28. GRACE, J.K., J. IRIAH and M.H.ZOBERI - **Evaluation of the use of termite attractants to synergize soil pesticide applications in structural pest control.**

The eastern subterranean termite, Reticulitermes flavipes (Kollar), is a serious pest of structures in southern Ontario, and is controlled by the application of pesticides to the soil. The purpose of this study is to evaluate the potential for integrating behaviour-modifying chemicals with soil pesticides to reduce the amount of pesticides that must be applied for control. Objectives for the first year are to (1) develop termite collection and behavioural bioassay techniques, (2) identify plants or fungi which may attract or aggregate foraging termites, and (3) extract and bioassay behaviourally active compounds.

Collection of termites and possible sources of behavioural chemicals began in June 1988 at field sites in Toronto, and Scarborough. Collections were also initiated at Kincardin later in the summer. Initially, white pine stakes (ca. 1.5 x 4 x 15 cm), each sheathed in a layer of moistened corrugated paper to aggregate termites, were placed throughout these sites (1158 in Scarborough, 461 in Toronto). Stakes were monitored at 1-2 week intervals. Where termite feeding was found, the stake was replaced by a collection trap, with adjacent traps installed no closer than two metres. These collection traps allowed nondestructive sampling of termite populations. The traps consisted of two 15 cm lengths of 4 cm ID plastic (ABS) pipe containing rolled corrugated paper, both placed within a 15 cm length of 10 cm ID pipe. This larger pipe was buried vertically just below the soil surface and the top capped. The outer pipe thus represented a permanent trap installation, while the two inner pipes could be readily removed and replaced.

Collection traps contained as many as 7,622 termites. These were maintained on corrugated paper and filter paper (Whatman No. 1) in the laboratory in plastic containers within an unlighted temperature ( 27 +/- 0.5$^{o}$ C) and humidity (90 +/- 5% RH) controlled cabinet. Termites, soil carried into the traps by termites, the paper in the traps, and wood at the field sites were cultured on the following natural media: malt extract agar, potato dextrose agar, nutrient agar, bacto yeast malt extract agar, starch agar, agar agar, cellulose agar, and filter paper malt extract agar.

Wood decayed by the brown-rot decay fungus Gloeophylum trabeum (Pers. ex

Fr.) Murr is reported to produce compounds, including (cis, cis, trans) 3,6,8-dodecatrien-1-ol, that aggregate subterranean termites. In early fall, E.E. Doyle and K. Seifert of Forintek Canada Corp. (Eastern Division, Ottawa) supplied us with red pine stakes decayed by 6 weeks exposure to G. trabeum. This wood was stored at 4° C prior to extraction with various solvents. Aqueous, hexane, and dichloromethane extracts were prepared by shaking 5 g wood shavings (40-mesh) in 50 ml solvent for 15 minutes, both with heat (50° C) and at room temperature (23° C), and filtering. Three bioassays were used to evaluate these extracts: (1) a straight-line trail-following assay, (2) an assay for attractance/repellence of individual termite workers, and (3) an assay for attractance/repellence of groups of ten termite workers.

In the trail-following assay, a straight 200 mm artificial trail is drawn on tracing paper with a syringe containing 4 microliters of the test solution. A single termite worker is placed on one end of the trail, and the distance traveled in 30 seconds recorded. This assay is repeated with 25 workers per treatment. In the attractance/repellence assays, 50 microliters of solution is applied to a 2.3 cm dia. Whatman No. 1 filter paper circle. This paper is aerated for 15 min. and paired with a solvent-treated control paper in a 5 cm dia. petri dish. Either an individual R. flavipes worker or a group of ten workers is placed in the dish and their positions recorded every 30 sec. for 20 minutes. Fifty individuals are tested in the individual assays, and 20 replicates in the group assays.

Table 1 lists genera of fungi isolated from field-collected materials. Species identifications are in progress, and solvent extractions and laboratory bioassays with selected isolates will follow.

Preliminary bioassays with the aqueous, hexane, and dichloromethane extracts of decayed red pine indicated that all were active in inducing trail-following behaviour. However, a time-series with the aqueous extract indicated that the orientation component was lost fairly rapidly (Table 2). This was consistent with the results of the attractance/repellence assays. As growth of microorganisms was rapid in the aqueous extract, we are currently concentrating on dichloromethane extracts.

Addition of antioxidants has been shown to prolong the field life of insect pheromones. Although the antioxidant BHA inactivated the dichloromethane decayed-wood extract, preliminary time-series assays with BHT added to the extract (1 mg/ml and 10 mg/l) were promising (Table 3). In the coming months we will continue work on approaches to stabilizing the active compound(s). We are also developing laboratory assays using a soil matrix to more closely approximate field conditions. In anticipation of the second year of this project, we have arranged to obtain samples of three pesticides currently used in soil treatments in North America (chlorpyrifos, isofenphos, and disodium octaborate tetrahydrate) to assay in combination with the attractant extracts.

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**Table 1.** Fungi isolated from termites, termite-infested wood, paper from collection traps containing termites, and soil carried into pipes within collection traps by termites.

| Genera | No. of species |
|---|---|
| Mucorales | |
| *Mucor* | 5 |
| *Cunninhamella* | 1 |
| *Absidia* | 1 |
| *Pirella* | 1 |
| *Rhizopus* | 1 |
| *Circinella* | 2 |
| *Actinomucor* | 1 |
| Hyphomycetes | |
| *Aspergillus* | 4 |
| *Penicillium* | 2 |
| *Trichoderma* | 3 |
| *Cephalosporium* | 1 |
| *Alternaria* | 1 |
| *Arthrobothrys* | 1 |
| *Gliomastix* | 1 |
| Unidentified | 3 |
| Dictyosteliales | |
| *Dictyostelium* | 1 |
| Actinomycetes | |
| Unidentified | 1 |
| Unidentified | 3 |

**Table 2.** Mean distances traveled by 25 *R. flavipes* workers on 200 mm artificial trails drawn with 4 µl of an aqueous extract of *G. trabeum* decayed pine.

| Aeration Time | Distance (mm ± SD) |
|---|---|
| 0.5 min | 160.56 ± 47.56 |
| 15.0 | 97.24 ± 76.93 |
| 30.0 | 58.76 ± 52.99 |
| 45.0 | 30.44 ± 35.20 |
| 60.0 | 41.44 ± 38.71 |
| 24 hours | 3.56 ± 5.54 |
| Solvent Control | 8.52 ± 12.05 |

**Table 3.** Mean distances traveled by 25 *R. flavipes* workers on 200 mm artificial trails drawn with 4 µl of a dichloromethane extract of *G. trabeum* decayed red pine.

| | Distance at Different Aeration Times (mm ± SD) | | |
|---|---|---|---|
| Treatment | 15 min | 45 min | 75 min |
| extract | 47.48±57.39 | 30.08±30.78 | 17.04±27.20 |
| extract + 1mg/ml BHT | 44.88±47.66 | 30.20±40.45 | 23.12±19.16 |
| extract + 10mg/ml BHT | 32.36±47.47 | 48.20±40.70 | 8.12±12.62 |
| extract + 1mg/ml BHA | 2.48± 6.25 | | |
| extract + 10mg/ml BHA | 0.00± 0.00 | | |
| solvent control | 3.12± 4.38 | | |
| solvent + 1mg/ml BHT | 0.00± 0.00 | | |
| solvent + 10mg/ml BHT | 1.52± 3.79 | | |
| solvent + 1mg/ml BHA | 0.72± 3.60 | | |
| solvent + 10mg/ml BHA | 4.40±16.09 | | |

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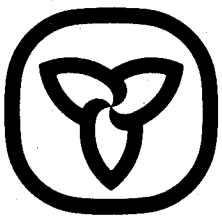

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