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# PRODUCTION OF SOLDIERS AND MAINTENANCE OF SOLDIER PROPORTIONS BY LABORATORY EXPERIMENTAL GROUPS OF *RETICULITERMES FLAVIPES* (KOLLAR) AND *RETICULITERMES VIRGINICUS* (BANKS) (ISOPTERA : *RHINOTERMITIDAE*)

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## *SUMMARY*

The effects of group size, time of year, and initial proportions of soldiers were explored in experimental groups of *Reticulitermes flavipes* (KOLLAR) and *R. virginicus* (BANKS). Groups were held in the laboratory for 12 weeks. Small groups (500 to 1000 workers) produced a smaller percentage of soldiers than did larger groups (1500 to 2500 workers). The number of soldiers produced in a group is highly correlated with the total number of termites surviving. The average percentage produced for all group sizes was 1.75 for both species.

Production of soldiers in *R. flavipes* was highest in colonies collected in April (2.0 %) and lowest in those collected in October (0.38 %). Bimonthly observations of increases and decreases in soldier production indicate a seasonal cycle in developmental potential.

Presence of soldiers in groups inhibited the production of new soldiers. An abnormally high percentage of soldiers did not precipitate extensive elimination of soldiers. In addition, overloading groups with soldiers (more than 1.75 %) did not adversely affect group survival.

## *RESUME*

**Production de soldats et maintien des proportions de soldats dans des Groupes Expérimentaux de** Reticulitermes flavipes **(Kollar) et de** Reticulitermes virginicus **(Banks) (Isoptères :** Rhinotermidae)

Les effets dûs à la taille du groupe, à l'époque de l'année et aux proportions initiales de soldats ont été explorés dans des groupes expérimentaux de *Reticulitermes flavipes*

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(KOLLAR) et de *Reticulitermes virginicus* (BANKS). On a élevé des groupes en laboratoire pendant 12 semaines. Les petits groupes (de 500 à 1000 ouvriers) ont produit un plus faible pourcentage de soldats que les grands groupes (de 1500 à 2000 ouvriers). Le nombre de soldats produits dans un groupe est hautement corrélé au nombre total de termites qui survivent. Le pourcentage moyen observé dans tous les groupes a été de 1,75 pour les 2 espèces étudiées.

La production de soldats chez *Reticulitermes flavipes* a été la plus élevée dans les colonies prélevées en avril (2 %) et la plus faible dans celles prélevées en octobre (0,38 %). Les observations bimensuelles des augmentations et des baisses de production de soldats montrent qu'il y a un cycle saisonnier du potentiel de développement.

La présence de soldats dans les groupes inhibe la production de nouveaux soldats. Un pourcentage anormalement élevé de soldats n'a pas provoqué l'élimination massive de soldats. De plus, la surcharge des groupes en soldats (plus de 1,75 %) n'a pas affecté de façon nuisible la survie du groupe.

## INTRODUCTION

The ecological and behavioral factors governing soldier production and regulation in termites are fundamental to evaluation of the effects of insect growth regulators (IGR's) on soldier production. Central to this study is the possibility that control of termites through the production of excess soldiers and the subsequent breakdown of normal caste proportions may be feasible. The Forest Service has begun a broad research program to study such factors in three species of economically important rhinotermitids *Reticulitermes flavipes* (KOLLAR), *R. virginicus* (BANKS) and *Coptotermes formosanus* SHIRAKI. The knowledge gained will, we hope, further our efforts at finding a practical termiticidal application for IGR's.

Our program includes both laboratory and field studies ; this report is the second arising from our laboratory studies. The first (HAVERTY, 1979a) set forth the experimental design used to evaluate the effects of group size and caste proportions on soldier differentiation and maintenance of soldier proportions in groups containing only workers and soldiers of *C. formosanus*. In this paper we examine similar relationships for *R. flavipes* and *R. virginicus*, and evaluate the influence of season upon soldier production in *R. flavipes*.

## METHODS AND MATERIALS

### Effects of Group Size on Production of Soldiers

Externally undifferentiated larvae of at least the third instar — subsequently referred to as workers — from two field colonies each of *R. flavipes* and *R. virginicus* were used. Termites were collected from fallen pine logs or from bolts of southern yellow pine placed around a log infested with a particular species of termite. All colonies were collected in southern Mississippi within 60 km of Gulfport, between late April and early May, 1976.

The initial group sizes were 500, 1000, 1500, 2000, and 2500 workers each. The experimental units were set up in screw-top canning jars of different sizes with tunneling

substrate at a ratio of 105 ml of sand and 21 ml of de-ionized water for each 500 termites. In addition, for each 100 termites a 2.0 × 2.0 × 1.5 cm block of oven-dried slash pine (*Pinus elliottii* Engelm. var. *elliottii*) was provided as food. All wood blocks were from the same tree. The jars containing the termites were incubated at 24 ± 1°C for 12 weeks. At the end of 12 weeks the units were disassembled and the termites counted by caste.

The data were subjected to analysis of variance using a randomized complete block design. Colony was the blocking factor to separate out error due to differences between colonies from experimental error. Each combination of treatment and colony was replicated three times. Response variables were percentages of soldiers (soldiers and presoldiers) in the surviving termites and survival. Before analysis all percentages were transformed to arcsin of the square root. Significance of treatment means was tested at the $\alpha = 0.05$ level. Means were ranked and significant differences tested with Student-Newman-Keuls' test.

The data were also analyzed by linear regression to evaluate the constancy of the total proportion. The total number of individuals in the group was the independent variable, and the number of soldiers and presoldiers was the dependent variable. Data from each colony were analyzed separately and together, by species.

## Effects of Time of Year on Soldier Differentiation in *R. flavipes*

Every 2 months, from December 1976 through October 1977, pine bait bolts were sampled from five randomly selected colonies of *R. flavipes* located in HARRISSON, JACKSON, and STONE counties in southern Mississippi. The bolts containing the termites were brought into the laboratory, all the termites were extracted, and workers randomly selected for use in this experiment. We used a mixture of sand and vermiculite to enhance survival over the 12-week test (HAVERTY, 1979b). Each half-pint (237 ml) canning jar was filled with *ca.* 53 ml of oven-dried sand, 6.0 g vermiculite, 50 ml of de-ionized water, and five 2.0 × 2.0 × 1.5 cm blocks of slash pine were provided as food. We added 500 workers to each jar. Jars were incubated at 24 ± 1°C for 12 weeks, then disassembled and the termites counted and identified to caste. Five groups of 500 workers were used from each source colony.

The data were subjected to analysis of variance utilizing a completely randomized design. Data from the five groups from each colony were pooled and the average percentage of each caste for each colony was used as the percentage for each replication. As a result, the values for each time period were replicated five times. The response variables were percent soldiers (soldiers and presoldiers) in the surviving termites and percent survival. Before analysis all percentages were transformed to arcsin of the square root. Significant treatment means were tested at the $\alpha = 0.05$ level. When there were significant differences between means they were ranked and significant differences tested with Student-Newman-Keuls' test.

## Effects of Variable Initial Proportions of Soldiers on Soldier Production, Elimination, and Maintenance

Workers and soldiers in various proportions were used from each of four colonies of *R. flavipes* or *R. virginicus*. Colonies were collected in the last 2 weeks of April 1976 in southern Mississippi. Experimental units each contained 500 workers and were set up as described earlier for the time-of-year experiment. The "normal" percentage of soldiers was assumed to be 1.75, the average percentage differentiation found after 12 weeks in the test of effects of group size (See Results and discussion). We set up groups of termites containing abnormal numbers of soldiers: 0, 0.44, 0.88, 3.50 and 8.75 percent soldiers; these numbers are 0, 0.25, 0.5, 2.0 and 5.0 times the "normal" soldier percentage.

The data were subjected to analysis of variance using a randomized complete block design. Colony was the blocking factor. Each combination of treatments (initial percen-

tage of soldiers x colony) was replicated three times. Response variables were percent survival, and number and percentage of each of the following in the surviving termites : presoldiers, soldiers, and total soldiers. Before analysis all numbers were transformed to the square root and percentages to the arcsin of the square root. Treatment means were tested for significant differences at the $\alpha = 0.05$ level, and significantly different means ranked and tested with Student-Newman-Keuls' test.

## RESULTS AND DISCUSSION

### Effects of Group Size on Production of Soldiers

Soldier differentiation after 12 weeks in groups of various sizes is shown in table I. There were no significant differences in group survival in either *R. flavipes* or *R. virginicus*. The average percentage of all soldiers (soldiers and presoldiers) produced for both species was 1.75. A significantly lower percentage of *R. flavipes* soldiers was produced in the smallest group as compared to the three largest groups. No such differences were found for *R. virginicus*, although there was a trend toward smaller soldier percentages with the smaller group sizes. A similar comparison of group size effects was reported earlier for *C. formosanus* (HAVERTY, 1979a) and there, too, over 12 weeks, a lower percentage of soldiers was produced in the smaller groups.

Table I. — Soldier differentiation (percent adult soldiers and presoldiers) and percent survival of *Reticulitermes flavipes* (KOLLAR) and *R. virginicus* (BANKS) after 12 weeks, in groups originally composed of 100 percent workers but varying in initial size (1).

Table I. — Différentiation des soldats (pourcentage de soldats adultes et de présoldats) et pourcentage de survie de *Reticulitermes flavipes* (KOLLAR) et de *Reticulitermes virginicus* (BANKS) après 12 semaines, dans des groupes qui étaient composés initialement de 100 % d'ouvriers de taille variable (1).

| Initial group size | *R. flavipes* | | *R. virginicus* | |
|---|---|---|---|---|
| | Soldiers | Survival | Soldiers | Survival |
| 500 | 1.2c | 69.1 | 1.3 | 63.5 |
| 1000 | 1.4bc | 62.4 | 1.4 | 68.9 |
| 1500 | 2.0a | 63.5 | 1.8 | 67.5 |
| 2000 | 1.8ab | 61.0 | 2.2 | 66.4 |
| 2500 | 2.4a | 63.8 | 2.1 | 68.4 |

(1) Mean of three replicates from each of two colonies. Means in a column followed by the same letter or not followed by any letter are not significantly different at the $\alpha = 0.05$ level (Student-Newman-Keuls' test).

The total number of soldiers produced was highly correlated ($\alpha < 0.01$) with the total number of surviving individuals in the group for both termite species ($r = 0.56$ for *R. flavipes* and $r = 0.88$ for *R. Virginicus*, fig. 1). Variation in the proportion of soldiers was found between colonies within each species. However, within each colony the number of soldiers was highly correlated with the total number of surviving individuals ($r = 0.91$ and 0.84 for *R. flavipes* and $r = 0.92$ and 0.84 for *R. virginicus*).

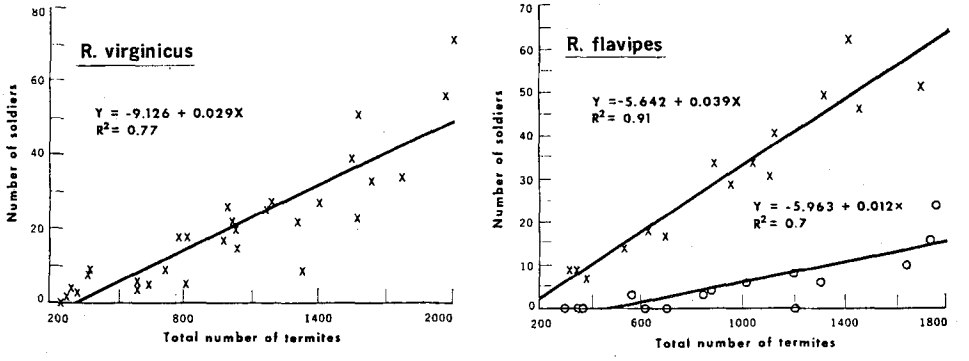


Fig. 1. — Relationship between the number of soldiers (adult soldiers and presoldiers) and the total number of termites in different sized groups of two species of *Reticulitermes*. In *R. flavipes* x and o represent separate colonies.

Fig. 1. — Relation entre le nombre de soldats (soldats et présoldats) et le nombre total de termites dans des groupes de taille différente des deux espèces de *Reticulitermes*.

## Effects of Time of Year on Soldier Differentiation in *R. flavipes*

There were significant differences in the percentages of soldiers (adult soldiers and presoldiers) differentiating from the workers collected at the six different times throughout one year (table II). The percentage of soldiers differentiating after 12 weeks varied from 0.38 to 2.00, with the highest

Table II. — Soldier (percent adult soldiers and presoldiers) differentiation and percent group survival after 12 weeks in laboratory groups initially composed of 500 *R. flavipes* workers collected from field colonies at 2-month intervals (1).

Table II. — Différentiation de soldats (pourcentage de soldats adultes et pré-soldats) et pourcentage de survie des groupes après 12 semaines dans des groupes de laboratoire qui étaient composés initialement de 500 ouvriers de *Reticulitermes flavipes* prélevés dans des colonies naturelles à intervalles de deux mois (1).

| Date of Collection | Soldiers | Survival |
|---|---|---|
| | Percent | |
| December 1976 | 0.76ab | 78.8 |
| February 1977 | 1.36ab | 65.8 |
| April 1977 | 2.00a | 71.7 |
| June 1977 | 0.99ab | 60.4 |
| August 1977 | 0.64ab | 67.1 |
| October 1977 | 0.38b | 58.6 |

(1) Mean of five colonies for each time period. Means in a column followed by the same letter or not followed by any letter are not significantly different at the $\alpha = 0.05$ level (Student-Newman-Keuls' test).

percentage resulting from April collections and the lowest from October collections. The decreases and increases in percentages indicate a consistent trend or cycle in soldier developmental potential. Soldier percentages were highest in the months (February and April) which precede the normal flight season (March though May) for *R. flavipes* (WEESNER, 1970).

**Effects of Variable Initial Proportions of Soldiers on Soldier Production, Elimination, and Maintenance**

The presence of soldiers in groups of *R. flavipes* and *R. virginicus* inhibited production of new soldiers as they did in *C. formosanus* (HAVERTY, 1979a). The extent of the inhibition increased as the number of adult soldiers in the initial mix increased (table III). The mean number of soldiers in

Table III. — Soldiers and presoldiers of *Reticulitermes flavipes* (KOLLAR) and *R. virginicus* (BANKS), and group survival after 12 weeks, in groups of 500 termites differing in initial mix of workers and soldiers (1) (2).

Table III. — Nombre et pourcentage de soldats et de pré soldats de *Reticulitermes flavipes* (KOLLAR) et de *Reticulitermes virginicus* (BANKS), et pourcentage de survie après 12 semaines dans des groupes de 500 termites qui avaient au départ des proportions variées d'ouvriers et de soldats (1) (2).

| Treatment | Initial | | | | After 12 weeks | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number | | Percent | | Number | | Percent | | Variation in number of SO & PS | Group survival (percent) |
| | WO | SO | WO | SO | PS | SO | PS | SO | | |
| | | | | | *R. flavipes* | | | | | |
| 1 | 500 | 0 | 100.0 | 0.0 | 1.2a | 2.6e | 0.4a | 0.7e | + 3.8 | 69.7b |
| 2 | 498 | 2 | 99.6 | 0.4 | 0.3b | 3.7de | 0.1b | 1.1de | + 2.0 | 67.8b |
| 3 | 496 | 4 | 99.1 | 0.9 | 0.3b | 5.1d | 0.1b | 1.4d | + 1.4 | 72.4ab |
| 4 | 491 | 9 | 98.2 | 1.8 | 0.3b | 8.3c | 0.1b | 2.3c | — 0.4 | 72.9ab |
| 5 | 483 | 17 | 96.5 | 3.5 | 0.1b | 16.4b | 0.0b | 4.5b | — 0.5 | 74.3a |
| 6 | 456 | 44 | 91.2 | 8.8 | 0.0b | 38.7a | 0.0b | 10.3a | — 6.2 | 75.5a |
| | | | | | *R. virginicus* | | | | | |
| 1 | 500 | 0 | 100.0 | 0.0 | 0.6a | 4.5d | 0.2a | 1.1d | + 5.1 | 79.0 |
| 2 | 498 | 2 | 99.6 | 0.4 | 0.8a | 4.9cd | 0.2a | 1.3cd | + 3.7 | 77.0 |
| 3 | 496 | 4 | 99.1 | 0.9 | 0.3b | 5.4cd | 0.1b | 1.5cd | + 1.7 | 75.2 |
| 4 | 491 | 9 | 98.2 | 1.8 | 0.3b | 6.5c | 0.1b | 1.7c | — 2.2 | 76.1 |
| 5 | 483 | 17 | 96.5 | 3.5 | 0.0b | 10.5b | 0.0b | 2.7b | — 6.5 | 76.7 |
| 6 | 456 | 44 | 91.2 | 8.8 | 0.0b | 22.8a | 0.0b | 6.3a | — 21.2 | 73.1 |

(1) Mean of the three replicates from each of four colonies. Means in a column followed by the same or not followed by any letter are not significantly different at the $\alpha = 0.05$ level by Student-Newman-Keuls' test.

(2) WO = worker, PS = presoldier, SO = soldier.

treatments 1 to 3 increased after 12 weeks for both species of *Reticulitermes*. The mean number of soldiers in treatments 4 to 6 decreased markedly in *R. virginicus*, but only slightly in *R. flavipes*.

In *R. flavipes* the percentages of soldiers in all treatments increased above their initial percentages ; however, treatments 1 to 3 had not yet reached 1.75 %, the assumed normal percentage of soldiers after 12 weeks (table III). With *R. virginicus*, treatments 1 to 3 had very similar soldier percentages while treatment 4 remained approximately the same as it was initially. The percentage of *R. virginicus* soldiers in treatments 5 and 6 decreased below the initial percentage. For both species the percentage of presoldiers and soldiers (1.1 and 1.3) in treatment 1 after 12 weeks was almost identical to that found in groups of 500 workers reported in table I.

The design of our experiment unfortunately does not allow us to assess whether, at the higher initial soldier percentages (treatments 4 to 6), production of new soldiers was completely suppressed, or rather, new soldiers were produced and new or old soldiers subsequently eliminated. What is clear, however, is that the termites did not initiate eliminations of soldiers extensive enough to bring their overall proportion down to a « normal » level. Such eliminations have been suggested as a mechanism for social homeostasis for other termites (STUART, 1972).

Contrary to the results with *Coptotermes formosanus* (HAVERTY, 1979a), overloading experimental groups of *R. flavipes* or *R. virginicus* with an abnormally high percentage of soldiers (5 times normal) did not adversely affect group survival (table III). With *C. formosanus*, which has a normal soldier percentage of 24.0 (HAVERTY, 1979a), a three-fold overload of soldiers means that 28 percent of the population (workers or pseudergates) suddenly must feed 72 percent of the population, whereas with the two *Reticulitermes* species the largest proportion of soldiers was 8.8 percent of the total. If one considers a normal *Reticulitermes* colony, when numerous larvae and nymphs are present, the workers are probably caring for more than 15 percent of the total population. Therefore, the trophic load placed on *R. flavipes* and *R. virginicus* in this study is nowhere near the trophic load that was placed on *C. formosanus* workers (HAVERTY, 1979a), and indeed may fall within the normal range for these *Reticulitermes* species.

It appears that although *R. flavipes* and *R. virginicus* do have an optimal percentage of soldiers (*ca.* 1.8-2.0 %), groups are able to tolerate much higher percentages without adverse effects on group survival, at least over a 12-week period. This suggests that if termite colonies are to be controlled by man by increasing their soldier percentage above that which they can feed, the percentage forced upon the colony needs to be well above that which is five times « normal » for either of these *Reticulitermes* species. Thus we would agree with LENZ (1976) that control of termites simply through the production of excess soldiers, and the subsequent breakdown of normal caste proportions,

may prove difficult unless the percentage of individuals differentiating to the soldier caste is extremely high-perhaps greater than 50 %. But, as our earlier work demonstrates (HOWARD and HAVERTY, 1978 ; HAVERTY and HOWARD, 1979), insect growth regulators, such as methoprene, not only increase soldier production, but also eliminate the termites' protozoan symbiotes. This additional burden might well allow for successful control, even though soldier proportions do not reach levels of 50 % or greater. Research is in progress to test this hypothesis.

## References

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

HAVERTY M. I., 1979b. — Selection of tunneling substrates for laboratory studies with three subterranean termite species. *Sociobiology, 4,* 315-320.

HAVERTY M. I., HOWARD R, W., 1979. — Effects of insect growth regulators in subterranean termites : induction of differentiation, defaunation, and starvation. *Ann. Entomol. Soc. Am., 12,* 503-508.

HOWARD R. W., HAVERTY M. I., 1978. — Defaunation, mortality and soldier differentiation : concentration effects of methoprene in a termite. *Sociobiology, 3,* 73-77.

LENZ M., 1976. — The dependence of hormone effects in termite caste determination on external factors. *In* Phase and caste determination in insects : Endocrine aspects, 73-89. Edited by M. LUSCHER, *Pergamon Press,* New York.

STUART A. M., 1972. — Behavioral regulatory mechanisms in the social homeostasis of termites (Isoptera). *Am. Zoologist, 12,* 589-594.

WEESNER F. M., 1970. — Termites of the Nearctic Region. *In* Biology of Termites, Vol. II, 477-525. Edited by K. KRISHNA and F. M. WEESNER, *Acad. Press,* New York and London.