Attraction of Subterranean Termites (Isoptera) to Carbon Dioxide

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ABSTRACT   Subterranean termites, *Reticulitermes* spp., were attracted to carbon dioxide (CO_2) in laboratory and field tests. In behavioral bioassays, *Reticulitermes flavipes* (Kollar), *Reticulitermes tibialis* Banks, and *Reticulitermes virginicus* Banks were attracted to CO_2 concentrations between 5 and 50 mmol/mol. In further bioassays, *R. tibialis* and *R. virginicus* were attracted to the headspace from polyisocyanurate construction foam that contained 10–12 mmol/mol CO_2. In soil bioassays in the laboratory, more termites foraged in chambers containing CO_2-generating formulations than in unbaited control chambers. In field tests, stations containing CO_2-generating baits attracted *R. tibialis* away from wooden fence posts at rangeland sites in Colorado. For all of the CO_2 formulations tested, termites foraged in significantly more bait stations at treatment fenceposts than in bait stations at the control fenceposts. By the end of the 8-wk study, the number of bait stations located by termites at treatment fenceposts ranged from 40 to 90%. At control fenceposts, termites foraged in only a single station and the one positive station was not located by termites until week 5 of the study. At treatment fenceposts, termites foraged equally in active stations (containing a CO_2-generating bait) and passive stations (with no CO_2-generating bait), indicating that bait stations may benefit passively from a proximal CO_2 source in the soil. CO_2 used as an attractant in current baiting systems could improve their effectiveness by allowing earlier exposure of termites to an insecticide.

KEY WORDS   termites, carbon dioxide, *Reticulitermes*, attractant

CARBON DIOXIDE (CO_2) IS an attractant for a number of soil-dwelling organisms, including numerous insect larvae (Klingler 1957; Moursi 1962; Paim and Beckel 1963b; Klingler 1965, 1966; Moursi 1970; Städler 1971; Meeking et al. 1974; Doane et al. 1975; Jones and Coaker 1977; Strnad et al. 1986; Bernklau and Bjostad 1998), insect adults (Moursi 1962, Paim and Beckel 1963), mites (Moursi 1962, 1970), chilopods (Moursi 1970), nematodes (Klingler 1961, 1965; Gaugler et al. 1980; Prot 1980; Dusenbery 1987; Johnson et al. 1993; Robinson 1995), and bacteria (Scher et al. 1985). Although subterranean termites are among the most abundant and widely distributed soil insects, they have apparently never been tested for attraction to CO_2.

Termite nest environments normally contain concentrations of CO_2 between 0.3 and 5% (Peakin and Josens 1978, Zimmerman et al. 1986), and levels as high as 15% have been reported (Peakin and Josens 1978, Zimmerman et al. 1986). The termite *Macrotermes natalensis* (Haviland) has been reported to rebuild porous walls when the CO_2 concentration in the nest increases to 2% (Ruelle 1964). The concentration of CO_2 in ambient soil atmosphere outside subterranean termite nests is much lower (0.04%) (Zimmerman et al. 1986) than inside the nest. A sensilla on the antennae of the termite *Schedorhinotermes lamanianus* Kolbe contains a neuron that responds specifically to CO_2 (Zeismann 1996). Zeismann (1996) demon-

strated that the sensitivity of this neuron to other odors is inhibited by exposure to CO_2, suggesting that the CO_2 concentration in the immediate environment may affect the sensory information being perceived by an individual termite.

Damp wood that is used as a food source by termites emits CO_2 in concentrations as high as 15% (Paim and Beckel 1964, Anderson and Ultsch 1987). We considered the possibility that termites might associate higher CO_2 levels with nest vicinity or food and that they may follow a CO_2 gradient to locate the source. The objective of the current study was to determine whether subterranean termites are attracted to CO_2. In addition, we tested a variety of CO_2-generating sources for behavioral effects in the laboratory and in the field.

Materials and Methods

Insects

Termites were collected in the field by using traps consisting of a square wood frame (15.2 by 15.2 cm, fir), containing several pieces of double-corrugated cardboard cut to fit the center of the frame and held in place by a 0.64-cm wire mesh. Traps were completely buried in a hole (30 cm in depth) dug at the base of a wooden fencepost. The traps were checked after 2 wk and termites found in the traps were placed

in covered plastic tubs (15 by 8 by 4 cm in height, Rubbermaid, Wooster, OH), along with some native soil ($\approx$400 g). *Reticulitermes flavipes* (Kollar) were collected from Michigan, *Reticulitermes tibialis* Banks were collected at rangeland sites in eastern Colorado, and *Reticulitermes virginicus* Banks were obtained from northern Virginia. Termites were kept in the sealed plastic tubs and moist cardboard (six pieces, corrugated, cut 10 by 10 cm and moistened by dipping in water) was added occasionally for food.

CO$_2$ Dose–Response Bioassays

Preparation of CO$_2$ Treatments. To prepare treatments, a 35-ml syringe (catalog no. 106-0490, Sherwood Medical, St. Louis, MO) was rinsed with distilled water and partially filled (5 ml) with ambient air. An amount of 100% CO$_2$ was obtained from a tank with a 100-ml Hamilton glass syringe and injected into the 35-ml syringe. Ambient air was then drawn into the 35-ml syringe to fill it and mix the gases by turbulence as the syringe was loaded. A second 35-ml polyethylene syringe was filled with ambient air (1 mmol/mol) for a control. Using this technique, treatments were prepared that contained a CO$_2$ concentration of 1, 2, 5, 10, 50, or 500 mmol/mol.

Choice-Test Bioassay Apparatus. The choice-test bioassay apparatus was constructed from a glass T-tube (5 mm i.d., 5-mm stem, with each branch 4.5 cm in length). Each branch of the T was bent downward (2.5 cm from the junction of the T) at a 45° angle to form a pitfall trap. A 5-mm NMR cap (catalog no. 100-0050, Drummond Scientific, Broomall, PA) with a 1-mm pinhole in it was firmly pushed over the end of each bent branch, and the cap was connected with a 25-cm length of Teflon tubing (0.8 mm i.d.) to a 35-ml polyethylene syringe. The two 35-ml syringes (containing mixtures of CO$_2$ and ambient air, prepared as described above) were clamped onto a syringe pump (Sage model 355, Fisher, Pittsburgh, PA) adjusted to provide airflow of 1 ml/min into each choice arm of the bioassay apparatus.

Bioassay Procedure. Before bioassays, five termite workers were placed in a small plastic holding container and held for 15 min. The holding container consisted of a 3-cm length of Teflon tubing (6 mm i.d.), capped on each end with an NMR cap that was perforated with three pinholes (to allow free air flow). To begin the test, the syringe pump was turned on, and after 3 min of pumping, the cap on one end of the holding container was removed and this end of the container was gently pushed onto the end of the central arm of the T-tube, allowing termites to crawl out and enter the apparatus. Termites crawled down the straight arm of the apparatus to the junction of the T where they made a choice to go either into the arm from which air with a specified concentration of CO$_2$ was flowing or into the control arm from which ambient air flowed. Upon reaching the bend in the choice arm (2.5 cm) of the apparatus, each termite fell down into the NMR cap (pitfall trap). Bioassays were conducted for 15 min, during which the syringe pump ran

continuously (at 1 ml/min). After 15 min, the number of termites in each pitfall (NMR cap) was recorded. For each replicate, a clean apparatus was used and the choice side was rotated. To clean the apparatus, all parts were washed with soap and water, rinsed with water, and the Teflon and glass parts were heated at 80°C in an oven for 30 min. Individual termites were not used for more than one replicate. Bioassays were conducted with *R. tibialis* and *R. flavipes* for 1, 2, 5, 10, 50, and 500 mmol/mol concentrations of CO$_2$ and with *R. virginicus* for 2, 5, 10, and 50 mmol/mol concentrations of CO$_2$.

CO$_2$ Measurements. The CO$_2$ concentration of the treatment and the control was measured for every bioassay conducted. Immediately before connecting the treatment syringes to the syringe pump, a 2-μl sample was removed from each polyethylene syringe by using a 10-μl Hamilton glass syringe. The CO$_2$ concentrations were measured using a Hewlett–Packard Series II 5890 gas chromatograph with a methyl silicone capillary column (30 m in length by 0.32 mm i.d., RSL-150, Alltech, Deerfield, IL), and interfaced with a Hewlett–Packard 5971 mass selective detector operated in selected ion monitoring mode (SIM) at m/z 44. A 10-mmol/mol mixture of CO$_2$ (a 300-ml glass bottle into which 3 ml of CO$_2$ was injected) was used as a standard to calculate the CO$_2$ concentrations of the unknown samples.

Statistical Analysis. A minimum of 10 replications were conducted with each termite species for every CO$_2$ concentration tested. For analysis, each replicate (group of five termites) was counted as a single experimental unit. Student's *t*-tests were conducted to determine significant differences between the number of termites recovered from the CO$_2$ side and the ambient air side of the apparatus for each concentration of CO$_2$ by using Minitab (Addison-Wesley Publishing Co. Inc., Reading, MA) with $\alpha = 0.05$.

Soil Bioassays

For choice-test bioassays in soil, the bioassay apparatus was constructed from three 4-ounce polyethylene jars with screw cap lids (catalog no. 42412KY, Consolidated Plastics Company, Inc., Twinsburg, OH). The jars were connected with 0.5-cm-diameter Tygon tubing inserted into holes drilled midway between the top and bottom. Each jar was filled two-thirds with clean soil (native sifted topsoil obtained from a local landscape supplier) mixed with distilled water to contain 15% moisture. The middle jar (harborage) and one of the outer foraging jars (control) contained soil, but no treatment. The other foraging jar (treatment) contained a CO$_2$-generating formulation that was mixed throughout the soil. Pinholes (four) were drilled into the bottom outer edge of each jar to allow CO$_2$ to diffuse out of the chamber. A wafer of yellow pine wood (6 by 6 by 0.5 cm in thickness, oven dried 48 h at 40°C, and weighed) was placed on top of the soil in each of the foraging chambers as a food source. A total of 200 termites (*R. tibialis* workers, with a few soldiers) were introduced into the middle

jar on day 0. The CO_2-generating materials tested included spent brewers grain (1 g, obtained from a local brewery and air-dried), processed corncob (1 g, 2 mm in diameter, Mt. Pulaski Co., Mt. Pulaski, IL), baking powder (1 g, Hulman & Company, Terre Houte, IN), whole malted barley (1 g, Beer Beer & More Beer, Concord, CA), activated charcoal (1 g, catalog no. C-5260, Sigma, St. Louis, MO), and effervescing tablets (1 g of Fizzi brand tablets, Premiere Innovations, Pacific Palisades, CA). The bioassays were stored in dim light at 25°C. CO_2 concentrations were measured in the treated soil at days 1, 7, and 14 (with day 1 being 24 h after introduction of the termites) by using gas chromatography-mass spectrometry-single ion monitoring (GS-MS-SIM) at m/z 44. A jig to measure CO_2 was constructed from a piece of glass tubing (5 cm in length by 1 mm i.d.) inserted through a hole drilled into the side of the jar (halfway between the top and bottom of the jar). The needle of a 10-μl Hamilton syringe was inserted into the glass tube, and a 2-μl sample of soil headspace was removed for analysis. On day 14, the bioassay apparatus was disassembled and the number of termites in each chamber was recorded. Termites recovered from either of the connecting tubes were counted as being in the center (harborage) chamber. The wood wafers were removed, oven-dried (48 h at 40°C), and weighed to determine the total amount consumed.

Statistical Analysis. The test was repeated five times for each treatment, and CO_2 concentrations were measured for three replications of each treatment. The percentages of termites recovered from the treatment chamber and the control chamber were calculated from the total number of termites recovered from all three jars of the bioassay apparatus. Statistical analysis was conducted to compare the percentage of termites recovered from the foraging chambers, and therefore termites recovered from the harborage (middle jar) were not included in the analysis. Experimental data (behavioral data and CO_2 concentration data) were transformed (square-root transformation) to normalize variances and then evaluated with analysis of variance (ANOVA) followed, when F values were significant ($P \leq 0.05$), by least significant difference (LSD) test (SAS version 8.2, SAS Institute, Cary, NC). For wood consumption, t-tests were conducted to compare the amount of wood (grams) consumed in the treated foraging chamber and the control foraging chamber by using Minitab (Addison-Wesley Publishing Co. Inc.) with $\alpha = 0.05$.

Field Tests

Field tests were conducted at two sites: the Central Plains Experimental Range located north of Nunn, CO, and a private ranch east of Wellington, CO. These sites contained long lines of old fenceposts, many of which were infested with termites (*R. tibialis*). To determine whether a post was infested with termites, wood frame traps (described previously) were completely buried in a hole (30 cm in depth) dug at the base of a wooden fencepost. The traps were checked
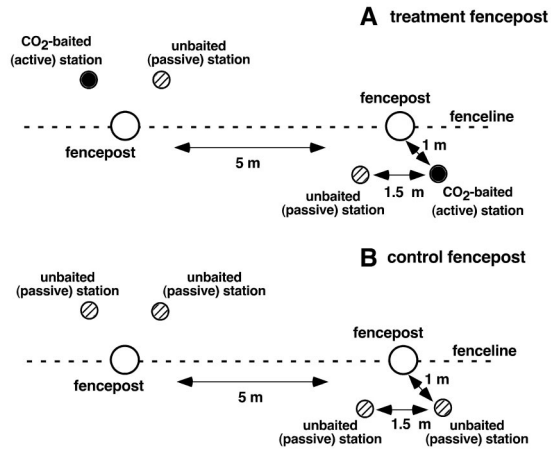

Fig. 1. Placement of bait stations in the field at wooden fenceposts for treatment fenceposts (**A**) and control fenceposts (**B**).

after 2 wk and, if termites were found in the trap, the post was determined to be infested and was used as a point source for the field experiments. Bait stations were 16-ounce polyethylene jars with screwcap lids (catalog no. 42416KY, Consolidated Plastics Company, Inc.). Each jar was perforated with 36 holes (3 mm) drilled around the circumference and was then filled to within 3 cm of the top with soil (300 g, sifted topsoil obtained from a local landscape supply company) containing 15% moisture. Treatments were mixed into the soil before filling the jars. Treatments included spent brewers grain (2 g, obtained from a local brewery and air-dried), processed corn cob (2 g, 2 mm in diameter, Mt. Pulaski Co.), activated charcoal (2 g, powdered charcoal, catalog no. C-5260, Sigma), and effervescing tablets (2 g, Fizzi brand tablets, Premiere Innovations).

In conducting this field test, we had two objectives. The first was to determine whether subterranean termites would be attracted away from wooden fenceposts to bait stations that were actively generating CO_2. Second, we wanted to determine whether unbaited termite stations would benefit passively from CO_2 in the nearby soil. At each treatment fencepost we placed two stations, one containing a CO_2-generating bait (active station) and one unbaited (passive station). The active and passive stations were buried in holes 30 cm in depth, 1 m away from the treatment fencepost and 1.5 m apart, with both stations on the same side of the fence line. At control fenceposts, two stations (both unbaited) were placed in the same manner (Fig. 1). The four treatments (active station and passive station) and the controls (passive stations only) were randomly distributed among the infested fenceposts.

All stations were checked once per week for 8 wk. To check a station, soil was removed from over the jar, the lid was removed, and the wood was gently lifted and examined for termites and termite feeding damage. The wood was then set back on the soil, the lid

replaced, and the jar was covered with soil as it had been found. A station was reported as positive if termites were present in the station, or if the wood showed new feeding damage. Feeding damage was evaluated visually on a scale from 1 to 10 in which a score of one represented consumption of at least 10% of the wood disk, two represented 20% consumed, three represented 30% consumed, etc. This method was used because termites were not always visible in the stations, yet obvious new feeding damage (10% increment increases) could be seen on the wood, indicating that termites were actively foraging in the station during that week. Results were reported as the total (percentage) stations visited at least once by termites up to that week of the study.

Statistical Analysis. Field data were analyzed using a logistic model with binary response (station positive or negative) at week 8 of the study. Planned contrasts between each treatment and the control were conducted using likelihood ratio confidence intervals with $\alpha = 0.05$ (SAS version 8.2, SAS Institute). Parameters defined in the model were formulation (spent brewers grain, $n = 20$; processed corncob, $n = 20$; activated charcoal, $n = 20$; effervescent tablets, $n = 20$; control, $n = 12$), station type (active or passive), and difference between station type and formulation.

Behavioral Bioassays with Foam

A piece of polyisocyanurate foam insulation was cut (0.25 g) from a large sheet by using a gasket punch. The foam was cut into four strips and all the strips were placed inside a 35-ml polyethylene syringe. Ambient air was then drawn into the 35-ml syringe to fill it and mix the gases by turbulence as the syringe was loaded. A second control syringe was loaded with ambient air only. After 30 min, 5 ml was ejected from each syringe (to relieve pressure), and a sample of headspace was removed from each syringe for CO_2 analysis (by using the method described previously). Behavioral bioassays were conducted with the foam and control syringes by using the glass T-tube bioassay described above. Bioassays were conducted with *R. tibialis* (19 replicates) and *R. virginicus* (10 replicates).

Statistical Analysis. For analysis, each replicate (group of five termites) was counted as a single experimental unit. Student's *t*-tests were conducted to determine significant differences between the number of termites recovered from the foam side and the ambient air side of the apparatus for each species of termite by using Minitab (Addison-Wesley Publishing Co. Inc.) with $\alpha = 0.05$.

Results

CO_2 Dose–Response Bioassays. Subterranean termite species *R. flavipes*, *R. virginicus*, and *R. tibialis* were attracted to a range of CO_2 concentrations between 5 and 50 mmol/mol (Fig. 2).

For *R. tibialis*, significantly more termites ($P \le 0.05$) were attracted to the CO_2 than to the control (ambient air) when the treatment side contained a CO_2
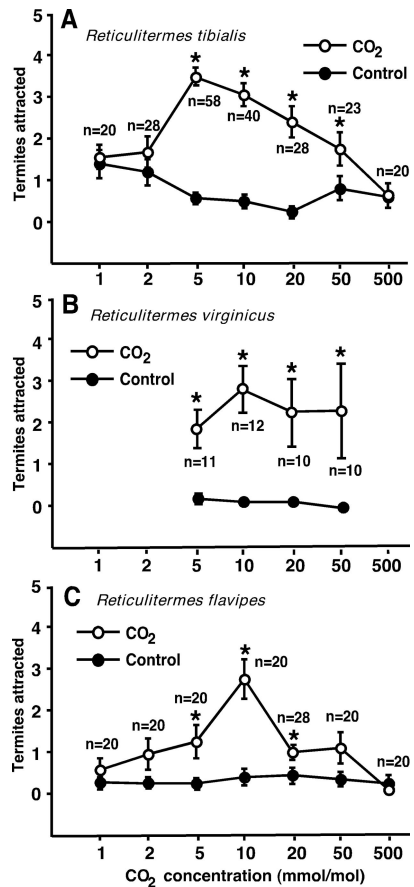

Fig. 2. Behavioral choice-test bioassays with termite workers in glass T-tube apparatus. Number of termites attracted to CO_2 for *R. tibialis* (A), *R. virginicus* (B), and *R. flavipes* (C). For all graphs, open circles represent the CO_2, and closed circles represent the ambient air control. Asterisk indicates significant difference between CO_2 and ambient air for the CO_2 concentration tested ($P \le 0.05$). Error bars represent standard errors (some standard error bars are too small to be seen).

concentration of 5 mmol/mol ($t = 244.11$; df = 1, 104; $P < 0.0001$), 10 mmol/mol ($t = 182.55$; df = 1, 78; $P < 0.0001$), 20 mol/mol ($t = 58.90$; df = 1, 54; $P < 0.0001$), or 50 mol/mol ($t = 8.51$; df = 1, 44; $P = 0.005$) (Fig. 2A). The greatest attraction was at 5 mol/mol CO_2 (3.68 ± 0.17 termites, mean $\pm$ SE, in the treatment and 0.49 ± 0.11 termites in the control).

For *R. virginicus*, significantly more termites were attracted to the CO_2 than to the control (ambient air) when the treatment side contained a CO_2 concentration of 5 mmol/mol ($t = 12.65$; df = 1, 20; $P = 0.001$), 10 mmol/mol ($t = 22.80$; df = 1, 22; $P < 0.0001$), 20 mol/mol ($t = 7.02$; df = 1, 18; $P = 0.016$), or 50 mmol/mol ($t = 20.16$; df = 1, 18; $P = 0.0002$) (Fig. 2B). The greatest attraction for *R. virginicus* was at 10 mol/mol CO_2 (2.75 ± 0.55 termites in the treatment and 0.10 ± 0.10 termites in the control).

For *R. flavipes*, significantly more termites were attracted to the CO_2 than to the control (ambient air)

when the treatment side contained a CO_2 concentration of 5 mmol/mol ($t = 5.46$; df $= 1, 38$; $P = 0.02$), 10 mmol/mol ($t = 21.29$; df $= 1, 38$; $P < 0.0001$), or 20 mol/mol ($t = 5.46$; df $= 1, 54$; $P = 0.02$) (Fig. 2C). The greatest attraction was at 10 mol/mol CO_2 (2.75 ± 0.47 termites in the treatment and 0.40 ± 0.20 termites in the control).

Soil Bioassays. At the end of the test, significantly more termites ($P \leq 0.05$) were recovered from the CO_2-generating chamber than from the control chamber for the spent brewers grain ($F = 7.688$; df $= 1, 4$; $P = 0.024$), effervescent tablet ($F = 21.381$; df $= 1, 4$; $P = 0.001$), malted barley ($F = 5.689$; df $= 1, 4$; $P = 0.02$), activated charcoal ($F = 133.07$; df $= 1, 4$; $P < 0.001$), and processed corncob ($F = 938.96$; df $= 1, 4$; $P < 0.001$) treatments (Fig. 3A). Termites consumed significantly more wood ($P \leq 0.05$) in the treated chamber than in the control chamber for the activated charcoal treatment (0.16 ± 0.04 g, mean $\pm$ SE, in the treated chamber versus 0.09 ± 0.03 g in the control chamber) ($F = 8.57$; df $= 1, 4$; $P = 0.019$). There were no significant differences in wood consumption for the other treatments.

CO_2 concentrations in the control chambers ranged from 1.51 to 3.80 mmol/mol. Concentrations were always lowest on day 1 and highest on day 14. The highest concentration of 14.52 ± 3.35 mmol/mol (mean $\pm$ SE) was measured in the spent brewers grain treatment on day 1. The spent brewers grain treatment produced the highest and most consistent concentration of CO_2 over the 2-wk period, dropping from 14.52 ± 3.35 mmol/mol on day 1 to 9.48 ± 2.21 mmol/mol on day 7, and then sustaining that approximate level (8.31 ± 0.19 mmol/mol) to day 14 (Fig. 3B).

Field Tests. Termite foraging in bait stations (active and passive) at treatment fenceposts was significantly greater ($P \leq 0.05$) than foraging in bait stations at control fenceposts ($\chi^2 = 27.73$, df $= 4$, $P < 0.0001$). Foraging was significantly greater for every CO_2 formulation tested. At week 8, the total positive stations (active and/or passive) were 90% for processed cob ($\chi^2 = 21.40$, df $= 1$, $P < 0.0001$), 75% for effervescent tablets ($\chi^2 = 16.19$, df $= 1$, $P < 0.0001$), 70% for activated charcoal ($\chi^2 = 14.06$, df $= 1$, $P = 0.0002$) and 40% for spent brewers grain ($\chi^2 = 5.28$, df $= 1$, $P = 0.0215$). In the first week of the study, termites were foraging in at least 10% of the stations (active and passive) at treatment fenceposts for every bait tested, but in none (0%) of the stations at control fenceposts. For the control fenceposts, the first positive station was reported at week 5, and this one station (representing 8% of all control stations) was the only station at control fenceposts that termites foraged in throughout the study (Fig. 4). There was no significant difference ($P \leq 0.05$) between active and passive stations for any of the treatments ($\chi^2 = 2.08$, df $= 4$, $P = 0.7206$).

Behavioral Bioassays with Foam. Significantly more termites ($P \leq 0.05$) were attracted to the foam headspace than to the ambient air control for both species tested (Fig. 5). For *R. tibialis,* 3.68 ± 0.25 termites
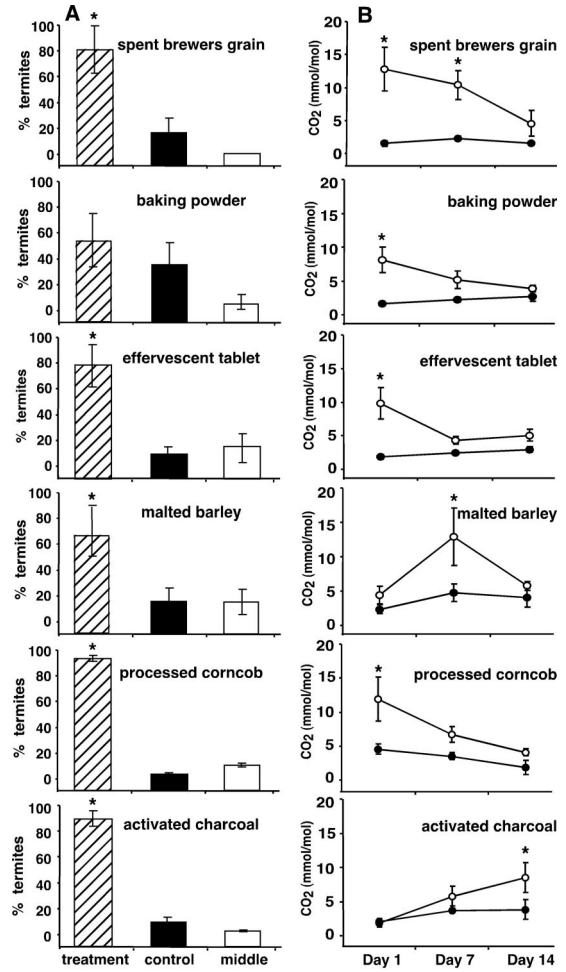

Fig. 3. Behavioral bioassays with termites in soil. (A) Percentage of termites recovered from treated foraging chamber containing a CO_2-generating bait versus number of termites recovered from unbaited control foraging chamber. (B) CO_2 concentrations in treated foraging chamber (containing a CO_2-generating bait) and in untreated control foraging chamber (unbaited) as measured by GC-MS-SIM. For CO_2 graphs, open circles represent the treated chamber (containing the CO_2 bait) and closed circles represent the control chamber (unbaited). Asterisk indicates significant difference ($P \leq 0.05$) between treated foraging chamber and control foraging chamber. Error bars represent standard errors (some standard error bars are too small to be seen).

were attracted to the foam, and 0.74 ± 0.24 termites were attracted to the control ($F = 71.09$; df $= 1, 37$; $P < 0.001$). The CO_2 concentration in the foam headspace was 10.02 ± 0.49 mmol/mol, and the concentration in the control was 1.14 ± 0.05 mmol/mol. For *R. virginicus,* 2.50 ± 0.65 termites were attracted to the foam and 0.40 ± 0.16 termites were attracted to the control ($F = 9.70$; df $= 1, 19$; $P = 0.005$). The CO_2 concentration in the foam headspace was 12.90 ± 0.71 mmol/mol, and the concentration in the control was 1.06 ± 0.05 mmol/mol.
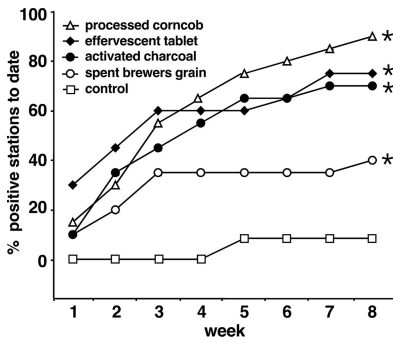
Fig. 4. Termite foraging for weeks 1 through 8 in bait stations at treatment fenceposts and control (untreated) fenceposts. Points on graph are the total percentage of stations visited by termites up to that time. Asterisk indicates a significant difference between a treatment and the control ($P \leq 0.05$), as determined at week 8 of the study.

Discussion

The response of subterranean termites to CO_2 in the behavioral bioassay is consistent with the known attraction for other soil-dwelling arthropods. Soil-dwelling arthropods that exhibit an oriented response to CO_2 include mites (Moursi 1962, 1970), Collembola (Klingler 1959; Moursi 1962, 1970), wireworm larvae (Klingler 1957, 1958, 1959; Moursi 1962; Klingler 1965; Moursi 1970; Doane et al. 1975), longhorn beetle larvae (Paim and Beckel 1964), carrot fly larvae, *Psila rosae* (F.) (Jones and Coaker 1977, 1979), and rootworm larvae (Strnad et al. 1986, Hibbard and Bjostad 1988, Jewett 1995, Bernklau and Bjostad 1998). In the current study, subterranean termites were attracted to CO_2 concentrations from 5 to 50 mmol/mol (0.5–5% CO_2). The most attractive concentration of CO_2 was 5 mmol/mol for *R. tibialis* and 10 mmol/mol for *R. flavipes* and *R. virginicus.* Wireworm larvae are attracted to CO_2 concentrations between 0.036 and 1.5% and respond to CO_2 differences as low as 0.02% (Klingler 1958, Doane et al. 1975). Larvae of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, are able to detect differences in CO_2 concentration as small as 12% (Bernklau and Bjostad 1998, Bernklau 2003). In behavioral bioassays, neonate rootworm larvae were attracted to a wide range of CO_2 concentrations from 1.25 to 100 mmol/mol, and they responded optimally to concentrations between 2 and 30 mmol/mol (Bernklau and Bjostad 1998). Termites, however, are negatively affected by higher concentrations of CO_2. When testing CO_2 as a possible fumigant for control of termites, Delate et al. (1995) reported significant termite mortality when the insects were exposed to CO_2 concentrations of 95% or greater for 60 h, and mortality as high as 70% was obtained with exposure to 50% CO_2 for 70 h. In the current study, termites were not attracted to concentrations of 500 mmol/mol.

Compared with ambient air, which contains ≈0.035% CO_2, surface soils of arable land may contain from 0.15 to 1.6% CO_2 (Doane et al. 1975, Brady 1990).

CO_2 is a small molecule that readily diffuses through micropores in the soil. A point source of CO_2 in the soil would generate a three-dimensional gradient that could serve as a behavioral cue for insects that are able to detect CO_2. A CO_2 gradient emitted by respiring plant roots serves as a nonspecific host location cue for polyphagous soil-dwelling organisms (Nicolas and Sillans 1989). The ability of a soil organism to orient toward a CO_2 source eliminates the need for random searching and increases the organism's chance of locating a suitable host plant. Similarly, subterranean termites may use CO_2 to orient toward a source of decaying wood in the soil. Damp wood that is used as food by termites emits CO_2 concentrations as high as 15% (Paim and Beckel 1964). In addition, termites may use CO_2 gradients to locate the nest when returning from foraging. CO_2 concentrations inside termite nests have been measured from 0.3 to 5% (Luscher 1961, Matsumoto 1977, Peakin and Josens 1978, Zimmerman et al. 1986), which is consistent with the attractive range demonstrated in our behavioral bioassays.

Recent studies show that, in desert ecosystems, sharp increases in soil CO_2 concentrations occur soon after rain events, as the soil saturation begins to decline (Zobitz and Bowling 2004). These surges in measurable CO_2 are due in part to the rapid diffusion of CO_2 previously immobilized in the soil pore spaces by water and are also a result of sudden increases in microbial activity stimulated by the moisture (Zobitz and Bowling 2004). Subterranean termites would be
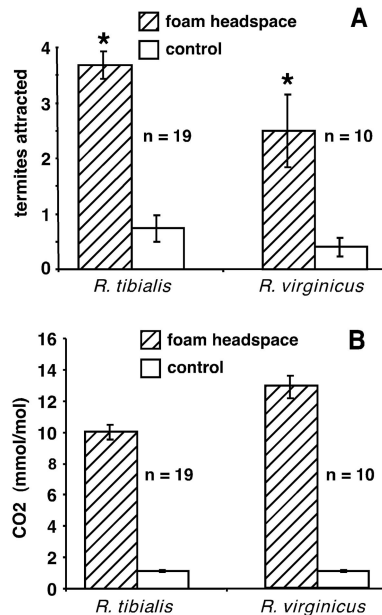

Fig. 5. Behavioral bioassays with foam headspace. (A) Number of termites attracted to foam headspace versus ambient air control. (B) CO_2 concentrations of foam headspace and ambient air control. Asterisk indicates significant difference between the treatment (foam) and control ($P \leq 0.05$). Error bars represent standard errors.

able to detect significant (at least 5 mmol/mol) CO_2 gradients produced by pockets of soil microbes, suggesting that CO_2 could serve as a cue to help termites locate sources of moisture in the soil.

In soil bioassays in the laboratory, termites demonstrated a preference for foraging in containers with elevated CO_2 concentrations. After 14 d, more termites were recovered from the chambers with CO_2-generating treatments than in the control chambers for five of the six treatments tested (spent brewers grain, effervescent tablets, malted barley, activated charcoal, and processed corn cob) (Fig. 3). Of these, only the charcoal treatment resulted in more wood being consumed in the treatment than in the control chamber. It is possible that the termites were feeding on some of the treatments. In preliminary tests (individual treatments placed in a closed petri dish containing termites), termites fed readily on the spent brewer's grain and minimally on malted barley, but they did not feed on the processed corn cob, baking powder, or charcoal treatments. At the end of the 8-wk field test, the processed corn cob and charcoal treatments were still intact in the soil jar, but the spent brewer's grain and malted barley seemed to be partially consumed. Such feeding could account for the lack of differential wood consumption in the treatment chambers during the soil bioassays. The baking powder was not considered to be a good candidate for subsequent field tests because, in preliminary tests, the powder dissolved too easily during short periods of water saturation in the soil. The malted barley treatment was also eliminated as a field treatment because, during the soil bioassays, excessive mold was observed growing in the soil chamber.

In the field study, all of the CO_2 formulations tested produced significantly more termite foraging activity than the untreated controls. Termites located and foraged in 90% of stations for the processed cob treatment, 75% of stations for the effervescent tablets, 70% of stations for the activated charcoal treatment, and 40% of stations for the spent brewers grain treatment (Fig. 4). In comparison, at control fenceposts (where no CO_2 treatment was placed), termites only foraged in a single station over the entire season, and the insects required 5 wk to locate this one station. Based on the attraction to CO_2 demonstrated in the dose–response bioassay, it is possible that termites were attracted from the fencepost directly to the bait station by CO_2 emitting from the station into the soil. CO_2 is a small molecule that moves readily through micropores in the soil. Although it is possible that CO_2 acted over a long distance (1 m) to attract termites from the fencepost to the CO_2-generating station, short-range attraction to CO_2 may provide a more plausible explanation for the higher rates of termite foraging in the treated stations. A short-range CO_2 gradient would increase the active radius of a bait station by effectively shortening the distance from the point source (fencepost) to the soil CO_2 gradient, thereby increasing the likelihood of a station being intercepted by termites during normal foraging.

A second objective of this study was to determine whether termite bait stations would benefit passively from CO_2 in the nearby soil. Toward this goal, two stations, one containing a CO_2-generating bait (active station) and one unbaited (passive station), were placed at each treatment fencepost. Both the active and passive stations were hit equally for every CO_2-generating bait tested. When only passive stations were placed, there was dramatically less termite activity (only one station hit throughout the season). The results of this test show that the passive stations did benefit from the CO_2-generating bait in the active station nearby, but the mechanism of this behavioral effect is not clear. Based on the results of the laboratory bioassays, we can attribute the termite activity in the active stations to CO_2 (through either long-range or short-range attraction), but more information (measurements of CO_2 concentrations in the soil) is needed to explain the termite foraging in the passive stations. In the current study, the CO_2-generating baits aided termites in locating the active bait stations and also affected an increase in foraging in unbaited stations nearby. Consequently, stations placed in proximity to a soil CO_2 gradient (within 1.5 m) need not be baited to be located quickly by foraging termites.

The economic impact of termites may exceed $11 billion each year in the United States (Su 2002) and $40 billion worldwide (Wiseman and Eggleton 1994). The majority of damage to homes and other structures is caused by subterranean termites, the group that includes *R. flavipes, R. virginicus,* and *R. tibialis* (Su 1990). Baiting strategies for termite control have recently gained popularity due to the withdrawal of chlordane, chlorpyrifos, and other termiticides from the market (Kard 1999, Su and Scheffrahn 2000). The development of insect growth regulators and slow-acting toxicants, and the subsequent incorporation of these materials into termite baits, has led to the introduction of commercial products such as the Sentricon Colony Elimination System (Dow Agrosciences, Indianapolis, IN), Outpost (Bayer, Manheim an Rhein, Germany), Exterra (Ensystex, Fayetteville, NC), Firstline (FMC Corp., Philadelphia, PA), Terminate (Spectracide, Spectrum Brands, St. Louis, MO), and Subterfuge (BASF Corp., Research Triangle Park, NC). Researchers have mixed opinions about the success of baiting techniques (Lewis et al. 1998, Potter et al. 2001, Lax and Osbrink 2003, Su 2003). Current efforts are focused on improving specific aspects of these systems, including the addition of attractants and/or bait enhancers (Pawson and Gold 1996, Lewis et al. 1998, Potter et al. 2001, Lax and Osbrink 2003). In a baiting system, the active ingredient is typically introduced into a station only after termites are detected in that station, and depending on the species, weeks may pass before termites locate a station and begin to feed (Lewis et al. 1998, Potter et al. 2001). Our field test results indicate that CO_2 can be used as an attractant to bring termites quickly to a point source. An attractant such as CO_2 has the potential to improve the effectiveness of a baiting system by reducing the time interval between station place-

ment and introduction of the active ingredient. In the current study, we also demonstrated that a point source of CO_2 causes an increase in termite foraging in the surrounding area of CO_2-enriched soil and that a station placed in proximity to a CO_2-baited station need not contain a CO_2 bait itself to be located by termites. Consequently, the performance of a baiting system could be enhanced by adding a CO_2 treatment to only a few bait stations around an infested structure, thereby minimizing disturbance to the stations and termites.

Environmental concerns have prompted the withdrawal of foam insulation products that are manufactured using chlorofluorocarbons (CFCs), methylene chloride, or 1,1,1-trichloroethane. Replacements for the CFC insulation products include rigid board polyurethane foams that are manufactured using liquid carbon dioxide as an expanding agent (Kim and Youn 2000). In the current study, termites were attracted to the headspace from pieces of polyisocyanurate construction foam. This foam headspace contained CO_2 concentrations of 10–12 mmol/mol, which is within the attractive range for subterranean termites (5–50 mmol/mol).

Although rigid board products are relatively inexpensive and provide good thermal insulation, buildings constructed with these materials may be prone to termite infestation (Smith and Zungoli 1995). With normal installation, the insulation is in direct contact with the ground and it often extends below ground as far as the structure footings. This degree of installation makes termite inspection difficult and allows easy access to the structure for subterranean termites (Smith and Zungoli 1995). Foam products manufactured with the new CO_2 process contain carbon dioxide gas that, with improper installation, could be released into the soil over time, thereby increasing the risk of termite attack.

Acknowledgments

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