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Role of (3*Z*,6*Z*,8*E*)-Dodecatrien-1-ol in Trail Following, Feeding, and Mating Behavior of *Reticulitermes hesperus*

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Abstract Trail pheromones mediate communication among western subterranean termites, *Reticulitermes hesperus* Banks. Repetitive passages of ≥28 termites were required to establish a pheromone trail and trails needed to be reinforced because they lasted <48 hr. The minimal threshold concentration for inducing responses from termite workers and secondary reproductives was between 0.01 and 0.1 fg/cm of (3*Z*,6*Z*,8*E*)-dodecatrien-1-ol (henceforth, dodecatrienol). Workers showed optimal trail-following behavior to dodecatrienol at a concentration of 10 fg/cm. Trails with concentrations >10 pg/cm were repellent to workers. Workers did not detect pheromone gradients, responding equally to increasing or decreasing gradients of dodecatrienol, and termite workers were not able to differentiate between different concentrations of dodecatrienol. Termites preferred dodecatrienol trails to 2-phenoxyethanol trails. Antennae played a key role in trail pheromone perception. Dodecatrienol acted as an arrestant for worker termites (10 fg/cm^2) and male alates (5 ng/cm^2), whereas sternal gland extracts from females attracted male alates. Workers and alates, upon contact with filter paper disks treated with higher doses (10 fg/cm^2 and 5 ng/cm^2, respectively) of dodecatrienol, were highly excited (increased antennation and palpation) and repeatedly returned to the treated disks. Dodecatrienol did not act as a phagostimulant when offered on a paper towel disk. *Reticulitermes hesperus* is highly responsive to dodecatrienol, and it may play an important role in orientation of workers and alates.

Keywords Antennae · Arrestant · (3*Z*,6*Z*,8*E*)-Dodecatrien-1-ol · Phagostimulant · Trail pheromone

Introduction

Semiochemicals play important roles in termite colonies, where they mediate caste regulation, reproduction, nestmate recognition, and behaviors associated with defense and foraging (Prestwich, 1988; Pasteels and Bordereau, 1998). In the subterranean termites *Reticulitermes*

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virginicus Banks, *Reticulitermes lucifugus grassei* Clement, *Reticulitermes speratus* Kolbe, *Reticulitermes santonensis* Feytaud, and *Coptotermes formosanus* Shiraki, the trail pheromone or at least one of its components has been identified as (3*Z*,6*Z*,8*E*)-dodecatrien-1-ol (henceforth, dodecatrienol) (Matsumura et al., 1968, 1969; Honda et al., 1975; Laduguie et al., 1994a; Tokoro et al., 1994; Wobst et al., 1999). In addition, wood degraded by the fungus *Gloeophyllum trabeum* Persoon is reported to be attractive to subterranean termites (Watanabe and Casida, 1963; Matsumura et al., 1969; Rust et al., 1996; Su, 2005), and extracts of the infested wood were found to contain dodecatrienol (Matsumura et al., 1969). Termites other than *Reticulitermes* spp. display trail-following behavior in response to dodecatrienol (Matsumura et al., 1972; Howard et al., 1976; Yamaoka et al., 1987; Tokoro et al., 1989, 1994; Bordereau et al., 1991; Laduguie et al., 1994a, b; Batista-Pereira et al., 2004) and its aromatic analogs (Tai et al., 1971; Prestwich et al., 1984). *Coptotermes formosanus* and *Reticulitermes* spp. also exhibit trail-following behavior toward 2-phenoxyethanol (Chen et al., 1998), a component in ballpoint pen ink. However, the importance of this compound to termites in their natural habitats remains unknown.

After fractionation of extracts of the fourth and fifth sternites of *Reticulitermes hesperus* workers, Grace et al. (1995) demonstrated that a single major component was responsible both for induction of trail following and for orientation of termites on the trail. Studies suggest that other minor components in trail pheromones may lead to qualitative and quantitative differences in the actual trail laid down by worker termites (Howard et al., 1976; Kaib et al., 1982; Runcie, 1987; Tokoro et al., 1994; Cornelius and Bland, 2001; Arab et al., 2004). It has been suggested that time, number of termites laying the trail, and presence or absence of a food source also has an effect on trail quality and longevity (Runcie, 1987; Cornelius and Bland, 2001). Different aspects of termite trail-following behavior have been studied using trails made from natural pheromone (sternal gland extracts [SGE]), synthetic dodecatrienol, and 2-phenoxyethanol (Traniello, 1982; Grace et al., 1988, 1995; Grace, 1991; Chen et al., 1998; Appel and Hu, 2005; Fe et al., 2005). Minor components might also provide the basis for species- and caste-specific responses (Affolter and Leuthold, 2000; Gessner and Leuthold, 2001), and quantitative differences in the concentration of trail pheromone were proposed for differential response in the recruitment behavior of soldiers and worker termites in *Nasutitermes costalis* Holmgren (Traniello and Busher, 1985).

Trail pheromones have been reported to have secondary roles as sex pheromones in various termite species. In *R. santonensis* Feytaud (Laduguie et al., 1994b) and *Cornitermes bequaerti* Emerson (Bordereau et al., 2002), it has been suggested that dodecatrienol is a component of the alate female's sex pheromone. The analogous alcohol, (3*Z*,6*Z*)-dodecadien-1-ol, has been identified as a sex and trail pheromone in *Ancistrotermes pakistanicus* Ahmad (Robert et al., 2004). So far, dodecatrienol has not been shown to be either a trail or sex pheromone of *R. hesperus*, and we did not detect dodecatrienol by GC/mass spectroscopy (MS) analysis of solid phase microextraction extracts of workers and female alates of *R. hesperus* (unpublished data). However, *R. hesperus* has been shown to be very responsive to dodecatrienol, which has been reported to elicit trail-following behavior at a threshold of 6 fg/cm (Matsumura et al., 1972). Grace (1986) was able to isolate trail pheromone from excised sternites by sequential high-performance liquid chromatography fractionation. Based on a GC and subsequent MS analysis of the active fraction and synthetic standards [(6*E*, 8*Z*- and 6*E*,8*E*)-dodecadien-1-ol], Grace (1986) concluded that an unsaturated dodecatrienol was present in the active fraction. However, its structure could not be elucidated because of the minute quantities. We also were unable to

detect dodecatrienol in solvent extracts of sternal glands of workers by GC-MS analysis, almost certainly because of the minute quantities of dodecatrienol produced by and present in sternal glands. However, previous studies across several species of subterranean termites indicate that dodecatrienol elicits trail-following behavior and is likely a trail pheromone component. Thus, we tested behavioral responses of workers and males to dodecatrienol with the goal of investigating the role of dodecatrienol as a possible pheromonal signal for *R. hesperus*. Our specific objectives were: (1) to study the preferences and minimal thresholds for trails of natural pheromone (SGE), synthetic trail pheromone (dodecatrienol), and 2-phenoxyethanol; (2) to determine whether termites could detect increasing or decreasing gradients of dodecatrienol; (3) to determine the potential role of dodecatrienol as a feeding stimulant and arrestant; and (4) to assess the responses of alate and dealate adults to synthetic dodecatrienol and to SGE from females.

Methods and Materials

Termites

Western subterranean termites, *R. hesperus* Banks, were collected on the University of California, Riverside Campus in polyvinyl chloride (PVC) traps buried in the ground and provisioned with rolls of corrugated cardboard. Termites were gently removed and maintained in the laboratory in a plastic container (43.3×29.9×17.7 cm, Rubbermaid Inc., Wooster, OH, USA) at 24°C and 98% RH. Pieces of brown paper towel (Fort James Corp., Deerfield, IL, USA) were regularly provided as a food source. Undifferentiated larval termites [mean weight 1.6±0.44 mg (N=50)] were used for bioassays (Thorne, 1996). All worker and soldier termites used in bioassays were from the same colony.

Reticulitermes hesperus alates used in the study were collected from the PVC traps before they swarmed and during swarms. The wings of alates collected from the traps 2–3 d before a swarming event were still wet, grayish-white in color, and the alates were unable to fly. Alates collected from active swarms were kept in the dark inside a plastic petri dish provisioned with a moistened brown paper towel to avoid desiccation. Alates were allowed to acclimate for 1 hr, and individuals that shed their wings (dealates) were used for bioassays.

Termites were starved by confining them inside a plastic petri dish (5.0×0.9 cm, Falcon, BD Biosciences, Franklin Lakes, NJ, USA) containing 5 g of sand moistened with 1 ml of deionized water. One hundred termites were transferred to each of five dishes. Termites were held without food for 24, 48, 72, 96, and 120 hr. Control dishes were provisioned with brown paper towel disks (3.0 cm diam.) as a food source. All the petri dishes were transferred to a plastic container and held at 100% RH. Humidity indicator strips (Sude-Cheme Performance Packaging, Colton, CA, USA) were taped to the walls of the container to ensure that RH remained at 100% throughout the studies. To ensure that termites did not feed on dead individuals, we removed dead or moribund termites regularly during the starvation period.

Sternal Gland Extracts

Fifty worker termites were frozen at −20°C for 5 hr. Sternal glands were dissected from the fourth and fifth abdominal sternites under a binocular stereoscopic microscope (Grace et al., 1988). Dissected glands was transferred to a 2-ml amber glass vial with a Teflon-lined cap

(Wheaton, Millville, NJ, USA) kept at 0°C. The combined glands were extracted overnight in 1 ml *n*-hexane. The supernatant was used for bioassays, with 20 µl considered one termite SGE equivalent. Sternal gland extract from the female alates (N=50) was obtained following the same procedure.

Production of Natural Trails

To determine how many termites were required to establish a pheromone trail, individual termites were placed at one end (release end) of a Y-maze (described under "Choice Bioassays") and were allowed to walk on one arm of the maze. At the intersection, a surgical blade was inserted halfway (0.5 mm) inside one of the two Y-arms to ensure that termites walked only on one arm. To test for trail pheromone, the surgical blade was removed and a termite was placed at one end of the Y-maze and allowed to walk up the trail. Thirty termites were tested, recording the arm chosen by each individual. The test was repeated using trails laid by 5, 10, 15, 20, 25, and 30 termites on the treatment arm. The passage of 30 termites generated a significant response. To narrow the range, we repeated the tests by increasing the number of termites in increments of 1 after 25 termites. The procedure was repeated on the other arm of a new Y-maze to avoid any directional biases.

All Y-mazes treated with different treatments and naturally laid trails were stored separately in sealed plastic containers at room temperature to avoid any direct effects of light on the laid trails. The mazes were tested when the trails were 0.1, 24, 48, and 60 hr old.

Straight-line Bioassays

Straight-line bioassays were conducted to determine the concentration (fg/cm) of dodecatrienol to which termites exhibited optimal trail-following responses. Lines were drawn (27 cm long, <1 mm wide) with a 0.5-mm mechanical pencil on a piece of white printer paper (21.6×27.9 cm, Boise™ X-9™, Boise, ID, USA). The line was lightly marked with a pencil 1 cm from each end of the 27-cm line. This 25-cm-long section was used as the walking distance for each bioassay. These lines were drawn very lightly, and preliminary runs were conducted to ensure that termites did not use them as guidelines. A 10-µl syringe (Hamilton Company, Reno, NV, USA) was used to treat these lines (27 cm) with hexane solutions of test treatments at the desired concentration (wt/cm) of a particular treatment.

For each test run, 100 termites were transferred to a plastic petri dish (3.5×1.0 cm) provisioned with a moistened brown paper towel disk. Termites were allowed to acclimate for ~30 min. A small acrylic paintbrush (no. 3/0 Round Red Sable, M. Grumbacher, NJ, USA) was used to gently put a termite at one end of the treated line. A stopwatch was used to record the time taken by termites to reach the other end of the trail. Timing began as soon as the termite's abdomen crossed the marked starting point of the 25-cm treated section of the trail, and ended as soon as it crossed the opposite end point. The number of termites that did not walk to the end of the trail, left the trail, or reversed their direction was recorded. Time recordings of these termites were not included in the rate of movement analysis.

Seven concentrations of dodecatrienol (0.01 fg/cm, 0.1 fg/cm, 1 fg/cm, 10 fg/cm, 100 fg/cm, 1 pg/cm, and 10 pg/cm) were tested with 30 termites per concentration. To ensure that termites did not detect any natural pheromone from a previous run, a new sheet of paper and a dodecatrienol trail were used for every run.

Gradient Bioassays

To determine whether termites responded to increasing or decreasing dodecatrienol concentration gradients, trails were drawn on printer paper as described above. The 25-cm long section was further divided into five equal sections. We selected a range of concentrations between 1 and 10 fg/cm to construct the gradient trail. Each 5-cm section was treated with an increasing concentration of dodecatrienol (1, 2, 5, 8, and 10 fg/cm). A single termite was placed at either the low concentration (increasing gradient) or the high concentration end (decreasing gradient). The termite's walking speed, pauses, and reversals in direction and the number of termites leaving the trail were observed. A new gradient trail was constructed on a new sheet of paper for each run. Thirty termites were tested on increasing and decreasing gradient trails.

Choice Bioassays

To study different aspects of trail-following behavior of termites, natural trails (laid down by worker termites), SGE trails, and artificial trails with dodecatrienol were used. A Y-maze was designed from a cardboard sheet (1 mm thick), with each arm of the Y-maze being 120° apart, 5 cm in length, and 3 mm in width (Fig. 1). All the Y-mazes were cut on a die-cutting machine such that each maze had a well-perforated margin. A no. 7 insect mounting pin (Ward's Natural Science, Rochester, NY, USA) was gently inserted in the Y-maze at the center at the junction of the three arms. The other end of the pin was inserted into a Styrofoam block. The Styrofoam served as a base to hold the Y-maze stable so that the arms were level. A 10-µl syringe was used to treat the surface of one arm with solutions of the desired concentration (wt/cm) of a particular treatment. The other arm of the maze was treated with acetone or hexane only. Three concentrations of SGE (TE = termite equivalent) (0.002, 0.02, and 0.2 TE/cm) and seven concentrations of dodecatrienol (0.01 fg/cm, 0.1 fg/cm, 1 fg/cm, 1 pg/cm, 0.01 ng/cm, 0.1 ng/cm, and 1 ng/cm) were tested. In other studies, different concentrations of dodecatrienol were applied to the two arms of the Y-maze to determine termites' ability to discriminate between different concentrations. Similarly, 2-phenoxyethanol

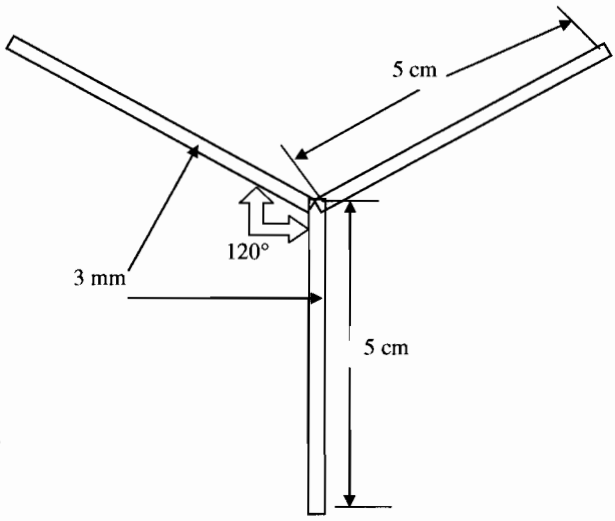

Fig. 1 Diagram of the Y-maze used for choice bioassays. Each arm is 120° apart, 5 cm long, and 3 mm wide

[ethylene glycol phenyl ether, (90+ percent purity), Aldrich Chemical Company, Inc., Milwaukee, WI, USA] trails were laid down using three concentrations (0.02 ng/cm, 0.2 ng/cm, and 10 μg/cm) on one arm and compared with the untreated arm as well as with different dodecatrienol concentrations.

The same acrylic paintbrush described above was used to gently put a termite at the end of one of the untreated arms, and the worker walked up to the intersection and chose between the treated or untreated arm. The run was considered complete when the termite walked to the end of one 5-cm arm. A new termite was used for each run, and 30 runs were made for each test. To control for directional biases, 15 runs were conducted with the left arm treated and 15 runs with the right arm treated. A new Y-maze was used for each run. All bioassays were performed in a room (6.70×3.65×3.35 m) illuminated by four 32-W white fluorescent tubes. We conducted preliminary runs in the absence of light and determined that light did not influence the trail following ability of *R. hesperus* workers (without functional eyes) and male and female dealates.

Detection of Trail Pheromone

To determine how termites detect trail pheromone, termites were tested on a bilevel Y-maze arena. A Y-maze with one arm treated as described above was inverted and rested upon three insect-mounting pins inserted in a Styrofoam sheet so that the Y-maze was level (Fig. 2). The arms of another Y-maze were cut such that each arm measured 4 cm. A no. 7 pin (cut to 3.7 cm in length) was inserted through a cork such that the pointed end was about 2 cm out on the other side. The pin and cork assembly was carefully pressed onto the center of the shorter Y-maze so that it securely held the shorter Y-maze. The shorter Y-maze was put directly under the inverted Y-maze (one arm treated) described above (Fig. 2). The distance between the untreated surface of the arms of the shorter Y-maze and the inverted Y-maze above it was ~1.0 cm. A single termite was placed on the end of the bottom Y-maze. The termite's behaviors were observed when it came to the intersection, specifically looking for any antennal response toward the treated arm above its head. The run was terminated when a termite walked to the end of the 4-cm arm of the shorter Y-maze (Fig. 2). For the series of assays, the distance (4 to 10 mm) between the treated inverted Y-maze and untreated smaller Y-maze surfaces was adjusted by inserting 1-mm-thick disks (2.5 cm diam.) of cardboard under the cork holding the smaller Y-maze.

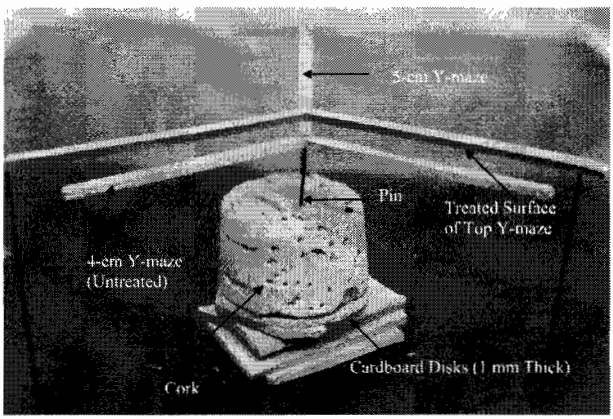

Fig. 2 A bilevel Y-maze used to determine whether the antennae or palpi detected the trail pheromone. One arm of the top Y-maze (each arm 5 cm long) was treated with acetone solutions of dodecatrienol and the other with acetone only. The arms of the shorter Y-maze were not treated. The distance between the top and bottom Y-mazes was adjusted by putting 1-mm-thick cardboard disks under the wooden cork

Arrestant Bioassays

To determine whether dodecatrienol or SGE arrested termite movement, four separate bioassays were conducted with workers and male dealates. The bottom of a plastic petri dish (9.0 cm diam.) was completely covered with a filter paper disk (Whatman no. 1, Whatman International Ltd., Maidstone, UK). For the first bioassay, a small filter paper disk (2.1 cm diam., Whatman no. 1) was treated with 10 µl of a solution of dodecatrienol in acetone (3.46 pg/ml) using a 10-µl syringe (Hamilton). The resulting concentration of dodecatrienol on the treated disk was 10 fg/cm^2. Another filter paper disk (2.1 cm diam.) was treated with 10 µl of acetone only. Both disks were allowed to dry for 5 min under a laminar flow hood and then glued to the bottom of the larger disk (9.0 cm diam.) so that they were 5 cm apart (between their center points). Individual workers were released in the center of the arena. The amount of time each termite (N=15) spent contacting each disk was recorded for 5 min. Their gnawing and palpation behaviors on the treated disk were also observed. New disks were used for each run.

In the second bioassay, the attractiveness of dodecatrienol to male dealates (N=15) was tested. Preliminary bioassays at different concentrations ranging from 10 fg/cm^2 to 10 ng/cm^2 were tested, and 5 ng/cm^2 was selected for a full experiment. The amount of time spent by male dealates contacting each disk was recorded for 5 min. Their palpation, excitement, and gnawing behaviors on treated vs. untreated disks were visually observed. The third bioassay was carried out using SGE from female dealates (1 female alate equivalent) on one disk and hexane (control) on the other disk. Male dealates (N=15) were released in the arena one at a time as described above. The amount of time each male spent contacting each disk was recorded for 5 min. Their palpation, excitement, and gnawing behaviors on treated vs. untreated discs were visually observed. The fourth bioassay was conducted to compare attractiveness of dodecatrienol with that of female SGE, for male dealates (N=15). The amount of time each male spent contacting each disk was recorded for 5 min. Their palpation, excitement, and gnawing behaviors on treated vs. untreated discs were visually observed. Termite workers used in the first bioassay were taken from the lab colony in cohorts of 50 termites, and were transferred to a plastic petri dish (3.5 cm diam.). The disk was provisioned with a moistened brown paper towel disk. Termites were allowed to acclimate for 30 min. Termites were used only once. The alates used were collected from an active swarm. They were immediately transferred to a plastic container provisioned with moistened brown paper towel, and dealates separated by sex were used for bioassays.

Feeding Bioassays

To determine whether dodecatrienol acted as a phagostimulant, workers were tested in a feeding choice bioassay. Small disks of brown paper towel (2.5 cm diam.) were cut in half, and both halves were weighed. One half was designated as treated (T) and the other as control (C). A calculated volume of dodecatrienol solution in acetone was applied to the treated half disk with an Eppendorf micropipetter to provide the desired concentration (pg/cm^2). The untreated half was treated with the same volume of acetone. Both disk halves were dried under a laminar flow hood for 5 min and were then weighed again. Concentrations of 0.01, 0.1, 1, and 10 pg/cm^2 and 1 ng/cm^2 were tested. After drying, treated and untreated half disks were stuck back together with a small strip of Scotch tape (3M®, St. Paul, MN, USA). The intact disk was placed in a plastic petri dish (3.5 cm diam.×1 cm, Falcon, BD Biosciences) with the taped side facing down. All the petri dishes with disks were then transferred to a plastic container (43.3×29.9×17.7 cm) and were maintained at 100% RH.

Forty termites of similar size and weight were transferred to each petri dish, the dishes were covered with lids to keep moisture high, and the dishes were held in the container for 5 d. Appropriate controls were used to check for mortality and amount of feeding on disks treated with acetone only. Five replicates were conducted for each concentration. At day 5, the termites were removed, and the two halves of the disk were separated, cleaned by gently brushing the fecal material and dirt from them, and dried in a laminar flow hood for 24 hr. The half disks were weighed again. Net feeding was calculated after subtracting amount of feeding on control halves from the feeding on treated halves.

Statistical Analyses

Statistical analyses of choice tests on Y-mazes were based on a binomial distribution, using a chi-square test ($P<0.05$) (SAS Institute, 1999). Results of termite feeding and straight-line bioassays were analyzed with the general linear model analysis of variance (ANOVA) procedure (SAS Institute, 1999). Means were separated using Tukey's honestly significant difference (HSD) test for significance at $P<0.05$. Arrestant bioassay data were analyzed by paired *t*-tests.

Synthesis of (3*Z*,6*Z*,8*E*)-Dodecatrien-1-ol (Fig. 3)

Unless otherwise stated, reactions were run under argon atmosphere. Tetrahydrofuran (THF) was dried by distillation from sodium-benzophenone ketyl. Solutions were dried over anhydrous Na_2SO_4 and concentrated with rotary evaporation under reduced pressure, unless otherwise stated. NMR spectra were taken on a Varian INOVA 400 NMR spectrometer, and mass spectra were taken with a Hewlett-Packard 6890 gas chromatograph interfaced to a Hewlett-Packard 5973 mass selective detector, using electron impact ionization at 70 eV. Reaction yields have not been optimized.

(E)-1-Iodo-1-pentene (3) Diisobutylaluminum hydride in hexanes (1 M, 100 ml, 100 mmol) was added dropwise to a solution of 1-pentyne **2** (7.5 g, 110 mmol) in hexanes chilled to −40°C. The resulting mixture was stirred 30 min, then slowly warmed to room temp over 3 hr, and stirred overnight. The mixture was warmed to ~50°C for 4 hr, then cooled to

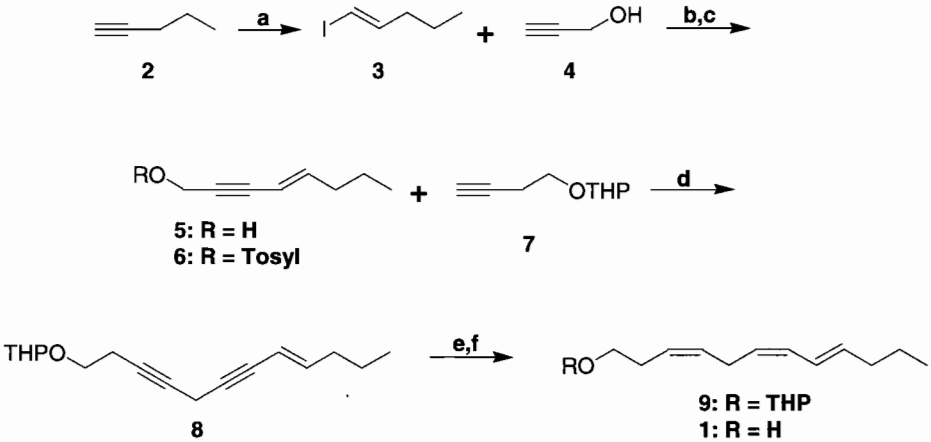

Fig. 3 Synthesis of dodecatrienol

−40°C, and a solution of iodine (25.4 g, 100 mmol) in THF (100 ml) was added dropwise over 30 min. The resulting dark brown suspension was allowed to warm to room temperature with stirring overnight, producing a clear pale yellow solution. The solution was cooled in an ice bath, and slowly quenched by dropwise addition of ice-cold H_2SO_4 (20%, 50 ml) (CAUTION: EXOTHERMIC) with vigorous stirring. After stirring for 30 min, the layers were separated, and the organic layer was washed with dilute $NaHSO_3$ solution and brine, and dried over $MgSO_4$. The resulting pale yellow solution was filtered through a 2.5-cm pad of silica, rinsing with pentane, then concentrated by rotary evaporation without heating. The resulting pale orange oil **3** (28.9 g, 88.8% pure) was contaminated with isobutyl iodide (8.7%), 1-iodo-1-pentyne (0.8%), and iodopentane (1.8%), but appeared to be isomerically pure. The crude material was used without further purification. MS: *m/z* (%): 196 (M+, 40), 167 (22), 154 (28), 127 (13), 69 (20), and 41 (100).

(E)-Oct-4-en-2-yn-1-ol (4) A 250-ml three-neck flask fitted with a thermometer was flushed with Ar and loaded with bis(triphenylphosphine)palladium (II) dichloride (1 g, 1.4 mmol), 0.5 g CuI (2.6 mmol), and pyrrolidine (50 ml). The mixture was stirred and cooled in an ice bath, and crude (*E*)-1-iodo-1-pentene **3** (14.5 g, ~50 mmol) was added dropwise, followed by dropwise addition of propargyl alcohol **4** (3.5 ml, 60 mmol). The mixture was slowly warmed to room temperature, at which point a rapid exothermic reaction commenced, necessitating external cooling with an ice bath. After cooling the mixture to room temperature, checking the mixture revealed a clean reaction with complete consumption of iodoalkene **3** and production of a single product. The reaction mixture was worked up by pouring into an ice-cold 1:1 mixture of saturated aqueous NH_4Cl and 2 M HCl (300 ml), and extracting several times with ether. The combined ether extracts were washed with a small portion of the NH_4Cl and HCl mixture and brine, dried and decolorized with a mixture of anhydrous Na_2SO_4 and decolorizing charcoal, filtered through a plug of celite, and concentrated. The resulting viscous orange oil was Kugelrohr-distilled (oven temp ~70°C, 1 mmHg), yielding 3.5 g of clear oil (56%). MS: 124 (M+, 96), 109 (15), 95 (42), 91 (40), 81 (100), 79 (40), 77 (34), 67 (40), 65 (41), 55 (28), 54 (26), 53 (31), 41 (48). ^{1}H NMR: 6.14 (dt, 1H, J=15.97, 7.04 Hz, H-5), 5.48 (dtt, 1H, J=15.97, 2.05, 1.5 Hz, H-4), 4.35 (d, 2H, J=2.05 Hz, H-1), 2.09 (overlapped tdd, 2H, J~7.5, 7.1, 1.5 Hz, H-6), 1.91 (br s, 1H, OH), 1.41 (overlapped qt, 2H, J~7.5, 7.3 Hz, H-7), and 0.89 (t, 3H, J =7.3 Hz, H-8). ^{13}C NMR: 145.8, 109.1, 85.8, 84.8, 51.8, 35.3, 22.1, and 13.8.

(E)-Oct-4-en-2-yn-1-ol Tosylate (6) Enynol **5** (3.1 g, 25 mmol) and *p*-toluenesulfonyl chloride (5.4 g, 28 mmol) were dissolved in 100 ml of ether, and the solution was cooled to ~−10°C in an ice–salt bath. With vigorous stirring, powdered KOH (16.8 g, 300 mmol) was added in portions over 30 min, and the resulting mixture was warmed to ~ 0°C and stirred until the starting alcohol had been completely consumed (~1 hr). The mixture was then poured into 100 ml of ice water, stirred for 10 min, and the layers were separated. The aqueous layer was extracted again with ether, and the combined ether extracts were washed with brine, dried, concentrated, and then pumped under high vacuum (~0.2 mmHg) for 1 hr to remove traces of solvent, yielding the tosylate as a gold oil (6.32 g). This was used immediately in the next step without further purification.

Isopropyl magnesium chloride (2 M, 16 ml, 32 mmol) was added dropwise at room temperature to a solution of THP-protected 3-butyn-1-ol **7** (4.62 g, 30 mmol) in 75 ml THF. The mixture was stirred for 2 hr at room temperature, then cooled to 0°C, and CuI (0.5 g,

2.6 mmol) was added. After stirring for 15 min, crude tosylate **6** (3.2 g, ~12.5 mmol) in 20 ml of THF was added dropwise. The mixture was warmed slowly to room temperature overnight in an insulated bath, then worked up by addition of saturated NH_4Cl and extraction with hexane. The hexane extract was washed with brine and dried over Na_2SO_4, filtered, concentrated, and Kugelrohr-distilled (oven temperature <90°C, 0.2 mmHg) to remove the excess THP-protected butynol. The residue was then purified by vacuum flash chromatography (7.5% ethyl acetate [EtOAc] in hexanes), yielding the protected endiynol **8** (1.06 g), which was used immediately in the next step.

A solution of dicyclohexylborane was prepared by addition of cyclohexene (2.43 ml, 24 mmol) dropwise to a THF solution (20 ml) of borane-dimethylsulfide complex (2 M, 6 ml, 12 mmol) maintained at <−10°C in an ice–salt bath. After the addition was complete, the mixture was slowly warmed to room temperature over 3 hr, yielding a white slurry. The mixture was cooled to <−10°C again, and the protected enediynol (0.78 g, 3 mmol) in 10 ml THF was added dropwise. The solution was slowly warmed to 0°C over 3 hr, and stirred at 0°C for a further 3 hr, yielding a cloudy orange solution. Acetic acid (3 ml, 50 mmol) was then added dropwise, and the solution was warmed to room temperature overnight. The next morning, the mixture was cooled to 0°C again, and a solution of 4 g NaOH in 20 ml H_2O was added over 10 min, followed by dropwise addition of 1.4 ml of 30% H_2O_2 (EXOTHERMIC). The resulting mixture was warmed to room temperature and stirred for 30 min, poured into water, and extracted with hexane. After washing with brine, drying over Na_2SO_4, and concentrating, the residue was purified by vacuum flash chromatography (5% EtOAc in hexane). The resulting yellow oil **9** was dissolved in MeOH (15 ml) containing ~50 mg *p*-toluenesulfonic acid, and stirred at room temperature for 3 hr. The mixture was then poured into aqueous $NaHCO_3$ and extracted twice with hexane. The combined hexane extracts were washed with saturated $NaHCO_3$ and brine, then dried over a mixture of anhydrous Na_2CO_3 and Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (22% EtOAc in hexane). The purified trienol **1** was Kugelrohr-distilled (oven temperature ~80–90°C, 0.2 mmHg), yielding 280 mg of trienol **1**, >99.5% pure by GC. MS: 180 (29), 137 (8), 133 (10), 119 (25), 105 (48), 93 (41), 91 (100), 79 (73), 77 (37), 67 (40), 55 (28), and 41 (30). ^{1}H NMR: 6.31 (ddq, 1H, J=15.1, 11.0, 1.3 Hz, H-8), 5.97 (br t, 1 H, J=11.1 Hz, H-7), 5.69 (dt, 1H, J=15.1, 7.0 Hz, H-9), 5.56 (ddt, 1H, J=10.7, 7.3, 1.5 Hz, H-4), 5.43 (ddt, 1H, J=10.7, 7.3, 1.5 Hz, H-3), 5.25 (dd, 1H, J=10.8, 7.5 Hz, H-6), 3.66 (br q, 2H, J=6.3 Hz, H-1), 2.95 (br t, 2H, J=7.3 Hz, H-5), 2.37 (br q, 2H, J=6.8 Hz, H-2), 2.09 (br q, 2H, J~ 7.1 Hz, H-10), 1.42 (overlapped tq, 2H, J~7.3, 7 Hz, H-11), and 0.91 (t, 3H, J=7.3 Hz, H-12). All spectra were in accord with literature values (Eya et al., 1990; Argenti et al., 1994).

Results

Termites (N=30) walked on the Y-maze at an average speed of 0.73±0.12 cm/sec on the untreated arm of the Y-maze where they were released. Once the termites reached the intersection of the maze and turned toward the dodecatrienol treated arm (1 fg/cm), they traveled significantly faster (t=8.08, df=29, P<0.05) with fewer delays toward the end than on nontreated controls. The average speed on the treated arm was 1.05±0.18 cm/sec (N=30).

Production of Natural Trails

Individual termites laid trails that could be detected by other termites in significant numbers only after about 28 termites walked along the given Y-maze treatment arm compared to the

other control arm (χ^2=13.34, *df*=1, *P*<0.001). This trail lasted for 1 d when the maze was held inside a sealed container at room temperature (Table 1). By day 2, the trail no longer elicited a significant response.

Straight-line Bioassays

Termites showed optimal responses to trails of 0.1 to 10 fg/cm dodecatrienol in our bioassays. When exposed to trails made with the various concentrations of dodecatrienol, they did not show significant differences in their mean walking speed on the trail except for the 10 fg/cm trail (*F*=6.91, *df*=5, 29, *P*<0.001; Table 2), on which they walked more quickly (1.15 cm/sec) than they did with most of the other concentrations. The percentage of termites that walked the entire distance of the treatment trails was lower at higher concentrations of dodecatrienol (*F*=17.91, *df*=5, 29, *P*<0.001; Table 2) compared with lower concentrations (≤10 fg/cm).

Gradient Bioassays

There was no difference in trail-following behavior of the termites when they were exposed to increasing or decreasing gradients of dodecatrienol. Their speed walking up or down gradients was not significantly different, with termites covering the distance in about 24 sec (increasing gradient 24.7±5.0 sec and decreasing gradient 23.7±4.5 sec). Similarly, no differences were observed in the percentages of termites (88 to 90%) that completed the gradient trails compared to nongradient trails.

Choice Bioassays

A minimum of 0.02 TE/cm (worker SGE) was required for trail following (Table 3). The threshold concentration of 2-phenoxyethanol to elicit significant levels of trail following on a Y-maze was 0.2 ng/cm. For dodecatrienol the threshold concentration was between 0.01

Table 1 The number of *R. hesperus* walking on the Y-maze arm before other termites followed the trail

No. of termites[a]	Control/Treated	Chi-square[b]
5	18/12	1.2
10	16/14	0.13
15	13/17	0.54
20	14/16	0.13
25	19/11	2.13
26	10/20	3.34
27	10/20	3.34
28	5/25	13.3
29	3/27	19.2
30	6/24	10.8
28 (24 hr)	9/21	4.8
28 (48 hr)	13/17	0.54

[a] Deposits fresh unless indicated by the number of hours later that the bioassay maze was tested after deposition.

[b] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between treated (traveled by termites) and control arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

Table 2 *Reticulitermes hesperus* worker responses to different concentrations of dodecatrienol on a 25-cm-long trail

Concentration	Percent of Termites Completing the Trail	Time (sec) Mean±SD
0.01 fg/cm	0.0a	0.0
0.1 fg/cm	75.0b	28.7±6.5a
1 fg/cm	90.9b	26.0±6.3ab
10 fg/cm	93.0b	21.7±3.8b
100 fg/cm	51.7c	29.5±6.2a
1 pg/cm	48.4cd	26.3±5.8a
10 pg/cm	38.5d	27.4±5.4a

Mean values with the same letter in a column are not significantly different (Tukey's HSD test, $P<0.05$).

and 0.1 fg/cm. Thus, *R. hesperus* workers were 10^7 times more responsive to dodecatrienol than they were to 2-phenoxyethanol (Table 3).

Sternal gland extracts at 0.2 TE/cm elicited significant trail following for only 24 hr after application (Table 3), whereas a 2-phenoxyethanol trail at 0.2 ng/cm on a Y-maze lasted for 24 hr, and a 1 fg/cm trail of dodecatrienol was active for at least 48 hr.

Threshold concentrations for trail following on dodecatrienol trails were similar for workers and secondary reproductives (Table 4). Soldiers and alate males of *R. hesperus* were not able to follow trails at 0.1 fg/cm dodecatrienol (Table 4). Both workers and soldiers followed the 2-phenoxyethanol trails at equivalent threshold levels (0.2 ng/cm). Workers,

Table 3 Responses of *R. hesperus* workers to trails of 2-phenoxyethanol, SGE, and synthetic dodecatrienol on a Y-maze

Compound	Concentration	Age (hr)	Control/Treated	Chi-square[a]
SGE	0.002 TE/cm	0.1	13/17	0.53
	0.02 TE/cm	0.1	3/27	19.2
	0.2 TE/cm	0.1	5/25	13.34
		24	7/23	8.54
		48	14/16	0.13
2-Phenoxyethanol	0.02 ng/cm	0.1	17/13	0.53
	0.2 ng/cm	0.1	4/26	16.13
	0.2 ng/cm	24	7/23	8.54
	0.2 ng/cm	48	10/20	3.34
	10 μg/cm	0.1	6/24	10.8
Dodecatrienol	0.01 fg/cm	0.1	14/16	0.13
	0.1 fg/cm	0.1	8/22	6.54
	1 fg/cm	0.1	1/29	26.1
	1 fg/cm	24	5/25	13.3
	1 fg/cm	48	4/26	16.13
	1 fg/cm	60	20/10	3.34
	1 pg/cm	0.1	6/24	10.8
	0.01 ng/cm	0.1	23/7	8.54
	0.1 ng/cm	0.1	22/8	6.54
	1.0 ng/cm	0.1	24/6	10.8

TE = termite equivalent.

[a] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between treated and control arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

Table 4 Responses of different castes of *R. hesperus* to SGE, 2-phenoxyethanol, and dodecatrienol on a Y-maze

Treatment	Caste	Control/Treatment	Chi-square[a]
SGE (Worker)	Worker	5/25	13.34
0.2 TE/cm	Soldier	3/27	19.2
	SR	4/26	16.13
0.02 TE/cm	Worker	7/23	8.54
	Soldier	9/21	4.8
	SR	7/23	8.54
0.002 TE/cm	Worker	13/17	0.53
	Soldier	16/14	0.13
	SR	14/16	0.13
SGE alate (F) (0.2 TE/cm)	Alate (M)	3/27	19.2
SGE alate (F) (0.02 TE/cm)	Alate (M)	17/13	0.53
2-Phenoxyethanol (0.2 ng/cm)	Worker	4/26	16.13
	Soldier	5/25	13.34
2-Phenoxyethanol (0.02 ng/cm)	Worker	16/14	0.13
	Soldier	13/17	0.53
Dodecatrienol	Worker	1/29	26.13
1 fg/cm	Soldier	3/27	19.2
	SR	2/28	22.53
	Alate (M)	5/25	13.34
0.1 fg/cm	Soldier	10/20	3.34
	SR	9/21	4.8
	Alate	12/18	1.2
0.01 fg/cm	Soldier	13/17	0.53
	SR	16/14	0.13
	Alate	12/18	1.2

SGE = sternal gland extract, TE = termite equivalent, SGE alate (F) = sternal gland extract of female alates, SR = secondary reproductive

[a] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between the control and treated arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

secondary reproductives, and soldiers readily followed the trails of 0.02 TE of SGE/cm of worker termites. Male alates followed the trails of 0.2 female alate SGE/cm (Table 4).

Starvation appeared to have no effect on the sensitivity of termites to dodecatrienol. Those starved for 120 hr were still capable of following 0.1 fg/cm dodecatrienol trails on the Y-maze (χ^2=6.54, *df*=1, *P*<0.013; Table 5). However, those starved up to 120 hr were not able to differentiate between 1 fg/cm vs. 10^4 fg/cm dodecatrienol trails (χ^2=2.13, *df*=1, *P*<0.18; Table 5).

Termites were not able to differentiate between different concentrations of fresh trails of dodecatrienol or SGE when offered simultaneously on each of the two arms of the Y-maze (Table 6). Even when the differences in concentrations of dodecatrienol were as great as 10^4, they did not show any preference. However, significantly more followed the higher concentration trail after it aged for 48 hr.

When given a choice between 2-phenoxyethanol and dodecatrienol, workers generally preferred the latter (Table 7). Only when the concentration of 2-phenoxyethanol was 10^{11} (0.1 mg/cm) or 10^{12} (1 mg/cm) times greater than dodecatrienol (1 fg/cm) did they show significantly higher preference for the 2-phenoxyethanol trails (Table 7). Unlike

Table 5 Trail-following behavior of *R. hesperus* workers starved for different durations

Starvation Period (hr)	Conc. (fg/cm)	Control/Treatment	Chi-square[a]
24	1.0	5/25	13.34
	0.1	7/23	8.54
	0.01	16/14	0.13
24	1.0 vs. 10^4	12/18	1.2
120	1.0	4/26	16.13
	0.1	8/22	6.54
	0.01	14/16	0.13
120	1.0 vs. 10^4	11/19	2.13

[a] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between treated and control arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

dodecatrienol (0.01 to 1 ng/cm, Table 3), at a higher concentration 2-phenoxyethanol (1 mg/cm, Table 7) was not repellent to termite workers.

Detection of Trail Pheromone

In this experiment, a treated Y-maze was placed directly above the untreated Y-maze upon which the test animal walked. Neither the antennae nor the mouthparts of the termites could touch the treated surface of the Y-maze above them. When the distance between the treated and untreated surfaces of the two Y-mazes was reduced to 4 mm, termites were able to detect and follow the treated arm (Table 7). When the distance between the two mazes was reduced to less than 4 mm, they were able to touch the upper Y-maze surface and in few cases were able to climb up the maze over their heads. Dodecatrienol has relatively low vapor pressure (estimated as 3.73×10^{-4} Torr at 25°C; Chemical Abstracts, 2005), and termites were only able to detect it when their antennae were about 1–2 mm from the trail. Increasing the concentration of dodecatrienol did not alter the distance over which it was perceived by the antennae (Table 8). Concentrations as high as 1 ng/cm were not repellent to workers in this setup.

Table 6 Ability of *R. hesperus* workers to differentiate between different concentrations of synthetic dodecatrienol and SGE on the Y-maze

Concentration/cm[a] of Arm A vs. Arm B	Time (hr)	A/B	Chi-square[b]
1 fg/cm vs. SGE 0.2 TE/cm	0.2	13/17	0.53
	24	14/16	0.13
	48	12/18	1.2
1 vs. 100 fg/cm	0.2	16/14	0.13
	24	11/19	2.13
	48	8/22	6.53
1 vs. 10^4 fg/cm	0.2	13/17	0.53
	24	12/18	1.2
	48	9/21	4.8

[a] TE = termite equivalent, Arm A treated with 1 fg/cm dodecatrienol

[b] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between two treated arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

Table 7 Ability of *R. hesperus* workers to differentiate between synthetic dodecatrienol and 2-phenoxyethanol trails

Concentration (fg/cm)			Chi-square[a]
Dodecatrienol	2-Phenoxyethanol	Dodecatrienol/2-P	
1	1	28/2	22.53
1	10^3	24/6	10.8
1	10^5	27/3	19.2
1	10^7	24/6	10.8
1	10^9	23/7	8.54
1	10^{10}	16/14	0.13
1	10^{11}	6/24	10.8
1	10^{12}	8/22	6.54

2-P = 2-phenoxyethanol.

[a] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between two treated arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

Arrestant Bioassays

Both worker termites and male dealates spent more time on disks treated with 10 fg/cm^2 and 5 ng/cm^2 dodecatrienol, respectively, than on control disks (Fig. 4A, t=4.45, df=14, P <0.05; Fig. 4B, t=3.61, df=14, P<0.05). Male dealates also spent more time (t=10.62, df= 14, P<0.05) on disks treated with female alate extract (SGE, 0.28 TE/cm^2) than on control disks (Fig. 4C). When female alate SGE was directly compared to dodecatrienol, male dealates spent more time (t=6.10, df=14, P<0.05) on SGE-treated disks (0.28 termite equivalent/cm^2) than disks treated with dodecatrienol (5 ng/cm^2, Fig. 4D).

Feeding Bioassays

The total amount of feeding on treated and untreated paper combined at various dodecatrienol concentrations was not significantly different (F=2.45, df=5, 24, P=0.06). Total feeding on the treated and control halves combined ranged from 5.9 to 3.9 mg 40 termites^{-1} 5 d^{-1}. Net feeding on the treatments was determined by subtracting the amount of feeding on the control half of the disk from feeding on the treated half. Net feeding on treated halves ranged from −1.6 to +1.9 mg 40 termites^{-1} 5 d^{-1}. No consistent trend of feeding on either the control or treated half was observed. There were no significant differences in the amount of net feeding on paper towel disk halves treated with different concentrations of dodecatrienol (F=1.92, df=5, 20, P=0.15).

Table 8 Detection of trail pheromone by *R. hesperus* workers on the double Y-maze arms

Weight/cm on Y-maze Arm	Distance Between Arms (mm)	Control/Treatment	Chi-square[a]
1 fg	10	12/18	1.2
1 fg	5	11/19	2.13
1 fg	4	6/24	10.8
1 ng	5	10/20	3.34
1 ng	4	7/23	8.54

[a] Chi-square (χ^2) analysis is based on a binomial probability of 1:1 for a null hypothesis of no difference between two treated arms, and χ^2 values less than 3.84 are not significant at the 0.05 level of significance.

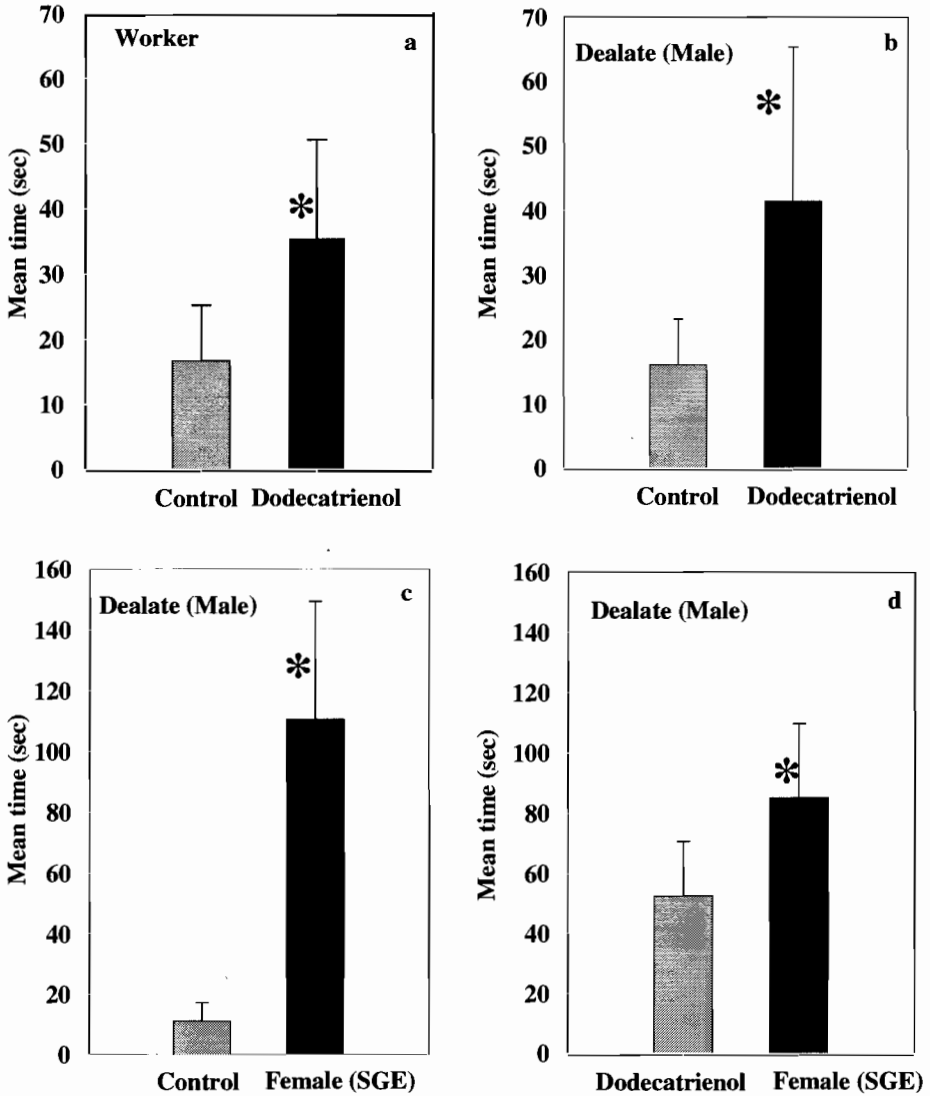

Fig. 4 Time spent (sec) on disks treated with dodecatrienol or female alate SGE by workers (**a**) and male dealates (**b**–**d**). Asterisk denotes means that are significantly different at $P<0.05$ (paired *t*-test)

Discussion

Individual termites, when allowed to walk on the Y-maze substrate, did not establish a trail that was recognized by conspecifics. Instead, it took a minimum of 28 termites walking over the same trail to establish a reliably detectable pheromone trail. This suggests that during its routine movements, a new recruit worker will prefer trails that have had considerable traffic, and these trails must be reinforced to remain effective. Grace et al. (1988) also suggested that *R. hesperus* termites are preferentially recruited to more well traveled trails.

Similar observations were made by Runcie (1987) concerning the trail-following behavior of *R. flavipes*, where trails in tubes with heavier termite traffic lasted longer than trails in tubes that were used less frequently. Single termite workers were allowed to lay trails in glass tubes and superimposed trails of as many as 50 workers remained active for only 5 min, whereas in our study trails superimposed by 28 termites remained active for 24 hr. These differences may be attributed to the properties of the substrate over which the trail was laid. In our study, the Y-maze was made of cardboard instead of a glass surface.

The concentrations of the trails did not affect the speed at which termites completed the trails. However, the concentration did affect the percentage that completed the trail bioassay. It took more than 30 termites to achieve 30 replicates in which the termite walked the entire length of the 25 cm trail because some veered off the trail before reaching the end. Termites exposed to 0.01 and 0.1 fg/cm trails walked slowly and stopped frequently, trying to sense the trail by actively waving their antennae (sampling substrate) on both sides of the trail. There was a limited range of dodecatrienol concentrations (0.1–10 fg/cm) to which the termites showed optimal trail-following behavior. The threshold concentration determined in our study for *R. hesperus* was ~60–600-fold lower than previously reported by Matsumura et al. (1972). The difference may be due to the purity of the synthetic dodecatrienol used in the two studies, or to the different substrates on which the trails were laid. In another study, the reported threshold concentration of dodecatrienol for *R. flavipes* was <0.01 pg/cm (Matsumura et al., 1968). On trails of higher concentration (>10 fg/cm), more termites failed to complete the trail or stopped occasionally and reversed direction, resulting in delays to reach the end of the treated line, than did termites on trails with between 1 and <10 fg/cm. Similar results have been reported by other researchers using different termite species (Grace, 1986; Laduguie et al., 1994b; Wobst et al., 1999; Peppuy et al., 2001; Arab et al., 2004; Robert et al., 2004). In our study, this effect manifested itself through a lower percentage of termites completing the trails at higher concentrations within the bioassay time period. Consequently, the walking speed of termites completing the trail was significantly greater for only one concentration (10 fg/cm), and there were no differences in the times taken to complete the trail among the other concentrations. As suggested by Prestwich et al. (1984), such differences may be related to the optimal response behavior of termites to the trail and have less to do with discriminatory ability.

On the Y-maze and the 25-cm long trail, significantly higher numbers of termites responded to 0.1 and 1 fg/cm treatments compared to untreated controls. The minimal threshold concentration of dodecatrienol eliciting trail following was between 0.01 and 0.1 fg/cm. The 1 fg/cm trail remained active for 48 hr. This may be because of the fact that lower concentrations volatilized from the substrate to the point where they were no longer detectable in the bioassay; higher concentrations probably still had some material "loaded" on the substrate. The natural trails and SGE trails (0.2 TE/cm) were active only for 24 hr. Thus, trails have to be reinforced by termites if they are to remain active. Runcie (1987) showed that *R. flavipes* trails leading to a food source were highly persistent compared to exploration trails. Affolter and Leuthold (2000) and Gessner and Leuthold (2001) reached a similar conclusion for trail-following behavior of *Macrotermes subhyalinus* Rambur. For another species, *Nasutitermes costalis*, extracts of 4-yr-old paves, aboveground guidelines formed by feces and debris by foragers, elicited orientation behavior (Traniello, 1982). However, *M. subhyalinus* and *N. costalis* have considerably different foraging and nesting behavior compared to *Reticulitermes* species, and observations on behavior of different species cannot be readily generalized. For example, termites starved for 120 hr were not able to discriminate between different concentrations of dodecatrienol, suggesting that *R.*

hesperus does not use concentration to differentiate between trails leading to food sources or for exploration. Under natural conditions, it seems more likely that the most highly used trails from the colony are those either leading directly to a food source, or foraging trails in a conducive soil environment.

Reticulitermes spp. rarely forage in the open, instead spending most of their time foraging underground in galleries constructed with a number of termites working on each tunnel. In nature, other factors such as tactile stimuli from workers returning from a food source, salivary secretions used to construct tunnels, moisture levels, and the age of the tunnel may provide additional signals.

Reinhard and Kaib (2001) showed that soldiers of *R. santonensis* responded only to trails of higher concentration from SGE of worker termites. In *N. costalis*, worker termites were recruited at higher concentrations compared to soldiers and third instar workers had larger sternal glands compared to soldiers, considered to be responsible for differential recruiting effects (Traniello and Busher, 1985). In our study, different castes of *R. hesperus* (worker, soldier, secondary reproductives, and male alates) responded equally well to dodecatrienol trails at 1 fg/cm. However, at 0.1 fg/cm, soldiers and male alates did not respond significantly more toward the treated arm, and 25% of workers tested failed to complete the 25-cm 0.1 fg/cm dodecatrienol trail.

Differences in threshold concentrations for various trail following chemicals have been reported in various subterranean termite genera and species (Matsumura et al., 1972; Arab et al., 2004). As suggested by other researchers (Howard et al., 1976; Kaib et al., 1982; Tokoro et al., 1994; Cornelius and Bland, 2001), termites of different genera and species may respond to dodecatrienol as a common component of a trail following signal, and there also may be other minor components involved, which may be responsible for species-specific signals.

We chose dodecatrienol concentrations of 1 fg/cm to compare with higher concentrations of dodecatrienol because >90% of the termites were able to follow 1 fg/cm trails. The fact that termites were not able to discriminate between different dodecatrienol concentrations on the two arms of the Y-maze suggests that in nature, trails contain largely qualitative information. Dodecatrienol appeared to act as a repellent at higher concentrations (≥0.01 ng/cm, Table 3). However, when the 0.01-ng/cm concentration was offered simultaneously with 1 fg/cm in a choice assay, termites did not discriminate between either concentration. Furthermore, when offered a choice between dodecatrienol (1 fg/cm) and SGE (1 TE/cm), termites were not able to discriminate between them. This suggests that dodecatrienol is a key component of the SGE signal.

Grace et al. (1988) reported that *R. hesperus* showed a differential response to a concentration gradient of SGE. In contrast, our studies indicated that *R. hesperus* did not respond to a concentration gradient and simply followed the trail. The concentrations selected for our gradient trails were based upon those used in the preliminary straight-line bioassays. Grace (1986) reported that to discriminate between increasing and decreasing gradients the difference on the treated sections must be a little over 10 times. The results suggested that termites showed optimal trail-following behavior at concentrations of between 1 and 10 fg/cm. This range seems more realistic and closer to the concentrations of trails laid by termites in nature. The ability of individual termites to either increase the production of trail pheromone or make it more concentrated so as to provide ≥100-fold differences in deposition on trails in nature seems to be unlikely. In insects such as pharaoh ants, *Monomorium pharaonis*, where trail pheromone is used for trail following, it has been demonstrated that trail geometry rather than a concentration gradient provides polarity to

ant foraging networks (Duncan et al., 2004). Their results showed that bifurcation angles similar to those found in natural networks (~60°) give the best reorientation ratios.

Termites were able to detect the dodecatrienol trails only when their antennae were very close to the trail (about 1–2 mm). The mouthparts apparently did not play a role in detecting the trail and initiating trail following. During normal trail following in termites, maxillary palpi are closer to the substrate than the antennae. When termites were allowed to walk on trail deposits as high as 1 ng/cm of dodecatrienol, they were repelled. In contrast, concentrations as high as 1 ng/cm were not repellent to termites in the bilevel Y-maze in which the antennae and palpi were not directly touching the treated surface. Together, these results suggest that the maxillary palpi may be involved in detecting repellent concentrations of dodecatrienol or perhaps the absence of repellency in the bilevel maze is just because of the additional distance of the antennae from the odor source.

In contrast, arrestant assays showed that male dealates were not repelled by dodecatrienol at relatively high concentrations, with dealates spending more time on treated disks than on controls. In other studies (Laduguie et al., 1994a, b; Robert et al., 2004), it has been suggested that at higher concentrations, dodecatrienol may have another role as a sex pheromone. However, male dealates spent more time on disks treated with extracts of female sternal glands than on disks treated with dodecatrienol, suggesting that other components are probably involved, which may act as synergists with dodecatrienol. On the basis of chemical analysis of extracts of *R. santonensis* (Laduguie et al., 1994a, b), female alates produced 10 to 100 pg of dodecatrienol, approximately 10 times more than the worker termites. Thus, dodecatrienol may be present as one of the components in female sex pheromone, serving to attract male alates for mating at higher concentrations, whereas at lower concentrations, it may act as a trail-following signal, helping in forming tandem pairs during mating.

Earlier studies (Matsumura et al., 1968; Esenther and Beal, 1979; Rust et al., 1996) suggested that wood degraded by *G. trabeum* was attractive to *Reticulitermes* spp., and dodecatrienol was isolated from fungus-degraded wood. Extracts of fungus-degraded wood can be used as arrestants in termite baits and have potential to increase bait station fidelity (Rust et al., 1996). In our feeding studies, we did not observe any phagostimulant or attractant properties of dodecatrienol applied to brown paper towel disks. When termites were transferred to petri dishes in which one half of the disk had been treated with dodecatrienol, they showed greater excitement and movement than termites in untreated controls, and it took longer for them to settle down. Thus, the attractive properties and increased feeding on wood degraded by *G. trabeum* may be due more to the effect of the degradation process on the wood composition or to other as yet unidentified compounds rather than the production of dodecatrienol.

Overall, our studies indicated that the context in which dodecatrienol was presented was critically important. When worker termites were offered a paper disk treated with a spot of dodecatrienol much larger than a termite rather than as a thin line, workers became excited and showed random movements, retouched the spot, and remained in contact with and around the treated disk. Workers perceived the stimulus, but they were not completely arrested by the stimulus. Termites exhibited normal trail-following behavior only when dodecatrienol was presented to them in its normal context, as a thin line. Concentration did not seem to play an important role in routine trail following but may have a role in mating behavior of alates.

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