

Protocol for Testing Effects of Microbial Pest Control Agents on Nontarget Subterranean Termites (Isoptera: Rhinotermitidae)

J. KENNETH GRACE

Department of Entomology, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822

J. Econ. Entomol. 87(2): 269-274 (1994)

ABSTRACT Subterranean termites (Isoptera: Rhinotermitidae) are ubiquitous in both wooded and desert regions of North America. They are important contributors to nutrient cycling, energy flow, and ecosystem productivity. As the use of microbiological pesticides or microbial pest control agents (MPCAs) increases in the United States, termites might suffer unintended exposure to these pathogenic agents. The Federal Insecticide, Fungicide, and Rodenticide Act requires that information on the susceptibility of nontarget species be developed as a condition of registration of MPCAs, and the United States Environmental Protection Agency (EPA) is in the process of drafting protocols to assess such effects. An interim protocol (drafted on behalf of EPA) to assess the lethal effects of MPCAs on nontarget subterranean termite species is described in this paper.

KEY WORDS insect pathogens, *Reticulitermes*, Environmental Protection Agency

IN THE UNITED STATES, the Environmental Protection Agency (EPA) is the primary federal agency responsible for registering and regulating chemical and microbial insecticides (Code of Federal Regulations [CFR], Title 40, Chapter 1). As the name of the agency indicates, protection of the environment (including nontarget organisms and human health), rather than product efficacy against target species, is the principal concern of EPA. As a practical matter, EPA develops and disseminates standard data collection protocols and reporting procedures to facilitate review of the voluminous amounts of data submitted to support requests for federal registration.

Exactly how such protocols and procedures are developed may not be entirely clear to biologists who are not employees of the federal government or, more specifically, employees of EPA. With limited personnel and resources, it would be unrealistic for EPA to develop all of these materials internally. Therefore, EPA researchers establish contacts with specialists employed outside of the agency and may contract for their assistance.

Recently, I was one of those contacted (and contracted) to assist in the development of interim protocols to test the lethal effects of microbial pest control agents (MPCAs) on specific groups of nontarget arthropods. In my case, effects of MPCAs on nontarget termite species was the topic of concern. I chose to limit the scope of the protocol to subterranean termites (Rhinotermitidae), the family with which I have had research experience (Zoberi & Grace 1990a, b; Grace 1991, 1993; Grace & Zoberi 1992).

The EPA considers an *interim* protocol to be an untested protocol based on the best available information (B. Lighthart, personal communication). Simplicity in terms of the technical competence required to do the tests and general availability of the test materials are desirable in such a protocol. EPA is currently interested in receiving suggestions for other interim protocols for testing the effects of MPCAs on other nontarget terrestrial arthropods, especially beneficial arthropods. It is preferable that test species be representative of a larger group; surrogates for rare and endangered species are a high priority (B. Lighthart, personal communication).

This paper contains the interim termite protocol, as accepted by the EPA. The protocol followed a predetermined format, which had been provided to me in the form of several anonymous interim protocols for evaluating the effects of MPCAs on other insects. Before the protocol was accepted, the draft was reviewed by the contact EPA researcher, by the quality assurance staff of an environmental technology firm for compliance with current federal guidelines and accepted laboratory practices, and by four peer referees.

I have two goals in publishing this interim protocol in the *Journal of Economic Entomology*. The first is to clarify the governmental processes, which may be poorly understood by those outside of government, and to share a particular approach to laboratory evaluation of MPCAs. The second goal is to stimulate critical consideration of this approach and of this specific protocol by a larger audience of informed re-

searchers. This publication should lead to comments and suggestions (either to the author or the regulatory agency) that will be useful in moving this and related documents from the stage of interim protocols, drafted and reviewed by a few individuals, to that of validated and generally accepted protocols for MPCA evaluations. In summary, open review by the entomological community and subsequent group consensus can and should be part of the process of developing regulatory procedures.

Introduction to Protocol¹

Subdivision M of the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR, Part 158, section 158.170) requires that information on the susceptibility of nontarget species be developed as a condition of registration of MPCAs. Although subterranean termites are frequent targets of control measures around buildings and other human constructions, they are also ubiquitous in both wooded and desert regions of North America and are likely to suffer unintended exposure to MPCAs. Worldwide, subterranean termites contribute substantially to nutrient cycling, energy flow, and ecosystem productivity by (1) consumption of dead vegetation and conversion of lignocellulosic materials to termite tissue and fecal matter; (2) mixing and aeration of the soil through their extensive tunneling activities; (3) seasonal availability of swarming alates as prey for birds, lizards, and other insectivores; and (4) creation of cavities in trees suitable for use as nesting sites by birds and other vertebrates (Lee & Wood 1971, Wood & Sands 1978, McMahan 1986, Ewart 1991, Whitford 1991).

There are few reports of pathogens isolated from termites and little information available on the toxic effects of MPCAs. A brief, but thorough, summary of this information from the point of view of termite control is given by Logan et al. (1990). A possible virus was isolated from termites by Gibbs et al. (1970), a strain of *Bacillus thuringiensis* Berliner was identified by Khan et al. (1977), a *Beauveria bassiana* (Bals.) Vuill. strain was described by Zoberi & Grace (1990b), and a microsporidian was described by Jafri et al. (1976). Naturally occurring nematode infections, particularly by *Rhabditis* spp., have been reported somewhat more frequently (Poinar 1975). Microbial control efforts with termites have largely focused on the use of nematodes (e.g., Georgis et al. 1982, Epsky & Capinera 1988) or fungi (e.g., Lai et al. 1982, Hanel & Watson 1983, Grace 1991, Suzuki 1991).

Criteria for Selection of Test Species

Approximately 2,200 species of termites are known; of these, most are found only in the tropics (Wilson 1971, Wood & Johnson 1986). Termites generally are classified as the order Isoptera, although an alternative classification as the suborder Termitodea of the order Dictyoptera (including mantids, cockroaches and termites) is found in some Canadian literature (e.g., Vickery & Kevan 1985). According to Wood & Johnson (1986), the distribution of described species in the seven families (number of species) of the order Isoptera is as follows: Mastotermitidae (1), Kalotermitidae (350), Termopsidae (17), Hodotermitidae (17), Rhinotermitidae (206), Serritermitidae (1), and Termitidae (1639). The Mastotermitidae are limited to northern Australia (*Mastotermes darwiniensis* Froggatt) and the Serritermitidae to central South America (*Serritermes serrifer* [Bates]).

The Rhinotermitidae are referred to as subterranean termites, so-called because they usually nest in the soil and feed on woody materials at (or above) the soil surface. The holarctic genus *Reticulitermes* Holmgren is the predominant and most broadly distributed Rhinotermitid genus in North America, with the exception of the southwestern desert regions where it is displaced in part by the ecologically similar *Heterotermes* Froggatt (Haverty & Nutting 1976, Weesner 1965, 1969). The keys provided by Weesner (1965) remain most useful in identifying North American termites to genus. The keys and descriptions of Banks & Snyder (1920) and Banks (1946) are useful in identifying species of *Reticulitermes*, although the taxonomy of this genus is difficult and controversial. As a result of this difficulty in species identification and their ecological equivalency, some authors and commercial sources refer only to *Reticulitermes* spp.

Zootermopsis (Termopsidae) is commercially available (Carolina Biological Supply, 2700 York Road, Burlington, NC) and is a popular laboratory experimental animal because of its relatively large size (20–30 mm). However, its limited distribution in damp and decayed wood (e.g., stumps and logs) in the west and northwest, its exceptionally large body size for North American termites, and its very small colony size in comparison with more broadly distributed genera mitigate against its use as a generalized test organism for MPCAs. If *Zootermopsis* is considered the termite genus most likely to be exposed to a particular MPCA application and if the generalized test species has already been determined as susceptible to the MPCA, then toxicity testing of *Zootermopsis* should also be performed. Results obtained with *Zootermopsis* may also be more applicable to drywood termites (Kalotermitidae), which have a similar caste structure and also nest in small colonies directly

¹ This interim protocol was developed on the basis of current knowledge of testing the toxicity of chemical pesticides. Modifications may be required as experience in testing microbial pest control agents is acquired.

within above-ground wood, than test results with subterranean termites.

Reticulitermes flavipes (Kollar), the eastern subterranean termite, is recommended as a test species in this protocol because of its very broad distribution in the United States, Canada, and parts of Europe. As a result of the taxonomic difficulties mentioned above, this recommendation may be further generalized to *Reticulitermes* spp., although the actual location (and date) of collection should always be specified and type specimens should always be maintained. As of the date of this publication, *Reticulitermes* spp. are commercially available from Carolina Biological Supply. Whether or not the test insects are obtained from a commercial source, the date and place of location, as well as the length of time that the termites have been maintained under laboratory conditions should be determined and recorded. Preserved (70% alcohol) specimens of the soldier and, if possible, winged alate reproductive castes from each termite collection should either be retained by the testing laboratory or be deposited in an appropriate entomological collection at a university, museum, or government facility.

Although not a subterranean termite, *Zootermopsis* spp. should be considered as alternative, secondary, test species. This termite is an important degrader of logs and stumps in western North America, it is simple to maintain and handle in the laboratory, and specimens can be obtained commercially. Its humid living environment certainly would favor the activity of many MPCAs. Test results obtained with *Zootermopsis* spp. are likely to be qualitatively, but not quantitatively, similar to those obtained with *Reticulitermes* spp.

For the purposes of this evaluation, test results obtained with *Reticulitermes* spp. can reasonably be assumed to be generally applicable to other subterranean Rhinotermitid (e.g., *Heterotermes* spp.) and Termitid species in North America. However, if another termite species is known to occur in the geographic region or ecological community for which the MPCA is intended or is considered to be particularly vulnerable to MPCA exposure as a result of the method of application or proximity to the intended target, then efforts should be made to test the MPCA against that particular species. Although other termite species (e.g., *Coptotermes formosanus* Shiraki) are not available commercially, testing can frequently be performed through the cooperation of academic and government termite researchers in the region of interest. In addition to a search of the current literature or of presentations at insect pathology or other entomological meetings, membership directories of two societies may be particularly useful in identifying these researchers: the International Isoptera Society (c/o M.I. Haverty [Treasurer], USDA For-

est Service, Pacific Southwest Experiment Station, P.O. Box 245, Berkeley, CA 94701) and the International Research Group on Wood Preservation (IRG Secretariat, Box 5607, S-114 86 Stockholm, Sweden).

Test Procedures

Summary of Test Procedures. Chemical insecticide toxicity evaluation with subterranean termites generally proceeds in three stages: (1) topical toxicity, by micro-application of a drop of the unformulated active ingredient in an appropriate solvent to the insect's abdomen; (2) oral toxicity, by providing the insects with a food source in the form of cellulose filter paper or wood slices impregnated with the unformulated insecticide; and (3) contact toxicity, by placing the insects on insecticide-treated sand or soil in a petri dish for a prescribed period of time. However, this sequence is not particularly suited to evaluation of the hazards of nontarget exposure of termites to MPCAs.

Termites are social insects that groom each other and share food both orally and anally. Subterranean termites not only contact soil with their tarsi and body surfaces but also manipulate and carry soil particles in their mouthparts as they tunnel. Thus, infective MPCAs are afforded opportunities for both direct penetration of the cuticle and invasion via body openings. The following three tests are suggested to assess MPCA toxicity/pathogenicity to subterranean termites: (1) contact/oral toxicity to individual termite workers; (2) contact/oral toxicity to groups of termite workers; and (3) contact/oral toxicity of the formulated MPCA in soil application. MPCA hazard to termites is actually a function of MPCA toxicity/pathogenicity, field use pattern (including application method), and termite field exposure to the MPCA (including defensive behavioral responses). Tests addressing these hazard factors should be developed by manufacturers and researchers to supplement the toxicity/pathogenicity test protocol. However, such tests must be developed to fit specific circumstances and are difficult to express as a generic protocol.

As described here, these test procedures are generally suitable for evaluation of MPCAs based upon pathogenic fungi and other pathogens with which mortality may reasonably be expected to occur within a 15-d period. If a longer pattern of mortality is anticipated (e.g., caused by the use of protozoa or slow-release formulation), then the test period may need to be extended.

Test 1. Contact/Oral Toxicity of MPCAs to Individual Subterranean Termite Workers. Workers of *Reticulitermes flavipes* or other termite workers (pseudergates, or externally undifferentiated individuals) are placed individually on cellulose filter paper disks (i.e., No. 1 or No. 2,

Whatman Paper, Maidstone, England) or pads (Gelman Instrument, Ann Harbor, MI) in the wells of a disposable flat-bottomed well tissue culture or enzyme-linked immunosorbent assay (ELISA) plate. I recommend that a 96-well plate be used. A droplet of unformulated MPCA in distilled water at either 10 \times , 1 \times , 0.1 \times , or 0 \times (water controls) field application rate is applied by pipet to the top of each well. Spreading agents such as polyethylene glycol *p-tert*-octylphenyl ether (Triton X-100, Rohm & Haas, Philadelphia, PA) or polysorbate 80 (Tween 80, ICI Americas, Wilmington, DE) should be used sparingly to suspend the MPCA in solution for application because these adjuvants may interfere with the adhesion of MPCAs such as fungi to the insect cuticle. A hemacytometer may be useful in determining solution concentration (e.g., propagules per unit volume).

Dose should be expressed in terms of both MPCA units per volume of solution (solution concentration, equivalent to field rate) and MPCA units per termite (actual dose). The average (wet) mass of the termite workers tested should be reported as determined by weighing a subsample of termite workers from the same collection from which the test insects were selected.

Each test should contain at least 20 termite workers per treatment (each individual in a separate well), and the test should be replicated at least three times. The well plates should be incubated under constant conditions, and termite mortality should be recorded daily for 15 d. Incubation at 25–27°C and \approx 80–95% RH is recommended for *Reticulitermes* spp., with slightly higher temperatures for *Coptotermes* or *Heterotermes* spp. (Lenz et al. 1987). Incubation in the dark, with use of light as needed to monitor the tests, is recommended. Vibration or other physical disturbance of the test units should be avoided.

Initially, analysis of variance (ANOVA) of transformed (arcsine of the square root) percentage mortalities may be used to determine whether treatments differ from the water controls (SAS Institute 1987). This can also be tested by linear regression (Robertson & Preisler 1992). If treatment effects exist, a suitable range of concentrations should then be tested to estimate the LC₅₀. These mortality data should be analyzed by an appropriate model (e.g., probit, logit, or complementary log–log [CLL] [Robertson & Preisler 1992]), after correcting for control mortality by Abbott's (1925) formula, to estimate the LC₅₀ for specific time intervals and time to reach 50% (LT₅₀) mortality.

Test 2. Contact/Oral Toxicity of the MPCA to Groups of Termite Workers. Differing results, attributed to termite social interactions, have been reported from MPCA efficacy tests with individual termites and with groups (e.g., Tru-

deau 1989). In this test, a filter-paper disk or paper pad is placed in a petri dish and saturated with the unformulated MPCA in distilled water at 10 \times , 1 \times , 0.1 \times , or 0 \times (water controls) field application rate. Dose should be expressed in terms of both MPCA units per volume of solution and MPCA units per unit of filter-paper surface area. A group of 30–100 termite workers (pseudergates) is placed on the moist paper in the dish. At least three replicates of each treatment should be included in the test. The dishes are incubated as previously described, and termite mortality should be recorded 7, 14, and 28 d after the test begins. Mortality data are analyzed and reported as described above.

Test 3. Contact/Oral Toxicity of the Formulated MPCA in Soil Application. Acetone-washed and autoclaved silica sand is saturated by mixing it into an excess of the formulated MPCA at 10 \times , 1 \times , 0.1 \times , and 0 \times (water controls) the recommended concentration for field application. Sawdust should be substituted for sand in tests with *Zootermopsis* spp. Excess solution is decanted and \approx 1 cm of the damp sand is placed on top of, and completely covering, a filter paper disk in a petri dish. A group of 30–100 termite workers is placed on the sand surface, and the dishes are incubated as previously described. At least three replicates of each treatment for each separate sampling period (independent samples) should be included in the test. Termite mortality is recorded 7, 14, and 28 d after initiation. Data are analyzed and reported as described above.

Supplemental Tests. As mentioned above, simulation of field use of the MPCA or of the anticipated pattern of field exposure of termites to the MPCA may be desirable to evaluate hazard more accurately and may mitigate direct toxicity/pathogenicity of the MPCA. Choice tests in which termites are provided with a refuge from contact with MPCA-contaminated sand, sawdust, or cellulosic food material, generally by provision of several nest containers connected by plastic or glass tubing are recommended. However, such tests must be developed to fit specific circumstances and are difficult to generalize.

Conclusions. I suggest that test procedures and results be reported as outlined in the Appendix. This reporting method provides sufficient detail to permit accurate replication and, if necessary, later re-interpretation of the experimental results to reflect any new advances in termite or MPCA biology or systematics.

As written, this interim protocol meets the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act to develop information on the susceptibility of nontarget species as a condition of registration of MPCAs. The tests described above should permit an accurate assessment of the lethal effects of MPCAs on nontarget termite species. I recognize that these lethal effects may be mitigated by application

methods or environmental factors that would limit termite exposure. Hopefully, as we become more experienced with MPCAs, the protocols currently being developed by the EPA will be modified as necessary to reflect this experience.

Appendix. Reporting Method

Test data should be recorded as described above, with the report including:

1. name of the test, sponsor, test laboratory, study director, principal investigator, personnel performing each activity, and dates of testing;
2. a detailed description of the test MPCA, including (a) the formulation and concentration, (b) lot or batch number, and (c) the type of dilutions carried out;
3. detailed information about the test insects, including the species (and source of the species identification) or genus, location where specimens (in alcohol) are deposited, source of the insects, collection locale and original date of collection, average (wet) mass of the termite workers, laboratory maintenance conditions, and length of time the insects were maintained in laboratory culture before the test; test and incubation conditions (temperature, duration, relative humidity) and the exact method of exposure to the MPCA should be included;
4. description of the test substrates, dimensions, number of termites per replicate in each test, and number of replicates per dilution of MPCA (for test 1, dose should be given both in terms of MPCA units per volume of solution and in terms of MPCA units per termite; for test 2, dose should be given both as MPCA units per volume of solution and as MPCA units per unit of filter paper surface area);
5. percentage mortality of the test insects at each dose after each observation period should be reported; control mortality should be reported, even if Abbott's [1925] formula is used to correct the treatment mortality for statistical analyses;
6. for tests 2 and 3, proportions (if any) of the test insects showing external evidence of developmental changes, such as wing pad development or molting into presoldiers, should be noted because such changes could indicate sublethal effects of the MPCA;
7. any supplemental test procedures (such as choice tests) should be fully described, including size, shape, and configuration of test containers or arena, termite numbers, substrate volume and moisture content, pathogen concentration, and duration of test;
8. the type of statistical test, and name of any computerized statistical procedures, should be reported, with confidence intervals, slope,

and intercept reported along with any LC_{50} and LT_{50} values; mean percentage mortalities reported should include the standard deviation or standard error of the mean;

9. any deviation from the test protocol should be reported, as well as any unusual events such as temperature fluctuation, disease occurrence, high control mortality or great variation in mortality among replicates.

Acknowledgments

I am grateful to the United States Environmental Protection Agency for supporting the writing of this interim protocol and to the Ontario Ministry of the Environment and USDA-ARS Specific Cooperative Agreement 58-6615-9-012 for financial support of research on microbial pest control agents. W. E. Jones, M. Tamashiro, and M. H. Zoberi collaborated in that research and in developing some of the methods discussed in this protocol. I thank B. Lighthart (United States Environmental Protection Agency, Environmental Research Laboratory, 200 S.W. 35th St., Corvallis, OR) for helpful information and reviews and thank D. Coffey (ManTech Environmental Technology, Corvallis, OR), B. T. Forschler (University of Georgia, Griffin, GA), M. I. Haverty (USDA Forest Service, Berkeley, CA), F. J. Genthner (US EPA, Gulf Breeze, FL), M. Tamashiro (Univ. of Hawaii, Honolulu, HI), and an anonymous reviewer for critical reviews. This is Journal Series No. 3836 of the Hawaii Institute of Tropical Agriculture and Human Resources.

References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Banks, F. A. 1946. Species distinction in *Reticulitermes* (Isoptera: Rhinotermitidae). Ph.D. dissertation, University Chicago.
- Banks, N. & T. E. Snyder. 1920. A revision of the nearctic termites. *U.S. Nat. Mus. Bull.* 108.
- Epsky, N. D. & J. L. Capinera. 1988. Efficacy of the entomogenous nematode *Steinernema feltiae* against a subterranean termite, *Reticulitermes tibialis* (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 81: 1313-1317.
- Ewart, D. McC. 1991. Termites and forest management in Australia, pp. 10-14. In M. I. Haverty & W. W. Wilcox [eds.], *Proceedings, Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use*. U.S. Dep. Agric. For. Serv., Pac. Southwest Res. St. Gen. Tech. Rep. PSW-128.
- Georgis, R., G. O. Poinar & A. P. Wilson. 1982. Susceptibility of damp-wood termites and soil and wood-dwelling termites to the entomogenous nematode *Neoaeplectana carpocapsae*. *IRCS (Int. Res. Commun. Syst.) Med. Sci.* 10: 563.
- Gibbs, A. J., F. J. Gay & A. H. Wetherly. 1970. A possible paralysis virus of termites. *Virology* 40: 1063-1065.
- Grace, J. K. 1991. Termite-fungal associations and manipulations for termite control, p. 29 (abstract no. 16). In *Program and Abstracts, 24th Annual Meet-*

- ing, Society for Invertebrate Pathology, Flagstaff, AZ.
1993. Microbial termite control: effects of entomogenous fungi on the Formosan subterranean termite (Isoptera: Rhinotermitidae), p. 474 (abstract). In K.B. Willey & W.H. Robinson [eds.], Proceedings, First International Conference on Insect Pests in the Urban Environment, St. John's College, Cambridge.
- Grace, J. K. & M. H. Zoberi. 1992. Experimental evidence for transmission of *Beauveria bassiana* by *Reticulitermes flavipes* workers. (Isoptera: Rhinotermitidae). Sociobiology 20: 23-28.
- Hanel, H. & J.A.L. Watson. 1983. Preliminary field tests on the use of *Metarhizium anisopliae* for the control of *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). Bull. Entomol. Res. 73: 305-313.
- Haverty, M. I. & W. L. Nutting. 1976. Environmental factors affecting the geographical distribution of two ecologically equivalent termite species in Arizona. Am. Mid. Nat. 95: 20-27.
- Jafri, R. H., M. Ahmad & K. Idrees. 1976. Microsporidian infection in the workers of termite, *Microcerotermes championi*. Pak. J. Zool. 8: 234-236.
- Khan, K. I., Q. Fazal & R. H. Jafri. 1977. Pathogenicity of locally discovered *Bacillus thuringiensis* strain to the termites: *Heterotermes indicola* (Wassman) and *Microcerotermes championi* (Snyder). Pak. J. Sci. Res. 29: 12-13.
- Lai, P.-Y., M. Tamashiro & J. K. Fujii. 1982. Pathogenicity of six strains of entomogenous fungi to *Coptotermes formosanus*. J. Invert. Pathol. 39: 1-5.
- Lee, K. E. & T. G. Wood. 1971. Termites and Soils. Academic, New York.
- Lenz, M., T. L. Amburgey, D. Zi-Rong, H. Kuhne, J. K. Mauldin, A. F. Preston & M. Westcott. 1987. Interlaboratory studies on termite-wood decay fungi associations: 1. Determination of maintenance conditions for several species of termites (Isoptera: Mastotermitidae, Termopsidae, Rhinotermitidae, Termitidae). Sociobiology 13: 1-56.
- Logan, J. W. M., R. H. Cowie & T. G. Wood. 1990. Termite (Isoptera) control in agriculture and forestry by non-chemical methods: a review. Bull. Entomol. Res. 80: 309-330.
- McMahan, E. A. 1986. Beneficial aspects of termites, pp. 144-164. In S.B. Vinson [ed.], Economic impact and control of social insects. Praeger, New York.
- Poinar, G. O. 1975. Entomogenous nematodes. Brill, Leiden, The Netherlands.
- Robertson, J. L. & H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.
- SAS Institute. 1987. SAS/STAT guide for personal computers, version 6 ed. SAS Institute, Cary, NC.
- Suzuki, K. 1991. Laboratory trial of biological control agents against subterranean termites. International Research Group on Wood Preservation document no. IRG/WP/1475.
- Trudeau, D. 1989. Selection of entomophilic nematodes for control of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). M.S. thesis, University of Toronto, Ontario, Canada.
- Vickery, V. R. & D. K. McE. Kevan. 1985. The insects and arachnids of Canada, part 14. Agric. Can. Publ. 1777.
- Weesner, F. M. 1965. The termites of the United States—a handbook. National Pest Control Association, Elizabeth, NJ.
1969. Termites of the nearctic region, pp. 477-525. In K. Krishna & F. M. Weesner [eds.], Biology of termites, vol. II. Academic, New York.
- Whitford, W. G. 1991. Subterranean termites and long-term productivity of desert rangelands. Sociobiology 19: 235-243.
- Wilson, E. O. 1971. The insect societies. Belknap, Cambridge, MA.
- Wood, T. G. & R. A. Johnson. 1986. The biology, physiology, and ecology of termites, pp. 1-68. In S. B. Vinson [ed.], Economic impact and control of social insects. Praeger, New York.
- Wood, T. G. & W. A. Sands. 1978. The role of termites in ecosystems, pp. 245-292. In M. V. Brian [ed.], Production ecology of ants and termites. Cambridge University Press, Cambridge, England.
- Zoberi, M. H., & J. K. Grace. 1990a. Fungi associated with the subterranean termite *Reticulitermes flavipes* in Ontario. Mycologia 82: 289-294.
- 1990b. Isolation of the pathogen *Beauveria bassiana* from *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 16: 289-296.

Received for publication 8 July 1993; accepted 5 October 1993.