

Variation in Acetylene-reduction (Nitrogen-fixation) Rates in *Reticulitermes* spp. (Isoptera; Rhinotermitidae)

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ABSTRACT

Using the acetylene-reduction assay, we examined nitrogen fixation in ten colonies of species of *Reticulitermes* Holmgren in Virginia. Colonies varied significantly in rates of acetylene reduction, but there was no clear association between rate and termite biomass. Ethylene production (nitrogen fixation) was significantly greater during the first 30 minutes of the assay than for subsequent periods, indicating that samples should be tested within the first hour of incubation. Based on acetylene-reduction rates measured in this study and on colony size determinations by Grace (1990), we estimate that *Reticulitermes* colonies are capable of fixing 0.01-0.04 g nitrogen/m²/year, or 125.5 - 445.3 g nitrogen/ha/year.

INTRODUCTION

Termites may be a significant source of nitrogen in the terrestrial environment because of their hindgut bacteria that fix atmospheric nitrogen (Breznak et al., 1973; Potrikus and Breznak, 1977; Waughman et al., 1981). Bentley (1984) demonstrated that fixed nitrogen is incorporated into termite tissues, but it is unknown whether the amount of nitrogen fixed is substantial enough to contribute to termite nitrogen budgets. Nitrogen fixation activity varies widely among termite species (Breznak, 1984; Waller and La Fage, 1987), termite castes (Prestwich et al., 1980; Hewitt et al., 1987), termites of different biomass (Waller et al., 1989) and levels of dietary nitrogen (Breznak et al., 1973; Prestwich et al., 1980).

Some of the variation reported for nitrogenase levels is related to assay timing, because fixation rates decrease soon after collection for some species. Prestwich et al. (1980) noted that nasute termites (Nasutitermitinae) fix significantly less nitrogen when confined with nest materials in a plastic bag for 24 hours. Lovelock et al. (1985) found that nitrogen fixation rates decrease in nasutes both in the laboratory and in containers at the nest site. Nitrogen fixation rates may also decline during the assay, but the decrease has not been quantified for any termite species.

In this study we investigated nitrogen fixation in the subterranean termite species of the genus *Reticulitermes* (Rhinotermitidae), important economic pests in the United States (Su and Scheffrahn, 1990). We examined variation in fixation rates among colonies by using the acetylene-reduction assay, a common method for measuring nitrogenase activity (Hardy et al., 1973; Breznak et al., 1973; Prestwich et al., 1980). In this assay, termites are incubated with acetylene, which is reduced to ethylene by the nitrogenase enzyme. Ethylene production is therefore a measure of nitrogenase activity. We examined nitrogenase activity several times

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during the assay to quantify variation in fixation rates during the incubation period. From the results we determined the most appropriate sampling regime and made a preliminary estimate of the amounts of nitrogen that can be fixed by *Reticulitermes* colonies.

METHODS AND MATERIALS

Termites

Reticulitermes-infested logs were collected from ten different locations near Norfolk, Virginia, during May and June 1990. Logs were placed in plastic bags and maintained in the laboratory at 22-24°C. Time constraints made it impossible to collect termites and perform bioassays on the same day. Therefore, nitrogenase activity may have been reduced during storage. Termites were removed from the logs immediately before the nitrogenase assays.

Acetylene-reduction assay

Three replicate samples from each termite colony were assayed using the acetylene-reduction technique. For each sample, fifty termite workers were isolated from the log and weighed to 0.1 mg, using an analytical balance. The termites were then placed in an 8.5 ml serum vial with an air-tight septum sleeve cap, and 1.0 ml air was removed from the vial. We then added 1.0 ml reagent-grade acetylene (C_2H_2) to produce an atmosphere of 12% C_2H_2 . Vials were incubated at room temperature and 200 μ l gas aliquots were removed at different times for analysis with a Varian® 3600 gas chromatograph equipped with flame ionization detector and a Porapak® N column which was maintained at 70°C with N_2 as the carrier gas. Analyses were performed immediately following sample collection. For six of the colonies, gas samples were removed after 30 minutes, 240 minutes, and 330 minutes. For four of the colonies, gas samples were removed after 30 minutes, 185 minutes and 270 minutes. By sampling two groups of colonies at different times we were able to measure acetylene-reduction rates over five incubation periods. It was not possible to sample all ten colonies for each incubation period because of the time required to perform the bioassays. Pilot studies indicated that incubation periods of under 30 minutes frequently did not yield measurable amounts of ethylene. Amounts of ethylene produced in these assays served as a measure of nitrogen fixation rates, because the nitrogenase enzyme reduces acetylene to ethylene (C_2H_4) at a rate three times greater than it reduces dinitrogen to ammonia (Bentley, 1984). For each colony, one sample with termites but no acetylene was examined for spontaneous production of ethylene, and vials containing acetylene but no organisms were sampled for ethylene contamination. Ethylene production was calculated as μ g C_2H_4 per gram termite fresh biomass per day.

Analysis

Variation in nitrogenase activity among colonies and over time (30 minutes and 240-270 minutes) was analyzed with repeated measures analysis of variance (STAT-VIEW). The association between termite biomass and the rate of ethylene production at 30 minutes and at 240-270 minutes was examined with regression analysis.

RESULTS

Colonies varied significantly in rates of nitrogen fixation for all sample periods ($p = 0.0001$) (Figs. 1, 2). There were significant differences in fixation rate over

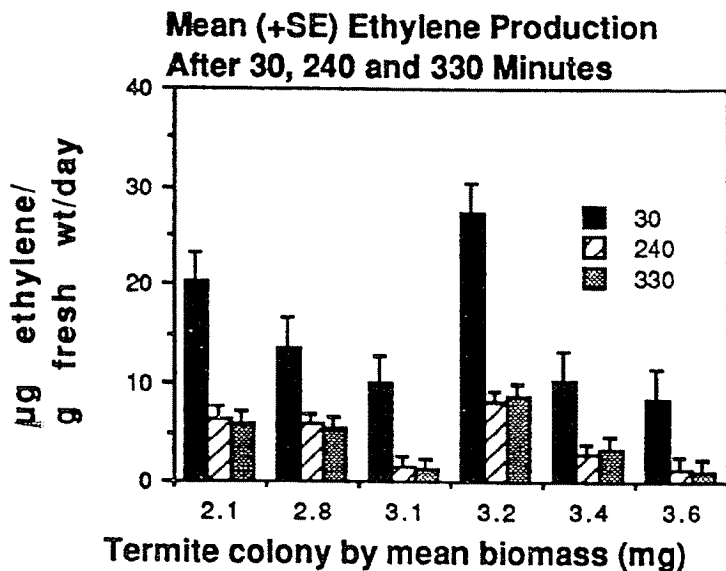


FIGURE 1. Ethylene production by six *Reticulitermes* colonies after incubation with acetylene for 30, 240, and 330 minutes. Mean worker biomass is listed for each colony.

time ($p = 0.0001$), and a significant interaction between colony source and sample timing ($p = 0.0014$). The acetylene-reduction rates measured at 30 minutes were almost four times greater than those at 240-270 minutes for some colonies (Figs. 1, 2). Fixation rates decreased after 185 minutes for the four colonies tested then (Fig. 2), and remained constant through the 330 minute sample for the other colonies (Fig. 1).

There was no association between termite biomass and nitrogenase activity at 30 minutes ($p = 0.1901$, $R^2 = 0.094$), and only a weak association at 240-270 minutes ($p = 0.0137$, $R^2 = 0.293$) (Figs. 1, 2).

We calculated the potential amount of nitrogen fixed by *Reticulitermes* by using the estimate of Grace (1990) of almost one million *R. flavipes* individuals per colony in Toronto. With our measure of *Reticulitermes* fresh biomass at approximately 3.5 mg per individual, termites could fix 3.6-12.7 g nitrogen/colony/year at rates measured at 30 minutes in our study. Grace (1990) found that one colony covered 285 m² in Toronto; at this density, termites could fix 0.01-0.04 g nitrogen/m²/year, or 125.5-445.3 g nitrogen/ha/year. This estimate reflects potential rates based on the highest rates measured in our study. Natural levels of nitrogenase activity are still unknown.

DISCUSSION

This study documents significant variation in nitrogen fixation rates among *Reticulitermes* colonies in Virginia. Rates differed over three-fold for some colonies. Waller et al. (1989) also found significant variation in nitrogen fixation rates among colonies of *Coptotermes formosanus* Shiraki (Rhinotermitidae) in Louisiana. In both studies, colony variation might have been related to differences in the food or in colony age or vitality. In addition, the taxonomy of *Reticulitermes*

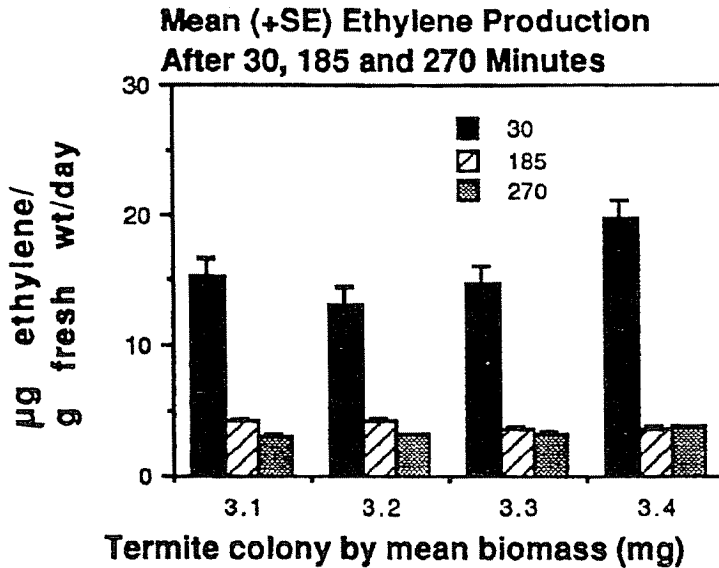


FIGURE 2. Ethylene production by four *Reticulitermes* colonies after incubation with acetylene for 30, 185, and 270 minutes. Mean worker biomass is listed for each colony.

warrants revision (Margaret Collins, personal communication), and there may have been genetic differences among the colonies tested in the present study. There was no association between termite biomass and nitrogen fixation rate after 30 minutes of incubation with acetylene. A weak negative association was evident after 240 minutes, but termite biomass explained less than 30% of the observed variation. In contrast, *C. formosanus* nitrogen fixation rates increase with mean termite biomass (Waller et al., 1989). Termite size differences may be related to genetic differences, termite age or nutritional status. The importance of termite size in the magnitude of nitrogen fixation remains unclear but requires further study if valid estimates of termite nitrogen contributions are to be made.

Rates of ethylene production dropped rapidly after 30 minutes of incubation with acetylene, possibly as a result of decreased termite vitality when confined with acetylene. Our results indicate that future assays should be made within the first hour of incubation, and efforts should be made to assay the termites under the most natural conditions possible (Prestwich and Bentley, 1981).

Our estimate of the potential amounts of nitrogen that can be fixed by *Reticulitermes* suggests these insects may be an important source of nitrogen in forest habitats. Estimated contributions by *Reticulitermes* of 125.5-445.3 g nitrogen/ha/year are higher than those reported for the desert termite *Gnathamitermes tubiformans* (Buckley) (Termitidae) of 66 g/ha/year (Schaefer and Whitford, 1981), but we did not consider seasonal variation in our study. Our estimates are conservative, because we did not measure nitrogen fixation directly in the field, and natural nitrogen fixation rates may be higher. Earlier measures of nitrogen fixation by *Reticulitermes flavipes* Kollar were much lower than those obtained in our study (Breznak et al., 1973; Breznak, 1984), perhaps in part because the termites were

not freshly collected. Our estimates rely on the assumption that colony size in Virginia is similar to those reported for Toronto (Grace et al., 1989; Grace, 1990). Grace et al. (1989) found *R. flavipes* colony size to be 2.1-3.2 million, with average foraging range of 266 m². Grace (1990) measured colony size at 722,679-943,237 with a foraging range of 285 m². Accurate estimates of termite colony size and density throughout their range, along with measures of natural nitrogen fixation rates throughout the year, are needed to provide reliable estimates of termite nitrogen contributions.

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LITERATURE CITED

- Bentley, B. L. 1984. Nitrogen fixation in termites: Fate of newly fixed nitrogen. *Journal of Insect Physiology* 30:653-655.
- Breznak, J. A. 1984. Biochemical aspects of symbiosis between termites and their intestinal microbiota. In Anderson, J. M., Rayner, A. D. M. (eds.) *Insect-Microbial Interactions*. Cambridge Univer. Press, Cambridge, U.K. pp. 173-203.
- Breznak, J. A., W. J. Brill, J. W. Mertins and H. C. Coppel. 1973. Nitrogen fixation in termites. *Nature* 244:577-580.
- Grace, J. K. 1990. Mark-recapture studies with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* 16:297-303.
- Grace, J. K., A. Abdallay and K. R. Farr. 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. *Canadian Entomologist* 121:551-556.
- Hardy, R. F. W., R. L. Burns and R. D. Holsten. 1973. Applications of the acetylene-reduction assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry* 5:47-81.
- Hewitt, P. H., M. C. Van der Westhuizen, T. C. De K. Van der Linde and R. A. Adam. 1987. Acetylene reduction by the harvester termite *Hodotermes mossambicus* (Hagen). *Journal of the Entomological Society of South Africa* 50:513-520.
- Lovelock, M., R. W. O'Brien and M. Slaytor. 1985. Effects of laboratory containment on the nitrogen metabolism of termites. *Insect Biochemistry* 15: 503-510.
- Potrikus, C. J. and J. A. Breznak. 1977. Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites. *Applied Environmental Microbiology* 33:392-399.
- Prestwich, G. D. and B. L. Bentley. 1981. Nitrogen fixation in intact colonies of the termite *Nasutitermes corniger*. *Oecologia* 49:249-251.
- Prestwich, G. D., B. L. Bentley and E. J. Carpenter. 1980. Nitrogen sources for neotropical nasute termites: Fixation and selective foraging. *Oecologia* 46: 397-401.

- Schaefer, D.A. and W.G. Whitford. 1981. Nutrient cycling by the subterranean termite *Gnathamitermes tubiformans* in a Chihuahuan desert ecosystem. *Oecologia* 48:277-283.
- Su, N. Y. and R. H. Scheffrahn. 1990. Economically important termites in the United States and their control. *Sociobiology* 17:77-94.
- Waller, D. A. and J. P. La Fage. 1987. Nutritional ecology of termites. In F. Slansky, Jr. and J. G. Rodriguez (eds.) *The Nutritional Ecology of Insects, Mites and Spiders*. John Wiley and Sons, New York. pp. 487-532.
- Waller, D. A., G. A. Breitenbeck and J. P. La Fage. 1989. Variation in acetylene reduction by *Coptotermes formosanus* (Isoptera: Rhinotermitidae) related to colony source and termite size. *Sociobiology* 16:191-196.
- Waughman, G. J., J. R. J. French and K. Jones. 1981. Nitrogen fixation in some terrestrial environments. In J. W. Broughton (ed.) *Nitrogen Fixation*. Vol 1, Clarendon Press, Oxford. pp. 104-134.