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EFFECT OF ANTIOXIDANTS ON EASTERN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) ORIENTATION TO FUNGAL EXTRACT

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Abstract.—Dichloromethane and cyclohexane extracts of wood decayed by *Gloeophyllum trabeum* induce trail-following, arrestment, and/or aggregation of *Reticulitermes flavipes* in behavioral bioassays. In trail-following assays with *R. flavipes* workers, addition of the antioxidant BHA completely suppressed termite response to the fungal extracts. Addition of the antioxidant BHT did not eliminate termite responses to the extracts, but concentration-dependent repellency was noted in orientation (preference) assays with individual termites and groups of workers. As measured by *R. flavipes* behavioral response, addition of BHT at the concentrations tested did not increase the longevity of the active semiochemicals in *G. trabeum* extracts.

Key Words: *Reticulitermes,* termite behavior, *Gloeophyllum,* decay fungus, semiochemicals

Wood decayed by the fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. (Basidiomycetes: Polyporaceae) contains the compound (*Z,Z,E*)-3,6,8-dodecatrien-1-ol (Matsumura et al. 1969) and other unidentified chemicals (Watanabe and Casida 1963, Ritter and Coenen-Saraber 1969) that affect the orientation behavior of subterranean termites (Isoptera: Rhinotermitidae). Solvent extracts of wood decayed by *G. trabeum* and other decay fungi (Grace and Wilcox 1988) elicit both trail-following and aggregation in *Reticulitermes* species (Esenther et al. 1961, Allen et al. 1964, Grace 1989b).

Currently, subterranean termites are excluded from buildings by the injection of large quantities of insecticides into the surrounding soil. An alternative approach is the development of toxic baits employing decayed wood to contaminate foraging termites and eradicate the colony through trophallaxis and grooming behavior (Esenther and Beal 1979). Natural or synthetic chemical termite "attractants" would offer more flexibility than decayed wood in developing such baits, and toxic analogues of dodecatrienol have been investigated by Carvalho and Prestwich (1984). In addition, subterranean termites are able to follow a chemical gradient (Clement et al. 1988, Grace et al. 1988), and compounds aggregating foragers might prove useful in enhancing the efficacy of pesticides applied to the soil for termite control.

The study reported here was undertaken to determine whether addition of the antioxidants BHA and BHT to solvent extracts of wood decayed by *G. trabeum* could increase the longevity of the compounds inducting a positive orientation response in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). These antioxidants

Table 1. Mean (±SE) distance traveled by *Reticulitermes flavipes* workers on artificial trails drawn with solvent (controls) and with dichloromethane extracts of *Gloeophyllum trabeum* decayed red pine containing the antioxidants BHT and BHA. Trails were air-dried 15 minutes, and each mean represents 25 individual assays. Means followed by the same letter are not significantly different at the 0.05 level (ANOVA, REGW multiple F test).

Treatment	Mean Distance (mm)
G. trabeum	35 ± 8b
G. t. + BHT (1 mg/ml)	48 ± 9ab
G. t. + BHT (10 mg/ml)	66 ± 12a
G. t. + BHA (1 mg/ml)	2 ± 1c
G. t. + BHA (10 mg/ml)	1 ± 1c
Dichloromethane Control	3 ± 1c
D.C. + BHT (1 mg/ml)	0 ± 0c
D.C. + BHT (10 mg/ml)	2 ± 1c
D.C. + BHA (1 mg/ml)	1 ± 1c
D.C. + BHA (10 mg/ml)	4 ± 3c

have been found to protect some semiochemicals with internal conjugated dienes from both oxidation and isomerization (Ideses and Shani 1988, Shani and Klug 1980) and thereby enhance their field life in pest management applications.

Methods

Termites.—Foraging eastern subterranean termites, *R. flavipes,* were collected from corrugated paper rolled within short lengths of plastic pipe buried just below the soil surface at a site in the city of Scarborough, Ontario (Grace 1989a). Prior to their use in bioassays, termites were maintained on corrugated paper and filter paper in plastic boxes in an unlighted incubator (27 ± 0.5°C, 90 ± 5% RH).

Fungus extracts.—Red pine, *Pinus resinosa* Ait., decayed for 6–8 weeks after inoculation with *G. trabeum* was provided by E. E. Doyle and K. Seifert, Forintek Canada Corp., Ottawa, Ontario. Ten grams of decayed wood, ground in a Wiley mill to pass a 40-mesh screen, were shaken in 100 ml dichloromethane or cyclohexane for 15 minutes at room temperature (24°C), and gravity-filtered through Whatman No. 1 filter paper to yield approximately 70 ml of filtrate. The antioxidants 3(2)-*tert*-butyl-4-hydroxyanoisole (BHA) and 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene, BHT) were purchased from Sigma Chemical Co., St. Louis, MO.

Trail-following assay.—Three bioassays were used to test the effects of *G. trabeum* extracts containing either 1, 10, or 100 mg/ml BHA or BHT on *R. flavipes* orientation behavior. In the trail-following assay, described by Grace and Wilcox (1988), a straight 20-cm line was drawn on tracing paper with 4 μl of solution, applied by a microliter syringe. This artificial trail was

Table 2. Mean (±SE) distance traveled by *Reticulitermes flavipes* workers on artificial trails drawn with dichloromethane and cyclohexane extracts of *Gloeophyllum trabeum* decayed red pine containing BHT. Trails were air-dried for different time intervals, and each mean represents 25 individual assays. Treatment means with the same solvent in each column followed by the same letter are not significantly different at the 0.05 level (*t* test, or ANOVA and REGW multiple F test).

		Trail Aeration Time (minutes)						
Solvent	Treatment	15	45	60	75	105	120	135
CH_2Cl_2	*G. trabeum*	47 ± 11a	30 ± 6a		17 ± 5a	15 ± 5a		10 ± 3a
	G. t. + BHT (1 mg/ml)	45 ± 10a	30 ± 8a		23 ± 4a	15 ± 4a		10 ± 3a
	G. t. + BHT (10 mg/ml)	32 ± 10a	48 ± 8a		8 ± 3b	13 ± 3a		9 ± 3a
C_6H_{12}	*G. trabeum*	18 ± 5a		15 ± 3b			11 ± 3a	
	G. t. + BHT (1 mg/ml)	22 ± 7a		19 ± 5a			6 ± 1b	

Table 3. Mean (±SE) number of *Reticulitermes flavipes* workers in contact with paper disks treated with dichloromethane extracts of decayed red pine containing BHT, during successive five-minute intervals. The positions of 50 workers, tested individually in separate petri dishes, were recorded every 30 seconds, with each mean representing 10 successive 30-second observations. Means within each column followed by the same letter are not significantly different at the 0.05 level (ANOVA, REGW multiple F test).

Treatment	Number of Termites on Treated Papers (Individual Assays)			
	0–5 min	5.5–10 min	10.5–15 min	15.5–20 min
G. trabeum	28 ± 1a	17 ± 1a	14 ± 1a	12 ± 1a
G. t. + BHT (1 mg/ml)	12 ± 1b	12 ± 1bc	7 ± 1c	7 ± 1b
G. t. + BHT (10 mg/ml)	23 ± 1b	14 ± 1abc	10 ± 1b	7 ± 1b
G. t. + BHT (100 mg/ml)	17 ± 1c	14 ± 1ab	10 ± 1b	9 ± 1b
Dichloromethane Control	11 ± 1d	11 ± 1c	12 ± 1ab	12 ± 1a

air-dried from 15 to 135 minutes, and an *R. flavipes* worker (pseudergate older than the third instar as determined by size) placed at one end. The forward distance traveled on the trail in 30 seconds by the worker was recorded, and the distance traveled by 25 workers on 25 such trails per treatment compared by *t* test, or analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch (REGW) multiple F test (SAS Institute 1987).

Orientation assays.—In addition to the trail-following assays, orientation assays using both individual workers and groups of ten workers were designed after those described by Grace (1989b) and Grace et al. (1989). In both individual and group assays, 100 μl of *G. trabeum* extract was applied by pipette to a 23 mm Whatman No. 3 filter paper disk. This disk was paired with a solvent-treated disk in a 5-cm diameter glass petri disk, and aired 15 minutes to evaporate the solvent. Either an individual termite worker or a group of ten workers was then placed in the dish, and their positions recorded every 30 seconds for 20 minutes.

In the individual assays, 50 workers were tested independently in separate petri dishes. The number of individuals in each treatment in contact with an extract-treated paper at each 30-second observation were compared for each five-minute interval (n = 10 observations per five-minute interval) by ANOVA and the REGW multiple F test (SAS Institute 1987). A series of control assays was included in which one of two equivalent solvent-treated disks was arbitrarily designated the "treatment" disk.

In the group orientation assays, 20 groups of ten workers were evaluated with each treatment. The 30-second observations were pooled over each five-minute interval (n =

Table 4. Mean (±SE) number of *Reticulitermes flavipes* workers in group assays in contact with paper disks treated with dichloromethane extracts of decayed red pine containing BHT, during successive five-minute intervals. The positions of 20 groups of 10 workers were recorded every 30 seconds and pooled for analysis, with each mean representing 200 observations. Means within each column followed by the same letter are not significantly different at the 0.05 level (ANOVA, REGW multiple F test).

Treatment	Number of Termites on Treated Papers (Group Assays)			
	0–5 min	5.5–10 min	10.5–15 min	15.5–20 min
G. trabeum	3.1 ± 0.1a	3.4 ± 0.2a	2.6 ± 0.2a	2.4 ± 0.1a
G. t. + BHT (1 mg/ml)	3.5 ± 0.1b	2.8 ± 0.1b	1.8 ± 0.1b	1.3 ± 0.1b
G. t. + BHT (10 mg/ml)	3.1 ± 0.1b	3.2 ± 0.1a	2.0 ± 0.1b	1.3 ± 0.1b
G. t. + BHT (100 mg/ml)	1.2 ± 0.1d	1.4 ± 0.1c	1.0 ± 0.1c	0.9 ± 0.1c
Dichloromethane Control	2.4 ± 0.1c	1.8 ± 0.1c	1.3 ± 0.1c	1.3 ± 0.1b

Table 5. Mean (±SE) number of *Reticulitermes flavipes* workers in contact with paper disks treated with a cyclohexane extract of decayed red pine or an extract containing 1 mg/ml BHT, during successive 5-minute intervals. Treated papers were aired either 15 or 60 minutes before the assay. The positions of 50 workers, tested individually in separate petri dishes, were recorded every 30 seconds, with each mean representing 10 successive 30-second observations. Treatment pairs (same time interval) within each column followed by an asterisk are significantly different at the 0.05 level (*t* test).

Time Interval	Treatment	Paper Aeration Time	
		15 min	60 min
0.5–5 min	*G. trabeum*	26 ± 2*	19 ± 1
	G. t. + BHT	20 ± 1*	20 ± 1
5.5–10 min	*G. trabeum*	18 ± 1*	15 ± 1
	G. t. + BHT	12 ± 1*	15 ± 1
10.5–15 min	*G. trabeum*	13 ± 1*	14 ± 1*
	G. t. + BHT	8 ± 1*	10 ± 1*
15.5–20 min	*G. trabeum*	15 ± 1*	14 ± 1*
	G. t. + BHT	6 ± 1*	8 ± 1*

200 observations per five-minute interval), and the mean numbers in contact with the extract-treated papers compared by ANOVA and the REGW multiple F test (SAS Institute 1987).

Both individual and group orientation assays, in which the test papers were air-dried 15 minutes, were performed with the dichloromethane *G. trabeum* extracts, containing either 0, 1, 10, or 100 mg/ml BHT. Cyclohexane *G. trabeum* extracts containing 0 or 1 mg/ml BHT were compared in individual assays in which the test papers were aired either 15 or 60 minutes. This latter procedure was adopted to preclude termite behavioral habituation to the test extracts (Grace 1989b) concealing differences between them.

Results and Discussion

Neither BHA nor BHT alone elicited trail-following by *R. flavipes,* and addition of BHA to the dichloromethane extract of wood decayed by *G. trabeum* completely suppressed trail-following (Table 1). Thus, BHA was not included in the individual and group orientation assays. With short aeration periods, addition of BHT did not affect the trail-following activity of the fungus extract (Table 2). Neither were any consistent effects on trail-following activity apparent with longer aeration periods. Addition of BHT did not arrest the observed decrease in extract trail-following activity with trail aeration time.

In the individual (Table 3) and group (Table 4) orientation assays, the BHT fortified extracts exhibited less activity than the dichloromethane *G. trabeum* extract, and decreased in activity over time. In several instances, the activity of the BHT extracts, particularly that containing 100 mg/ml BHT, fell below that of the solvent control, suggesting concentration-dependent repellency.

Results of the individual orientation assays with the cyclohexane extract (Table 5) were comparable to those obtained in the dichloromethane solvent system. Whether the test papers were aired 15 or 60 minutes, addition of 1 mg/ml BHT either made no difference in comparison to the activity of the stock *G. trabeum* extract, or resulted in a significant decrease in positive responses to the extract.

At the concentrations tested here, addition of BHA to *G. trabeum* extract resulted in a complete loss of behavioral activity towards *R. flavipes,* while BHT slightly decreased activity. Neither antioxidant appeared to provide protection to the fungal semiochemicals nor extend their longevity. However, the concentrations of behaviorally active compounds in the *G. trabeum* extracts were not determined, and very low concentrations of semiochemicals can elicit activity. More encouraging results might therefore be obtained with lower concentrations of antioxidants. This study indicates that BHT, which demonstrated a concentration-dependent repellency rather than complete suppression of activity, is the more

promising additive for use in such investigations.

Although studies with isomers and analogs of the identified dodecatrienol (Prestwich et al. 1984 and included citations) have demonstrated that the (*Z,Z*)-3,6-alkadien-1-ol functionality is more important than the conjugated 6,8-diene in eliciting trail-following, 6,8-dodecadien-1-ol also shows activity with *Reticulitermes* (J. K. Grace and M. Kim, unpublished results). Chemical protection of the identified semiochemical in *G. trabeum,* as well as other unidentified biologically active compounds in the solvent extracts, thus deserves further study as an approach to extending the field life of these potentially useful extracts.

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