Habituation in Termite Orientation Response to Fungal Semiochemicals

by

J.K. Grace[1]

ABSTRACT

Evidence is presented of behavioral habituation by *Reticulitermes flavipes* workers to a dichloromethane extract of wood decayed by *Gloeophyllum trabeum*. In a series of two-choice orientation assays, termite response to paper disks treated with *G. trabeum* extract decreased as the length of the exposure period increased. Subsequent assays indicated that this response decrement was not due to loss of the semiochemicals. Termites conditioned by prior exposure to the fungal extract did not demonstrate a positive orientation response.

INTRODUCTION

Wood infected by the brown-rot decay fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. (Basidiomycetes: Polyporaceae) contains (Z,Z,E)-3,6,8-dodecatrien-1-ol (Matsumura *et al.* 1969) and other semiochemicals (Ritter & Coenen-Saraber 1969) that elicit positive orientation (including trail-following) and arrestment responses in *Reticulitermes* spp. (Isoptera: Rhinotermitidae) (Esenther *et al.* 1961; Allen *et al.* 1964). Observations of these positive behavioral responses to fungal extracts inspired the bait-block method of termite control (Esenther & Coppel 1964), and subsequent investigations of toxic analogues of the dodecatrienol (Carvalho & Prestwich 1984) and other potential bait toxicants.

In this paper, I report evidence of behavioral habituation in *Reticulitermes flavipes* (Kollar) workers as the result of exposure to a dichloromethane extract of *G. trabeum*. Habituation, the simplest form of learning, refers to a decrease in response to a stimulus occurring as the result of repeated or continuous expo-

[1]Faculty of Forestry, University of Toronto, Toronto, Ontario, M5S 1A1, CANADA

sure to that stimulus (Hinde 1970; Manning 1972). The response decrement is generally associated with central nervous system activity, as opposed to sensory adaptation (e.g., saturation of pheromone receptor sites) or muscular fatigue. The results described here suggest that care must be taken in designing and interpreting bioassays with termite "attractants" and that learning behavior is a potential consideration in the application of semiochemicals in termite control.

MATERIALS AND METHODS

Termites. Eastern subterranean termites, *R. flavipes*, were collected in summer and fall 1988, in rolled corrugated cardboard within short lengths of plastic pipe placed on top of an infested maple stump and in the surrounding soil at a site in the City of Scarborough, Ontario (Grace 1989). Termites were maintained in plastic boxes in an unlighted growth chamber (27±0.5°C, 95±5% RH) for 4–6 months prior to the bioassays.

Fungal extract. Red pine, *Pinus resinosa* Ait., stakes decayed for 6–8 weeks after inoculation with *G. trabeum*, in covered trays under laboratory conditions, were supplied by E.E. Doyle and K. Seifert, Forintek Canada Corp., Ottawa, Ontario. Ten grams of decayed wood, ground in a Wiley mill (40-mesh screen), were shaken in 100ml dichloromethane for 15 minutes at room temperature (24°C), and then gravity-filtered through Whatman No. 1 filter paper to yield approx. 70ml of filtrate.

Unconditioned orientation assays – 20minute aeration. Orientation assays using both individual *R. flavipes* workers, older than the third instar, and groups of ten workers were designed after those described by Grace *et al.* (1989). In both types of assays, 100 microliters of the dichloromethane *G. trabeum* extract was applied by pipette to a 23mm Whatman No. 3 filter paper disk. This disk was paired with a solvent-treated disk in a 5cm diameter glass Petri dish, and aired for 20 minutes to evaporate the solvent. In the individual orientation assays, a single worker was then placed in the Petri dish, between the two paper disks located on either side of the dish, and its position recorded every 30 seconds for 20 minutes (a total of 40 observations). Fifty individuals were tested, using a new worker, new paper disks, and a clean dish for each assay. The proportion of the 50 individuals in contact

with an extract-treated paper and the proportion in contact with a solvent-treated paper at each 30-second observation were compared over each five minute interval (n = 10 observations per five minute interval) with a paired-comparisons *t* test (PROC MEANS), $\alpha \leq 0.05$ (SAS Institute 1987a).

The group orientation assays were prepared in the same manner as those with individual workers, except that ten workers were placed in the Petri dish and the number in contact with the extract-treated and the solvent-treated papers then recorded every 30 seconds over the 20-minute assay period. This assay was repeated with 20 groups of ten *R. flavipes* workers. Observations were pooled for analysis over each five minute interval (n=200 observations per five minute interval) and proportions compared with a paired-comparisons *t* test (PROC MEANS), $\alpha \leq 0.05$ (SAS Institute 1987a).

To test the assumption under the null hypothesis of equal distribution of termite workers on equivalent filter paper disks, individual and group assays were also performed in which both papers in each petri dish were treated only with solvent. Proportions were compared as described above.

Unconditioned orientation assays - 45 minute aeration. A second series of individual and group assays was performed to determine whether the decrease in termite response observed during the 20-minute orientation assays was due to semiochemical evaporation or degradation. Treated paper disks were aired 45 minutes, rather than 20 minutes, before each of these assays, and results analyzed as described above.

Conditioned orientation assays. As a test of the habituation hypothesis, a series of individual orientation assays was performed to evaluate the responses of "experienced" versus "unexperienced" *R. flavipes* workers to the *G. trabeum* extract. The individual assays were performed as described above, with the extract-treated and solvent-treated paper disks aired 20 minutes before the assay to evaporate the solvent. Prior to the assay, each "experienced" worker was conditioned by exposure to the same assay conditions (an extract-treated and a solvent-treated disk in a Petri dish) for 30 minutes, while each "unexperienced" worker was conditioned by exposure to a control assay situation (two solvent-treated disks) for the same 30-minute period. Following the conditioning period, the termite worker was gently tipped out

of the conditioning petri dish, and into the assay dish. Fifty "experienced" and fifty "unexperienced" workers were assayed, and the proportions of each test population in contact with the two disks over each five minute interval of a 10-minute assay (n=10 observations per five minute interval) compared with a *t* test (PROC TTEST), $\alpha \leq 0.05$ (SAS Institute 1987b).

RESULTS AND DISCUSSION

In the orientation assays with individual termites and those with groups of ten workers, positive responses to the *G. trabeum* extract-treated papers decreased over the 20-minute assay period (Fig. 1). The same trend of decreasing response was noted

Table 1. Mean (± SEM) percentage of termite test population in contact with *G. trabeum* extract-treated papers (T) air-dried for either 20 or 45 minutes, and with solvent-treated control papers (C) during successive 5 minute intervals of a 20 minute observation period. Treatment percentages indicated by an asterisk (*) are significantly different from the corresponding controls (paired-comparisons *t* test, $\alpha \leq 0.05$).

Drying Time	Assay Interval (minutes)	Individual Assays[a] T (%)	C (%)	Group Assays[b] T (%)	C(%)
20 min	0.5- 5	56±2*	22±2	48±1*	12±1
	5.5-10	35±2*	20±1	34±2*	11±1
	10.5-15	28±2*	20±1	26±2*	11±1
	15.5-20	24±2	23±1	24±1*	8±1
45 min	0.5- 5	53±4*	14±2	43±2*	11±1
	5.5-10	32±2	26±2	27±1*	12±1
	10.5-15	24±2	25±2	19±1*	10±1
	15.5-20	27±2	26±2	17±1*	10±1
Solvent Control[c]	0.5- 5	22±1	23±1	24±1	23±1
	5.5-10	21±1	26±2	18±1	18±1
	10.5-15	24±2	24±2	13±1	16±1
	15.5-20	25±2	20±1	13±1	14±1

[a]Positions of 50 individuals were recorded every 30 seconds. N=10 observations per five minute time interval.
[b]Positions of 20 groups of 10 workers were recorded every 30 seconds. N=200 observations per five minute time interval.
[c]"Treatment" and "Control" papers were equivalent in Solvent Control assays.

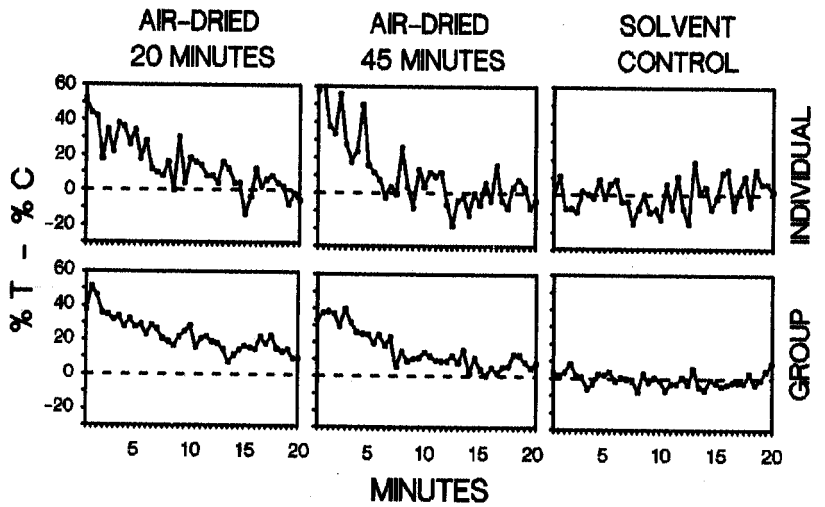

Fig. 1. Difference over a 20 minute period in the percentage of *R. flavipes* workers in contact with *G. trabeum* extract-treated paper disks (%T) dried either 20 or 45 minutes and the percentage in contact with solvent-treated papers (%C) in two-choice behavioral assays. In the "Solvent Control" assays, both disks were treated with solvent only. Each 30 second observation represents either 50 individual assays or the mean of 20 group assays.

whether the treated papers were aired 20 minutes or 45 minutes. The initial positive response to papers aired 45 minutes before assay strongly suggested that the observed response decrement was not caused by evaporation or degradation of the semiochemicals.

The same trend of decreasing response to the fungal extract over time was apparent in both individual and group assays. However, the proportion of termites in contact with extract-treated papers in the group assays remained slightly but significantly greater throughout the 20-minute assay period than the proportion contacting the solvent-treated papers (Table 1). In the individual assays, the differences in proportions were no longer significant by the end of the 20-minute assay. This indicates that habituation did not occur as rapidly in a group context. In behavioral assays with wood extractives, Grace *et al.* (1989) also noted that the results of individual and group assays were complimentary but not identical. Assays designed to track the movements of the individuals comprising the test group, rather than recording the

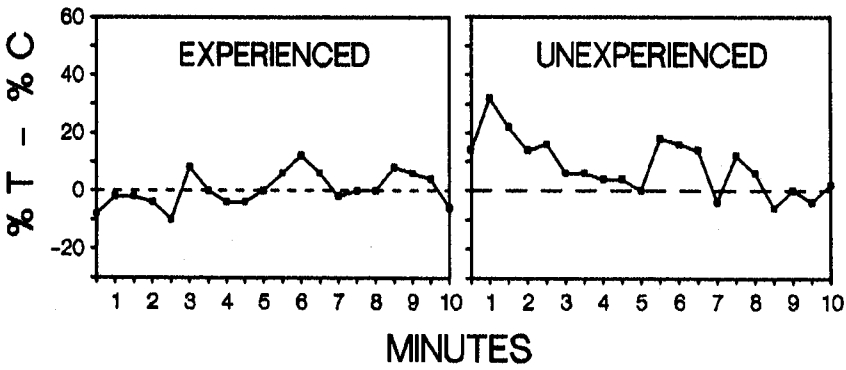

Fig. 2. Difference over a 10 minute period in the percentage of "experienced"and "unexperienced" *R. flavipes* workers in contact with *G. trabeum* extract-treated papers (%T) and solvent-treated papers (%C) in two-choice behavioral assays. "Experienced" workers were conditioned prior to assay by a 30 minute exposure to *G. trabeum* extract under the same assay conditions, while "unexperienced" workers were exposed only to solvent-treated papers during the conditioning period. Each 30 second observation represents 50 individual assays.

"average" position of the entire group, could provide insight into the effects of social situations on individual termite behavior.

When termites were conditioned by exposure to an extract-treated paper for 30 minutes before testing, they did not respond to the *G. trabeum* extract (Fig. 2). Unexperienced termites, conditioned only by exposure to solvent-treated papers, evidenced a significantly greater initial response (*t* test, P=0.001) to the fungal extract than the experienced test group, supporting the hypothesis of behavioral habituation to the extract. The response of the unexperienced workers decreased rapidly, however, and differences in the responses of the two groups did not differ significantly after the first five minute assay interval, raising the possibility of adverse physiological effects (effector fatigue, or desiccation) from an extended assay under laboratory conditions (24 °C, 15% RH).

Habituation is a simple form of learning, reported even in protozoa (Manning 1972). However, evidence of this limited capacity for learning in termites indicates that care should be taken in the design and interpretation of behavioral assays. As a form of behavioral resistance, habituation to arrestants or attractants may also be a consideration in developing behaviorally-based methods of subterranean termite control.

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