Pathogen Alarm Behavior in a Termite: A New Form of Communication in Social Insects

R.B. Rosengaus (✉), C. Jordan, M.L. Lefebvre, J.F.A. Traniello
Biology Department, Boston University, 5 Cummington Street, Boston, MA 02215, USA
e-mail: rrm@bio.bu.edu, Tel.: +1-617-3536977, Fax: +1-617-3536340

Received: 12 May 1999 / Accepted in revised form: 26 August 1999

Abstract Dampwood termites, *Zootermopsis angusticollis*, show an alarm response after detecting the presence of spores of the pathogenic fungus *Metarhizium anisopliae.* Termites in direct contact with a high concentration of spores (10^7 spores/ml) show a striking vibratory display which appears to convey information about the presence of pathogens to nearby unexposed nestmates through substrate vibration. Nestmates not directly in contact with spores that perceive the vibrational signal increase significantly their distance from the spore-exposed vibrating termites, apparently to escape from the source of infection. The fleeing response is not induced by the presence of the spores alone or by pheromones, and requires the perception of the vibrations propagated through the substrate. This "pathogen alarm behavior" appears to be a previously unrecognized communication mechanism that allows termites to reduce disease risks within the nest.

Termite communication systems generally involve chemical signals which regulate foraging organization (Traniello and Leuthold 2000; Traniello and Robson 1995), alarm behavior and defensive recruitment (Pasteels and Bordereau 1998; Traniello and Beshers 1985) and sexual attraction (Pasteels and Bordereau 1998). However, termites also communicate through substrate-borne vibrations by drumming and head-banging. This form of alarm communication has been described as a response to disturbances such as breaching the nest and exposure to light and air (Howse 1964 1965; Kirchner et al. 1994; Leis et al. 1994; Stuart 1969). We have observed that larvae, nymphs, and imagines of the dampwood termite *Zootermopsis angusticollis* Hagen show a striking vibratory alarm behavior during and after exposure to high concentrations of a fungal pathogen. The mechanical signals produced by the vibratory display appear to communicate information about the presence of pathogens and cause nestmates to respond adaptively by increasing their distance from the source of contagion.

Pathogen alarm behavior appears to be induced by direct contact with fungal spores. During the vibratory motor display the entire body of the termite lunges in an anteroposterior motion while the legs flex dorsoventrally. In our studies we elicited the response consistently by placing ten nymphs of *Z. angusticollis* in a petri dish (100×15 mm) lined with filter paper moistened with a solution of a lethal concentration (10^7 spores/ml) of the fungal pathogen *Metarhizium anisopliae* Metschnikoff (batch #93-09, media #325, ATCC 90448). The spore suspension was prepared by scraping conidia from several infected termite corpses using a flamed metallic loop and then transferring the spores into a 0.1% Tween 80 solution (Rosengaus and Traniello 1997; Rosengaus et al. 1998). Conidia viability was determined by plating 40 μl of the spore suspension on a thin layer of potato dextrose agar and subsequently recording germination at ×400 magnification after an 18 h/25° C incubation period (Rosengaus and Traniello 1997). In the present studies conidia viability was 89.0% ±1.7% ($n=30$ fields of vision).

The induction of alarm behavior is dependent upon the concentration of the fungal pathogen. In the first set of three replicates/concentration no vibratory or other displays were released by concentrations lower than 10^7 spores/ml, nor was alarm behavior elicited in any other context (Table 1). To determine whether the vibrational signal conveys information about the presence of pathogens from spore-exposed termites to unexposed nestmates, we established a second set of three replicates in which termites were observed inside a covered transparent Plexiglas® observation nest (16 cm length × 9 cm

Table 1. Average frequency ±SD of vibrational displays during three observation periods as a function of fungal spore concentration ($n = 3$ replicates/spore concentration). Controls consisted of exposure to a sporeless suspension medium

	Spore concentration (spores/ml)				
Time period after exposure (min)	Control (Tween 80)	7×10^1	7×10^4	7×10^7	P^a
0–10	0	0.3±0.6	0	7.0±1.0	<0.05
10–20	0	0	0.3±0.6	8.3±1.5	<0.05
20–30	0	0	0.3±0.6	11.0±4.3	<0.05
P^b	NS	NS	NS	NS	

Nymphs of *Z. angusticollis* engage in vibratory displays at frequencies that are dependent upon the concentration of *M. anisopliae* spores

[a] Comparison across spore concentrations

[b] Comparisons within concentrations

width × 3 cm height) divided into two equal central nest arenas by a fine mesh barrier (Fig. 1). Each arena was connected by a 3-cm plastic tube to a plastic petri dish (100 × 15 mm) lined with filter paper moistened with 1.0 ml sterile water. We hypothesized that one main effect of the vibratory signal is to cause termites to flee to avoid contact with pathogens. To quantify escape behavior ten individually color-coded nymphs (Testors® enamel paint) were placed in each arena, on opposite sides of the mesh barrier. The mesh barrier (approx. 32 × 12 threads/cm^2, 125-μm grid, L.M. Gerson) eliminated contact between the two groups of nymphs but permitted the transfer of cues related to the presence of fungal spores or signals produced by the termites themselves.

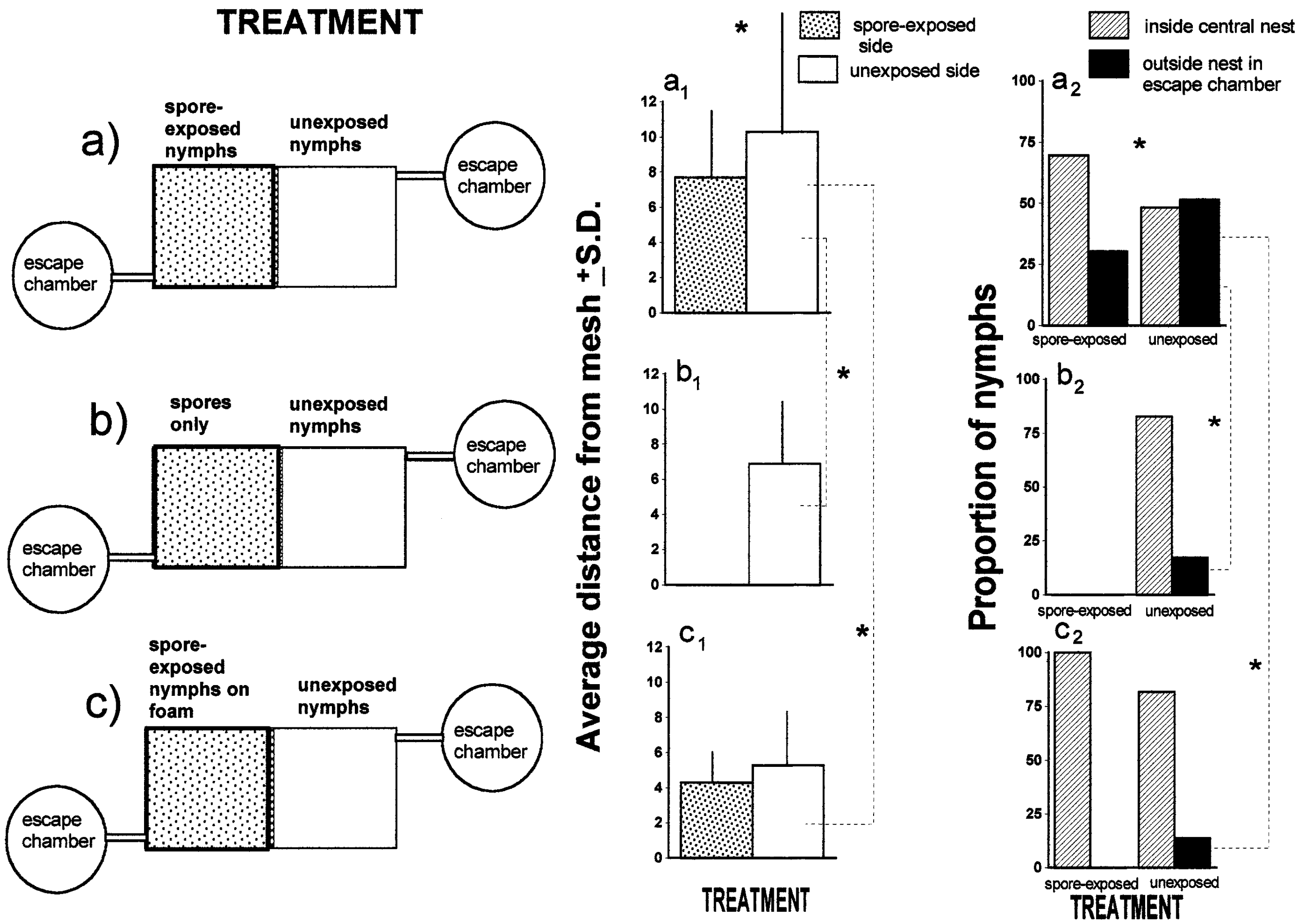

Fig. 1a–c. Average distance ±SD from the mesh barrier and proportion of termites found either inside or outside the main area of the nest (i.e., escape tube and/or escape chamber) for the various experimental designs (*a-c*). Each experimental design was replicated three times using naive nymphs. Data for the spore-exposed side in b_1 and b_2 is not presented because no termites were present in this arena. In treatment *c*, spore-exposed termites walking over the foam engaged in the typical vibrational alarm response. Because the foam covered the escape exit on the spore-exposure side, exposed termites could not escape. Nevertheless, spore-exposed termites did not attempt to escape (see a_1, a_2). Average distances were compared by Mann-Whitney *U* test. The frequencies with which spore-exposed and unexposed termites were found inside and outside the central nest chamber were compared by a 2×2 χ^2 test; $^*P<0.0001$

Termites were allowed to acclimate to the observation nest for 24 h prior to data collection. Nymphs on one side of the mesh were then exposed to spores of *M. anisopliae* by allowing the termites to walk freely over filter paper (Whatman #5) impregnated with 1.5 ml of a 3.5×10^7 spores/ml suspension. Nymphs on the opposite side of the mesh were concurrently allowed to walk over filter paper (Whatman #5) moistened with 1.5 ml of a 0.1% sporeless Tween 80 control solution. The location and distance of each termite from the mesh barrier was recorded every 5 min for 1 h; distance was measured from the location of the head of each termite. The frequency and duration of behavioral acts for termites on either side of the mesh barrier were also recorded in a third set of three replicates (6 h and 40 min per replicate). Termites on each side of the mesh barrier were observed during four time periods: preexposure (baseline), during exposure to spores, immediately after spore exposure, and 24 h postexposure. The behaviors most commonly recorded included locomotion, vibratory display, drumming (head banging), feeding, resting, self-grooming, allogrooming, and licking the nest (see Rosengaus and Traniello 1991, 1993 for a definition of behavioral acts).

Results showed that the spatial dispersion of nymphs was dependent upon the treatment. Termites that directly contacted spores did not evacuate their nest arena but remained in place. Unexposed nestmates on the opposite side of the mesh separating the spore-exposed and unexposed nymphs, however, significantly increased their distance from the mesh (Fig. $1a_1$). Also, the proportion of unexposed termites outside the central nest chamber was higher than the proportion found in the escape chamber of the spore-exposure side (51.6% vs. 30.3%, respectively; Fig. $1a_2$). Spore-exposed termites decreased the frequency and/or the time spent in exploratory and feeding behavior while vibratory displays and allogrooming were performed at significantly higher rates and/or for longer periods of time after nymphs contacted the spores directly (Fig. 2a,b). The frequency and duration of acts of concurrently observed nonexposed termites, in contrast, remained relatively constant across the four observation periods, exhibiting only a tendency toward increased allogrooming (Fig. 2c,d). Other acts such as nest licking, self-grooming, and head drumming remained relatively unchanged over time in both spore-exposed and nonexposed groups (Fig. 2a-d).

The movement away from the source of infection apparently required the presence of nymphs reacting to the spores because the average distance of unexposed termites from the mesh barrier and the proportion of unexposed termites found outside the nest when only spores (but no termites) were present in the adjacent area were significantly lower than when nymphs vibrated after contacting spores (Fig. $1b_1$, $1b_2$). No significant changes were observed in the frequency with which each act was performed relative to the baseline data ($P>0.05$, Kruskal-Wallis test, data not shown). This suggests that changes in the behavior of unexposed termites were not caused by a volatile chemical associated with the spores themselves but rather by the production of either a chemical alarm signal emitted by the spore-exposed termites or by the perception of the substrate vibrations produced by drumming. When the vibrational signal was attenuated by fitting the bottom of the spore-exposure arena with foam ($8.0 \times 9.0 \times 2.5$ cm) prior to allowing nymphs to walk over the spore-treated filter paper, the average distance from the mesh of unexposed nestmates was significantly lower than that when spore-exposed termites produced the vibratory displays directly on the nest floor (compare Fig. $1a_1$, $1c_1$). Also, the proportion of unexposed termites found outside the central nest chamber was significantly lower when the vibratory signals were not dampened by the foam-padded nest floor (compare Fig. $1c_2$, $1a_2$). These results suggest that substrate-borne vibratory signals, not a volatile alarm pheromone, convey information about the presence of pathogens to nearby unexposed nestmates, and that the perception of the vibratory signal is required to induce escape behavior in unexposed nestmates.

Previous work has noted that *Z. angusticollis* produce mechanical alarm signals (Howse 1964, 1965; Kirchner et al. 1994; Stuart 1969), but the association of motor and/or vibrational displays with pathogens has not been described. Although we lack information on the temporal structure and physical attributes of the pathogen alarm signal and thus cannot directly compare it to disturbance-alarm vibrational signals, we believe that the information conveyed by the typical motor patterns associated with nest disturbance differs from that of the pathogen alarm behavior signal. There appears to be important variation in the motor components of the displays: vibratory alarm responses associated with nest disturbance involve alternately striking the roof and floor of the nest galleries with the head, producing an audible sound. Pathogen alarm behavior, in contrast, involves simultaneous anteroposterior and dorsoventral motions, and audible sound does not appear to be produced. Also, termites flee in response to the pathogen alarm rather than aggregate at the source of the stimulus as they do in response to a disturbance alarm (Stuart 1969).

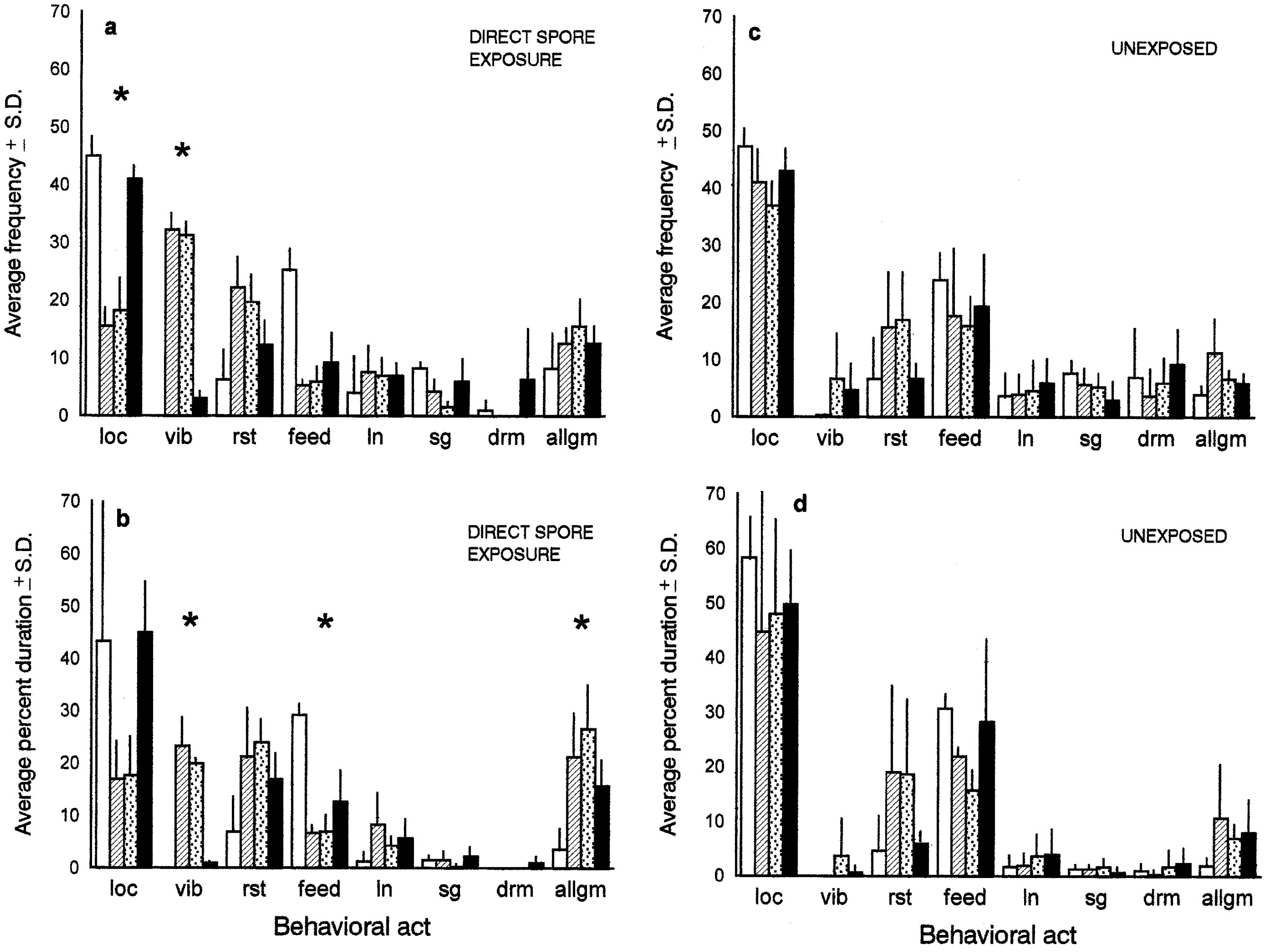

Fig. 2. Average frequency ±SD and average percentage duration ±SD of acts performed by nymphs exposed to 3.5×10^7 spores/ml solution (*a*,*b*) during the periods prior (baseline, *open bars*), during (*hatched bars*), immediately postexposure (*dotted bars*), and 24 h postexposure (*black bars*) to the spores. The average frequency and average percentage duration of acts performed by nonexposed nestmates in the area adjacent to the spore exposed termites (*c*,*d*, respectively) were recorded concurrently as the data for *a* and *b*. $^*P<0.05$ (Kruskal-Wallis test, SPSS 1990). Behavioral acts: *loc* locomotion; *vib* vibratory display; *rst* rest; *feed* feed; *ln* lick nest; *sg* self-grooming; *drm* head drumming; *allgm* allogrooming

The induction of pathogen-alarm behavior in *Z. angusticollis* is related to the presence of high fungal spore concentrations and may result from detecting by contact chemoreception the components of the conidial epispore. *Kalotermes flavicollis* nymphs and pseudergates react to the introduction of xenochemicals such as acetic acid, phenol, and acetone into the nest by engaging in a convulsive vibratory movement (Leis et al. 1994). This display appears to be unrelated to the longitudinal vibratory movement, each motor pattern having a different triggering stimulus and conferring different information to nestmates. However, it is unclear whether the perception of the *K. flavicollis* displays alters the behavior of nestmates (Leis et al. 1994). Recently Connétable et al. (1998) showed that the perception of head-banging displayed by *Pseudacanthotermes* after nest disturbance elicits escape behavior in workers.

The use of substrate vibration as a communication signal is not unique to termites. In other social and subsocial insects, stridulation, shaking, and head drumming appear to be associated with increasing nestmate activity (Seeley et al. 1998), recruitment (Baroni-Urbani et al. 1988; Roces and Hölldobler 1995, 1996), the modulation of responsiveness to other signals (Fuchs 1976; Hölldobler 1998; Salanga et al. 1998), the facilitation of leaf cutting (Roces and Hölldobler 1996; Roces et al. 1993; Tautz et al. 1995), and defense (Cocroft 1996). Vibrational communication thus has been described in several contexts in social insects, but not in the communication of information about disease. To the best of our knowledge, our results represent the first demonstration of pathogen alarm behavior in a social insect. We suggest that the pathogen-induced vibratory display of *Z. angusticollis* and its corresponding induction of an escape behavior in nestmates represents an adaptation to reduce infection risk within their microbially rich nests.

We thank the administrators of the Redwood East Bay Regional Park for allowing us to collect termites on their property, and the comments of two anonymous reviewers. This research was funded by grant IBN-9632134 from the National Science Foundation (J.F.A.T. and R.D. Karp, sponsors). The experiments described here comply with the current laws of the United States.

Baroni-Urbani C, Buser MW, Schilliger E (1988) Substrate vibration during recruitment in ant social organization. Insectes Soc 35:241–250

Cocroft RB (1996) Insect vibrational defence signals. Nature 382:679–680

Connétable S, Robert A, Bordereau C (1998) Vibratory alarm signaling in two higher termite species: *Pseudacanthotermes spiniger* and *P. militaris* (Termitidae, Macrotermitinae). In: Schwarz P, Hogendoorn K (eds) Social insects at the turn of the millennium. Proceedings of the XIII International Congress of IUSSI, Adelaide

Fuchs S (1976) An informational analysis of the alarm communication by drumming behavior in nests of Carpanter ants (*Camponotus*, Formicidae, Hymenoptera). Behav Ecol Sociobiol 1:315–336

Hölldobler B (1998) Multicomponent signals in ant communication. In: Schwarz P, Hogendoorn K (eds) Social insects at the turn of the millennium. Proceedings of the XIII International Congress of IUSSI, Adelaide

Hölldobler B, Wilson EO (1990) The Ants. Harvard University Press, Cambridge

Howse PE (1964) The significance of the sound produced by the termite *Zootermopsis angusticollis* (Hagen). Anim Behav 12:284–301

Howse PE (1965) On the significance of certain oscillatory movements of termites. Insectes Soc 12:335–346

Kirchner WH, Broecker I, Tautz J (1994) Vibrational alarm communication in the damp-wood termite *Zootermopsis nevadensis*. Physiol Entomol 19:187–190

Leis M, Angelini I, Sbrenna-Micciarelli A, Sbrenna G (1994) Further observations on intercaste communication in *Kalotermes flavicollis*: frequence of vibratory movements under different experimental conditions. Ethol Ecol Evol 3:11–16

Pasteels JM, Bordereau C (1998) Releaser pheromones in termites. In: Vander Meer RK, Breed MD, Winston ML, Espilie KE (eds) Pheromone communication in social insects. Westview, Boulder

Roces F, Hölldobler B (1995) Vibrational communication between hitchhikers and foragers in leaf-cutting ants (*Atta cephalotes*). Behav Ecol Sociobiol 37:297–302

Roces F, Hölldobler B (1996) Use of stridulation in foraging leaf-cutting ants: mechanical support during cutting or short-range recruitment signal? Behav Ecol Sociobiol 39:293–299

Roces F, Tautz J, Hölldobler B (1993) Stridulation in leaf-cutting ants: short-range recruitment through plant-borne vibrations. Naturwissenschaften 80:521–524

Rosengaus RB, Traniello JFA (1991) Biparental care in incipient colonies of the dampwood termite *Zootermopsis angusticollis* Hagen (Isoptera: Termopsidae). J Insect Behav 4:633–647

Rosengaus RB, Traniello JFA (1993) Temporal polyethism in incipient colonies of the primitive termite *Zootermopsis angusticollis*: a single multi-age caste. J Insect Behav 6:237–252

Rosengaus RB, Traniello JFA (1997) Pathobiology and disease transmission in dampwood termites [*Zootermopsis angusticollis* (Isoptera: Termopsidae)] infected with the fungus *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes). Sociobiology 30:185–195

Rosengaus RB, Maxmen AB, Coates LE, Traniello JFA (1998) Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). Behav Ecol Sociobiol 44:125–134

Salanga MC, Malina B, Robson SK (1998) Chemical and vibrational alarm communication in two species of nest-weaving ants, *Polyrachis queenslandica* and *P. yarrabahensis* (Formicinae). In: Schwarz P, Hogendoorn K (eds) Social insects at the turn of the millennium. Proceedings of the XIII International Congress of IUSSI, Adelaide

Seeley TD, Weidenmuller A, Kuhnholz S (1998) The shaking signal of the honey bee informs workers to prepare for greater activity. Ethology 104:10–26

Stuart AM (1969) Social behavior and communication. In: Krishna K, Weesner FM (eds) Biology of termites. Academic, New York

Tautz J, Roces F, Hölldobler B (1995) Use of a sound-based vibrotome by leaf-cutting ants. Science 267:84–87

Traniello JFA, Beshers SN (1985) Species-specific alarm/recruitment responses in a neotropical termite. Naturwissenschaften 72:491–492

Traniello JFA, Leuthold RH (2000) The behavior and ecology of foraging in termites. In: Bignell D, Abe T, Higashi M (eds) Termites: evolution, sociality, symbioses, ecology. Kwyler, the Hague (in press)

Traniello JFA, Robson S (1995) Trail and territorial pheromones in the social insects. In: Bell WJ, Carde RT (eds) Chemical ecology of insects, Chapman and Hall, New York