINERITER, S. A.: Marking techniques for recognizing individual insects. The Florida Entomol. *64* (1981) 18 - 29.

Addresses of the authors:

Dr. NAN-YAO SU
Dr. JEFFERY P. LA FAGE

Department of Entomology
Louisiana State University
402 Life Sciences Building
Baton Rouge, LA 70803
U.S.A.

Dr. GLENN R. ESENTHER

Forest Products Laboratory
Forest Service
U.S. Department of Agriculture
P.O. Box 5130
Madison, Wisconsin 53705
U.S.A.