

# *(E,E)*- $\alpha$ -Farnesene, an Alarm Pheromone of the Termite *Prorhinotermes canalifrons*

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**Abstract** The behavioral and electroantennographic responses of *Prorhinotermes canalifrons* to its soldier frontal gland secretion, and two separated major components of the secretion, (*E*)-1-nitropentadec-1-ene and (*E,E*)- $\alpha$ -farnesene, were studied in laboratory experiments. Behavioral experiments showed that both the frontal gland secretion and (*E,E*)- $\alpha$ -farnesene triggered alarm reactions in *P. canalifrons*, whereas (*E*)-1-nitropentadec-1-ene did not affect the behavior of termite groups. The alarm reactions were characterized by rapid walking of activated termites and efforts to alert and activate other members of the group. Behavioral responses to alarm pheromone differed between homogeneous and mixed groups, suggesting complex interactions. Antennae of both soldiers and pseudergates were sensitive to the frontal gland secretion and to (*E,E*)- $\alpha$ -farnesene, but soldiers showed stronger responses. The dose responses to (*E,E*)- $\alpha$ -farnesene were identical for both soldiers and pseudergates, suggesting that both castes use similar receptors to perceive (*E,E*)- $\alpha$ -farnesene. Our data confirm (*E,E*)- $\alpha$ -farnesene as an alarm pheromone of *P. canalifrons*.

**Keywords** Alarm behavior · Alarm pheromone · (*E,E*)- $\alpha$ -farnesene · Chemical communication · Termites · EAG · Isoptera · Rhinotermitidae

## Introduction

Termites, as creatures generally living in permanent darkness, largely rely upon chemical communication during intraspecific interactions. Trail-following pheromones produced by the sternal gland (Matsumura et al. 1968; Bordereau et al. 1991; Laduguie et al. 1994; Peppuy et al. 2001), or the food-marking pheromone produced by the labial glands (Reinhard et al. 2002) are well known. Other means of chemical communication include alarm pheromones produced by the frontal gland of soldiers (Vrkoč et al. 1978; Roisin et al. 1990; Reinhard and Clément 2002), and sexual pheromones produced by tergal (Bordereau et al. 2002), posterior sternal (Peppuy et al. 2004), or sternal glands in alate imagoes (McDowell and Oloo 1984; Bordereau et al. 1991; Laduguie et al. 1994). In addition, there are several other glands whose functions are still unknown or hypothetical, such as the mandibular glands (Cassier et al. 1977; Šobotník and Hubert 2003), tarsal glands (Bacchus 1979), or epidermal glands (Šobotník et al. 2003).

When disturbed, termites communicate alarm mechanically via vibrations (Howse 1964, 1965; Connétable et al. 1999; Röhrig et al. 1999), or with alarm pheromones. With some exceptions (Maschwitz and Müllhenberg 1972; Traniello et al. 1984), pheromones mediate alarm communication in termite species with a developed frontal gland, i.e., in the families Serritermitidae, Rhinotermitidae, and Termitidae (Noirot 1969; Costa-Leonardo and Kitayama 1991). Although the involvement of the frontal gland

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secretion in alarm communication has been proven for many species (Kriston et al. 1977; Kaib 1990; Roisin et al. 1990; Pasteels and Bordereau 1998; Reinhard and Clément 2002), a striking gap exists in our knowledge of the chemicals actually involved in alarm communication. Alarm pheromones have been identified only in three *Nasutitermes* species, including  $\alpha$ -pinene and limonene in *N. rippertii*, carene and limonene in *N. costalis* (Vrkoč et al. 1978), and  $\alpha$ -pinene in *N. princeps* (Roisin et al. 1990). The production of alarm pheromones may also be linked with soldier polymorphism: alarm pheromones are probably produced solely by small soldiers in *Schedorhinotermes lamanianus* (Kaib 1990) or by large soldiers in *Nasutitermes exitiosus* (Kriston et al. 1977).

The genus *Prorhinotermes* (Isoptera: Rhinotermitidae) is the only genus in the subfamily Prorhinotermitinae (Quennedey and Deligne 1975). Its crucial phylogenetic position within Rhinotermitidae as well as a peculiar combination of primitive and advanced characteristics makes it an attractive model to study. *Prorhinotermes* species have a linear ontogenetic scheme lacking a true worker caste; the work tasks are performed by temporarily specialized helpers, pseudergates, which retain capacity to become either alate imago (through a single nymphal instar) or neotenic reproductives (by a single molt; Roisin 1988). Soldiers in *Prorhinotermes* originate from pseudergates or larvae through an intermediary presoldier instar (Roisin 1988; Hanus et al. 2006). They are abundant (up to 22%), they guard inside the nest and on its peripheries, but also act as defenders when the nest is being moved (Haverty 1977, Roisin et al. 2001). Soldiers' defense includes their piercing mandibles and a powerful frontal chemical weapon; they also perform vibratory alarm movements in response to disturbance (Deligne et al. 1981; Hanus et al. 2005).

The frontal gland secretion in soldiers of the genus *Prorhinotermes* contains predominantly (*E*)-1-nitropentadec-1-ene, with small amounts of sesquiterpenes (Vrkoč and Ubik 1974; Hanus et al. 2006; Piskorski et al. 2007). On average, the frontal gland of *P. canalifrons* soldiers contains 153  $\mu$ g of (*E*)-1-nitropentadec-1-ene and 1.8  $\mu$ g of

(*E,E*)- $\alpha$ -farnesene, the latter representing about 65% of its sesquiterpenoid content (Piskorski et al. 2007). Whereas the defensive function of the frontal gland secretion, due to the large amounts of toxic (*E*)-1-nitropentadec-1-ene, is well established (Kuldová et al. 1999), its role in alarm communication and the contribution of (*E,E*)- $\alpha$ -farnesene remain hypothetical. The aims of the experiments described in this paper were as follows: (1) to determine whether the soldier frontal gland secretion is responsible for alarm communication in *P. canalifrons*; (2) to determine if (*E,E*)- $\alpha$ -farnesene mediates the alarm reaction; (3) to evaluate potential differences in alarm reaction among castes; (4) to verify the function of the putative alarm pheromone in conditions closer to natural ones, i.e., at the level of a whole colony. Thus, we studied the alarm reactions in homogeneous (formed by either pseudergates or soldiers) and heterogeneous termite groups (formed by pseudergates and soldiers), and subsequently in small complete colonies. As experimental stimuli, the whole frontal gland secretion, and solutions of synthesized (*E*)-1-nitropentadec-1-ene and (*E,E*)- $\alpha$ -farnesene were used. Electrophysiological responses of termite antennae were also recorded to investigate possible differences between castes in sensitivity to gland secretion, and (*E,E*)- $\alpha$ -farnesene.

## Materials and Methods

**Termites** The colonies of *Prorhinotermes canalifrons* (Sjöstedt 1904) used for experiments originated from Saint-Denis (Réunion). The termites were collected in 2001 and since then were reared in containers in permanent darkness at 27°C and elevated humidity. Wet fine sand served as substratum. Colonies were continuously provided with decayed birch wood. The experimental design described below is summarized in Table 1.

**Experimental Groups** Termite groups of 4 different caste compositions were tested: (A) 40 pseudergates +10 soldiers (close to natural proportions, Haverty 1977), (B) 50

**Table 1** The design of experiments: caste composition, stimuli used, number of repetitions, and number of individuals measured in experiments A–D

Experiment	Caste Composition	Stimuli	Individuals Measured per Repetition	No. of Repetitions per Stimulus	Individuals Measured per Stimulus	Individuals Measured
A	40 Ps+10 sold	bl, hex, npd, fg, far	10 Ps+5 sold	5	50 Ps+25 sold	250 Ps+125 sold
B	50 Ps	bl, hex, npd, fg, far	10 Ps	2	20	100 Ps
C	20 sold	bl, hex, npd, fg, far	10 sold	2	20	100 sold
D	Ps+sold+neo+pres+L	fg, far	Not evaluated	4	0	0

Ps pseudergate, sold soldier, neo neotenic, pres presoldier, L larva, bl blank control, hex hexane, npd (*E*)-1-nitropentadec-1-ene, fg frontal gland secretion, far (*E,E*)- $\alpha$ -farnesene

pseudergates, (C) 20 soldiers, and (D) incipient colonies. In experiments A and B, termites were transferred from the original colonies into experimental arenas 20–24 h prior to observation to allow to acclimatize after manipulation. This delay was reduced to 4–6 h only in experiment C because soldiers are care-dependent. The incipient colonies (experiment D) originated from groups of 60 pseudergates isolated and left to develop for 8 months. At the time of experimentation, these groups consisted of 25–35 pseudergates, 6–10 soldiers, 5–15 neotenic, 0–2 presoldiers, 0–5 larvae from the first to the third instar, and 0–8 eggs. The complete population of each small colony was carefully extracted from its original box and transferred to the experimental chamber inside the birch wood (see Fig. 1). The corridor connecting the chamber with the exterior was initially closed with a piece of filter paper, and was opened after 2 days. Behavioral tests were performed from the next day on.

**Experimental Design** All experiments were performed at 27°C under red light in Petri dishes (85 mm diameter) with wet filter paper as substratum (Fig. 1). A piece of birch wood served as a food and natural substrate. The test substance was loaded onto a piece of filter paper (7×3 mm) and introduced immediately into the Petri dish through a slit in the dish cap. The filter paper was hung out of reach of termites by a pin bridged over the slit.

The following stimuli were tested in experiments A–C: (1) untreated paper (blank control), (2) 1 µl of pure hexane (solvent control), (3) 31 µg of (*E*)-1-nitropentadec-1-ene (~0.2 frontal gland equivalent, FGE) in 1 µl of hexane, (4) one soldier frontal gland secretion (1 FGE), (5) 1.5 µg of (*E,E*)- $\alpha$ -farnesene (~1 FGE) in 1 µl of hexane. Each group was tested once for one stimulus and once for the control in random order. In experiment D, only two stimuli were

tested: (4) frontal gland secretion, and (5) (*E,E*)- $\alpha$ -farnesene in random order with at least 2-day intervals between exposures (each stimulus tested once in every group).

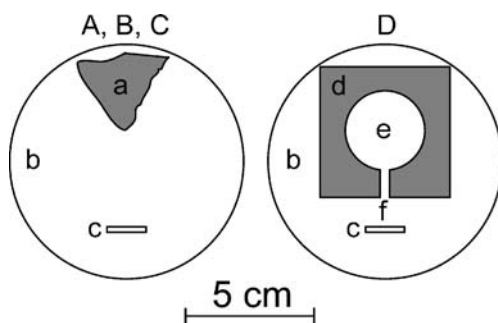
The frontal gland secretion of a soldier was obtained by decapitation, evacuation of the thoracic and abdominal part of the frontal gland reservoir with a slight pressure by forceps tip against the body, and then smashing the head on the test filter paper. For synthesis of (*E*)-1-nitropentadec-1-ene (>99%) (see Kuldová et al. 1999). (*E,E*)- $\alpha$ -farnesene (68%; from plant material; minor components incl.  $\alpha$ -caryophyllene, hydrocarbon and sesquiterpene oxidation products) was provided by Anna-Karin Borg-Karlson, KTH, Sweden. Minor components included  $\alpha$ -caryophyllene, sesquiterpene, and hydrocarbon oxidation products.

**Recording** The behavior of all termite groups was recorded with a Panasonic WV-CL920 camera and a Panasonic DVD hard drive recorder DMR-HS2. Each recording started 2 min before introduction of the stimulus and lasted for 7 min. The parameter evaluated in behavioral experiments was the speed of termite walking. New software, Mouse-Tracer, was developed to track the position of the mouse cursor. The cursor was controlled by the experimenter, and the selected termite was followed on the screen. The cursor position, recorded 20 times per second of observation, was initially expressed in pixels and subsequently converted into millimeters.

The selection of termites to track in all experiments was designed to include all possible termite statuses at the beginning of the experiment: individuals close and far from the introduction slit, in the center and at the periphery of a termite group, individuals resting or walking in the arena, etc.

**Statistics** Experiment A was repeated five times for each stimulus, B and C twice, and then the data were merged for a particular stimulus. In each repetition of experiment A, the tracks of ten pseudergates and five soldiers were analyzed; ten individuals were tracked in experiments B and C. Thus, 50 pseudergates and 25 soldiers were analyzed in A, 20 pseudergates in B, and 20 soldiers in C for each stimulus. Experiment D was performed with four colonies, but the data were not evaluated statistically because termites often walked on the vertical walls of the chamber, which prevented them from being tracked.

Kruskal–Wallis rank tests and multiple comparisons of non-parametric data were used to compare the speed of particular castes before and after insertion of the stimulus, as well as the antennal responses in electroantennography (EAG) experiments compared with controls. The walking speeds between A, B, and C and the latency in response to olfactory stimuli were compared by means of nonparametric Mann–Whitney *U* tests. The curves in Fig. 2 were

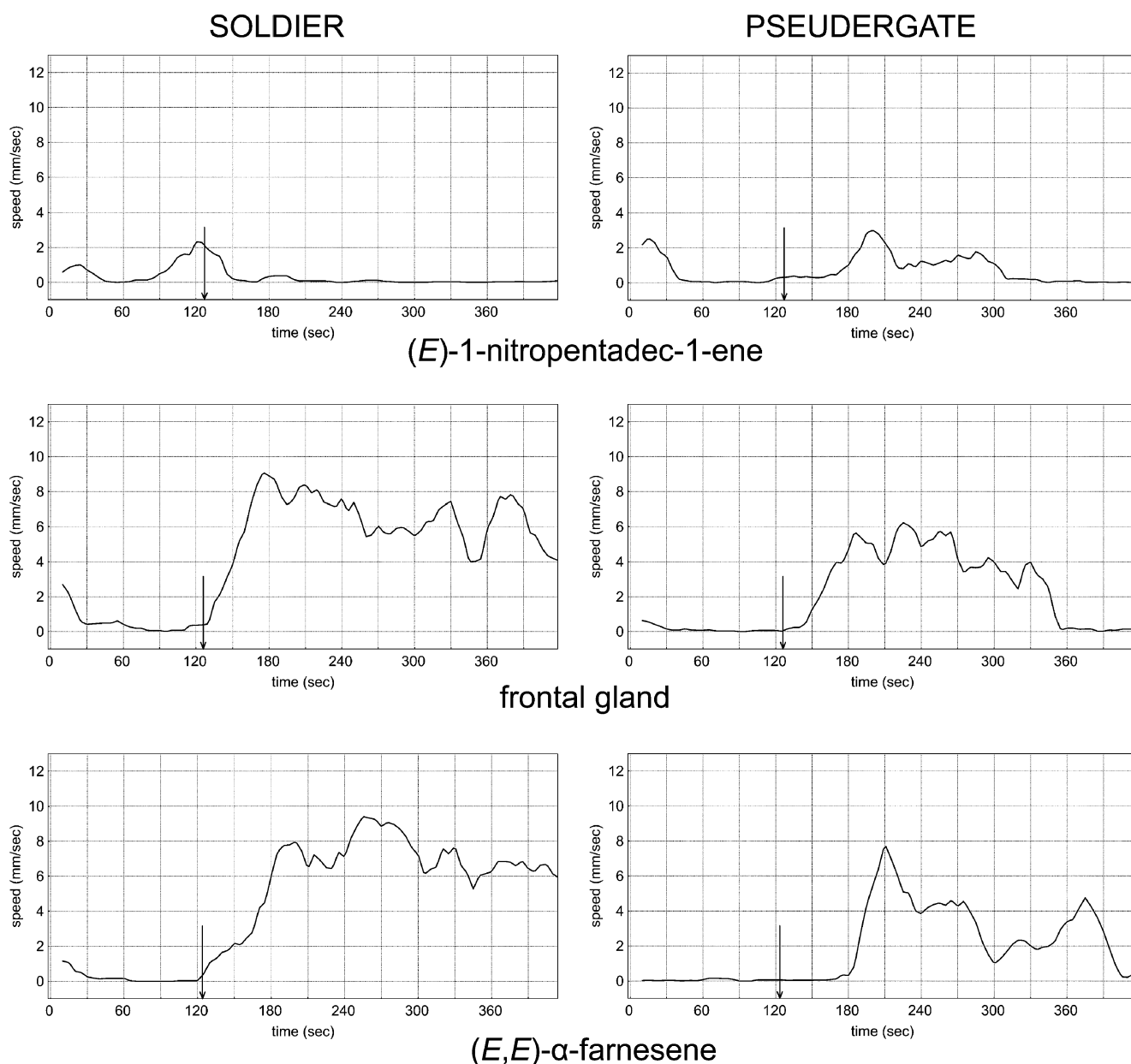


**Fig. 1** Schematic drawing of the experimental design of behavioral bioassays: *left*, experiments A–C; *right*, experiment D. The arenas are drawn to scale; *a*, piece of birch wood; *b*, filter paper; *c*, slit in the lid of the Petri dish through which filter paper loaded with test solution was hung; *d*, birch wood test chamber; *e*, central arena where whole incipient colonies were placed, reaching from bottom to top of the dish; *f*, narrow corridor, the only connection between the central arena and the exterior

obtained by using the moving average of the distances walked by the individual every 0.05 s, expressed as the average for every 5 s. Statistica® 7.1 was used for all calculations.

**Electroantennography** EAG recordings were performed by using individual antennae of pseudergates and soldiers with the antennal tip cut off. Glass Ag/AgCl microelectrodes filled with insect saline solution were used. Electrodes were connected to an amplifier (Syntech), and the tenfold amplified signal was fed to a PC via an IDAC 2 PC board (Syntech). (*E,E*)- $\alpha$ -farnesene was diluted in hexane in

decadic steps (10 pg–10  $\mu$ g/ $\mu$ l). From each concentration, 1  $\mu$ l of stimulus solution was loaded onto a 1  $\times$  0.5 cm piece of filter paper inserted in a Pasteur pipette. After solvent evaporation, the loaded odorant cartridges were sealed with parafilm and stored at  $-20^{\circ}\text{C}$  until used. Prior to each experiment, the cartridges were allowed to warm up for 1 h at room temperature. Stimuli were delivered by injection of an air pulse (1 s, air flow 0.8 l/min) through an odorant cartridge into a continual air stream directed towards the antenna. Standard stimuli, i.e. cartridges with filter paper loaded with 100 ng of (*E,E*)- $\alpha$ -farnesene, were applied at the beginning and the end of each experiment to determine



**Fig. 2** Typical dynamic recordings of walking speed of soldiers and pseudergates exposed to different olfactory stimuli (experiment A). The curves represent a moving average of distances walked scored second by second. Arrows mark the insertion of the stimulus

possible changes in antennal sensitivity during EAG recordings and to allow normalization between experiments. The antennal responses (maximal negative deflection during stimulation in mV) were normalized to these standard stimuli and expressed in percent. Each stimulus was tested once on each antenna, with 19 different antennae per stimulus used; thus, the data analyzed represent 19 independent measurements for each stimulus.

## Results

**General Features of Alarm Behavior** At the beginning of each experiment, most termites were quietly clustered near the piece of wood, and usually only a few individuals, predominantly soldiers, would slowly explore the arena. After insertion of an untreated filter paper, termite activity increased slightly for a while due to the disturbance, but decreased within a few seconds. Similar responses were elicited by hexane and (*E*)-1-nitropentadec-1-ene solution. On the other hand, insertion of a paper treated with frontal gland secretion or (*E,E*)- $\alpha$ -farnesene solution led to a long-lasting activation characterized by fast walking, scanning of the space with straightened antennae, zigzag searching for the odor source, mandibular opening and closing (in soldiers), and nestmate alerting. Activation led to marked change in termite distribution within the arena, namely the accumulation of termites below the odor source.

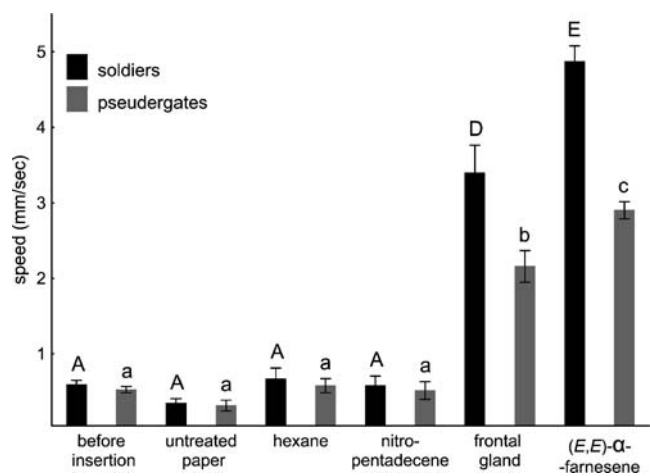
During nestmate activation, the alerting termites (usually soldiers, less commonly also pseudergates) searched for quiescent termites, touched them with their antennae, and subsequently performed a series of longitudinal vibrations. This behavior was usually repeated several times (most frequently 2–4 times during 3–10 s) until the alerting termite calmed down or until all nestmates were alerted. Soldiers reacted to alerting stimulation more easily than pseudergates: one alerting signal was usually enough to activate a soldier, whereas repeated alerting was often necessary to activate a pseudergate. The behavioral responses elicited by the frontal gland secretion did not differ qualitatively from those elicited by (*E,E*)- $\alpha$ -farnesene. Typical time courses of activation of soldiers and pseudergates are shown in Fig. 2.

**Heterogeneous Groups of 40 Pseudergates + 10 Soldiers—Experiment A** The results of Experiment A are summarized in Fig. 3. Exposure to frontal gland secretion increased the walking speed in both pseudergates and soldiers ( $P < 10^{-6}$  for both): the average speed increased from 0.53 to 2.15 mm/s in pseudergates, and from 0.58 to 3.39 mm/s in soldiers. The same was observed for (*E,E*)- $\alpha$ -farnesene, which increased the speed of walking to 2.9 mm/s in

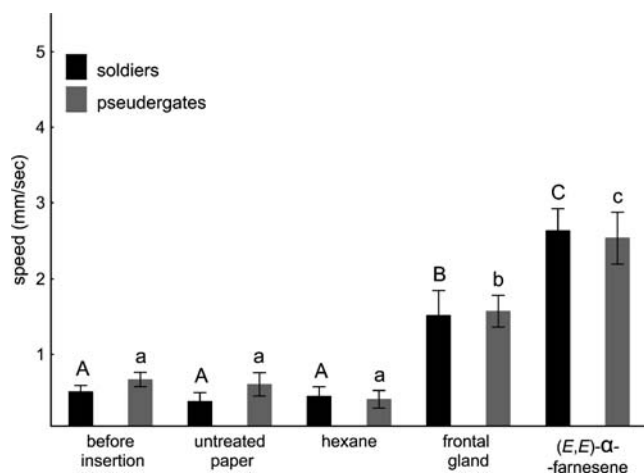
pseudergates, and to 4.87 mm/s in soldiers ( $P < 10^{-6}$  for both). (*E,E*)- $\alpha$ -farnesene at the dose used apparently represented a more effective stimulus than the frontal gland secretion for both pseudergates and soldiers.

Soldiers generally reacted more quickly than pseudergates, as documented by individual measurements of 12 soldiers and 14 pseudergates exposed to (*E,E*)- $\alpha$ -farnesene: median value of the first maximum in walking speed was 20 s in soldiers and 40 s in pseudergates ( $P < 7 \times 10^{-3}$ ). With the exception of differences in reaction times, the responses of pseudergates and soldiers were similar. About 1 min after the introduction of the paper treated with (*E,E*)- $\alpha$ -farnesene or frontal gland secretion, all termites ran vigorously in the arena.

**Homogeneous Groups of 50 Pseudergates or 20 Soldiers—Experiments B and C** The responses of homogeneous groups of either pseudergates or soldiers were qualitatively similar compared to the mixed groups in experiment A: hexane and nontreated paper elicited no significant responses (Fig. 4). In contrast, the locomotion of soldiers and pseudergates was significantly faster after exposure to the frontal gland secretion or (*E,E*)- $\alpha$ -farnesene. The increase in speed in pseudergates did not differ from that in mixed groups. In soldiers, on the other hand, the increase was lower for the frontal gland secretion (1.51 vs. 3.39 mm/s in mixed groups;  $P < 6 \times 10^{-4}$ ) as well as for (*E,E*)- $\alpha$ -farnesene (2.63 vs. 4.87 mm/s in mixed groups;  $P < 10^{-6}$ ) when compared to observations in heterogeneous groups.



**Fig. 3** Average walking speed of soldiers and pseudergates during behavioral bioassays with mixed groups (experiment A) exposed to various olfactory stimuli before (the two columns on the left,  $n = 125$  soldiers and 250 pseudergates) and after the insertion of the stimulus ( $n = 25$  soldiers and 50 pseudergates for each stimulus). Columns represent means, whiskers represent SEM. The columns marked with different letters are significantly different (Kruskal–Wallis test;  $P < 0.05$ ; multiple non-parametric comparison for unequal number of observations)



**Fig. 4** Average speed of soldiers and pseudergates from single-caste groups (experiments B or C) exposed to different olfactory stimuli during behavioral bioassays. Columns represent means, whiskers represent SEM. The columns marked with different letters are significantly different (Kruskal–Wallis test;  $P < 0.05$ ; multiple non-parametric comparison for unequal number of observations)

*Incipient Colonies—Experiment D* Prior to the introduction of the test substances, members of all castes of incipient colonies were randomly distributed within the chamber with at least one soldier patrolling the corridor that connected the chamber with the outside. Introduction of (*E,E*)- $\alpha$ -farnesene or frontal gland secretion strikingly affected the walking speed of termites. The alerting behavior was also performed, predominantly by soldiers. The most prominent effect was the accumulation of the soldiers in the narrow corridor or in its vicinity, whereas members of other castes revealed a tendency to move to the parts of the chamber located farthest from the entrance or to climb up the chamber walls. Soldiers (and never a member of any other caste) were often seen to leave the corridor and perform zigzag searching; one to three soldiers were observed out of the chamber in four out of eight experiments recorded.

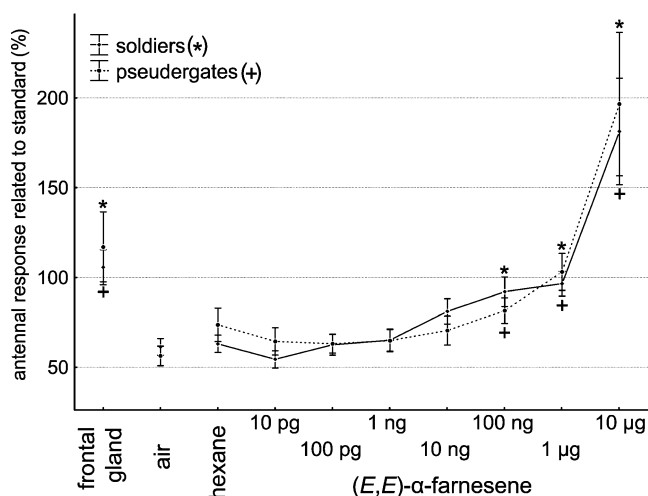
*Electroantennography* Antennae of both pseudergates and soldiers responded to frontal gland secretion and to (*E,E*)- $\alpha$ -farnesene (Fig. 5). The responses of soldiers to particular stimuli were slightly but significantly higher than that of pseudergates: the mean values for the standard stimulus were 0.253 mV in pseudergates and 0.327 mV in soldiers ( $P < 0.04$ ).

On the other hand, the responses of the two castes did not differ qualitatively: the dose response curves expressed as percent of the standard were identical for both castes (Fig. 5). The EAG response threshold to (*E,E*)- $\alpha$ -farnesene (compared to air control) was 100 ng for both castes. Above this threshold, the EAG response increased with the doses of (*E,E*)- $\alpha$ -farnesene: no EAG saturation was

observed for the biologically relevant concentrations tested. The EAG response to the frontal gland secretion from a single soldier was approximately equivalent to that elicited by 1  $\mu$ g of (*E,E*)- $\alpha$ -farnesene.

## Discussion

Our results show that the frontal gland secretion is involved in alarm signaling in *P. canalifrons*. However, the major constituent of the frontal gland secretion, (*E*)-1-nitropentadec-1-ene, has no alarm effect. The function of this highly toxic compound (see Kuldová et al. 1999) can, therefore, be considered as purely defensive. In contrast, (*E,E*)- $\alpha$ -farnesene triggered several changes in behavior including increase in walking speed, searching for the odor source (zigzag running), attraction of both soldiers and pseudergates to the source (experiments A, B, and C), or attraction of soldiers and withdrawal of members of other castes from it (experiment D), and alerting of nestmates, performed predominantly by soldiers. Thus, (*E,E*)- $\alpha$ -farnesene clearly functions as an alarm pheromone in *P. canalifrons*. Together with the dominant (*E*)-1-nitropentadec-1-ene, isomers of  $\alpha$ -farnesene also have been documented in species-specific mixtures in the frontal gland secretions of other *Protermiter* species, *P. simplex* and *P. inopinatus* (Piskorski et al. 2007). As in *P. canalifrons*, we predict that



**Fig. 5** EAG responses recorded from antennae of soldiers and pseudergates exposed to control stimuli (air and hexane), to the content of the soldier frontal gland, and to (*E,E*)- $\alpha$ -farnesene in seven doses. The responses are related to the value of response to a standard stimulus, i.e., 100 ng of (*E,E*)- $\alpha$ -farnesene. Nineteen measurements were performed for each stimulus. Points represent means, whiskers represent SEM. The values marked with asterisks (for soldiers) or crosses (for pseudergates) are significantly different from the air control (Kruskal–Wallis test;  $P < 0.05$ ; multiple non-parametric comparison)

these compounds will mediate alarm communication in these two species.

Behavioral effects of farnesene isomers have been recorded from numerous insect species. For example, (*E,E*) and/or (*Z,E*) isomers of  $\alpha$ -farnesene act as sexual or trail pheromones in several insect orders, including many ant genera (e.g., Cammaerts 1973; Attygalle and Morgan 1983; Van der Meer 1983; Detrain et al. 1987). (*E*)- $\beta$ -farnesene has been shown to be an alarm pheromone of aphids (Dawson et al. 1990) and a kairomone for their predators (ladybirds; Al Abassi et al. 2000; Francis et al. 2004). It has also been found in soldiers of *Reticulitermes* species (Quintana et al. 2003).

Although (*E,E*)- $\alpha$ -farnesene mediated an alarm response in both soldiers and pseudergates of *P. canalifrons*, soldiers were more sensitive, showing a stronger response in both behavioral tests and EAG. Similar EAG dose response curves suggests that both castes may use the same type of receptors. Thus, the difference in antennal sensitivity is probably based on differences in numbers of sensillae rather than on differences in sensitivity of olfactory receptor neurons tuned to (*E,E*)- $\alpha$ -farnesene. The numbers of sensillae between soldiers and pseudergates were not compared, but the soldiers' antennae are longer due to an increase in the number of antennal segments during soldier development from pseudergates (see Hanus et al. 2006).

The fact that soldiers are more sensitive to the alarm pheromone than pseudergates reflects their different social roles within termite societies. In this respect, the higher latency of response to chemical alarm in pseudergates can also be understood as caste specialization. The lower sensitivity and longer latency may ensure that pseudergates are not disturbed by stimuli that eventually appear as not dangerous, and rely upon soldiers to be alerted. The lower sensitivity of pseudergates is compensated by soldier nestmate alerting behavior mediated by mechanical means. Interestingly, the behavioral responses of soldiers to the alarm pheromone were more pronounced in heterogeneous groups containing both soldiers and pseudergates. In the absence of pseudergates, soldiers may exhibit lower activity simply because there are no other castes to alert.

A curious phenomenon that has been repeatedly observed in experiments with alarm pheromones is the accumulation of termites near the source of odor (Roisin et al. 1990, Reinhard and Clément 2002). We suggest that this response may be an artifact of the situation during testing because this was never observed when more natural conditions were used, i.e., in experiment D.

In both behavioral and EAG tests, the responses of both castes to the frontal gland content of one soldier were lower than to the equivalent dose of (*E,E*)- $\alpha$ -farnesene in hexane solution. This is probably due to (1) incomplete emptying of the gland on the tested paper or/and (2) different physical

properties of the two solutions: hexane, being more volatile than (*E*)-1-nitropentadec-1-ene (which constitutes over 90% of the secretion), may enhance the evaporation of (*E,E*)- $\alpha$ -farnesene and thus volatilize higher amounts of alarm pheromone during the experiment. When considering the lower threshold dose of the alarm pheromone for sensorial and behavioral responses of pseudergates and soldiers, approximately 10% of the total amount of (*E,E*)- $\alpha$ -farnesene in one soldier frontal gland, one has to keep in mind that many soldiers participate in the alarm communication in a colony. It can thus be initiated and mediated by only a partial emptying of the frontal gland reservoir of a number of soldiers.

Termite soldiers are specialized for fulfilling defensive tasks to such a degree that they are not able to feed themselves and are fully dependent on the pseudergates' care. Their fundamental importance in *Prorhinotermes*, documented by their high proportion in the colony compared to other termite species (7–22%; Haverty 1977), comprises a wide variety of functions that include guarding the nest and foraging groups, direct physical defense, initiation and mediation of alarm by both vibratory and chemical means, and egg evacuation after an attack on the nest (Roisin et al. 2001; Hanus et al. 2005). Nevertheless, the role of pseudergates (or true workers in other termite species) in colony defense should not be overlooked because in many cases, they play a crucial role in defense (Thorne and Haverty 1991; Shelton and Grace 1996; Clément and Bagnères 1998).

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