NORMALIZED MODEL SYSTEM FOR THE STUDY OF TERMITE MANAGEMENT

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Abstract: A set of partial working procedures has been designed for standardizing the laboratory setup modeling the control of harmful acitivity of termites in nature by affecting caste differentiation by a juvenogen. This set of verified procedures monitors the metabolism of the juvenogen in both the worker and the metamorphosis-derived soldier. The standardization permits an evaluation based on using only a single worker and a single soldier from the whole experimental group and increases thereby considerably the efficiency of the monitoring. The methodology is based on the use of radiolabeled juvenogen and transmission X-ray radiography of the selected termite.

Keywords: termite control, laboratory system, conditions of normalization

1. INTRODUCTION

Termites represent serious insect pests. The total annual amount spent by the consumers for subterranean termite control was estimated at 2.2 billion US dollars in the United States in 1999 without including the repair cost of damaged buildings [1].

A promising way in termite management is represented by the use of juvenile hormone (JH) or its analogues (JHA). JH is responsible for caste determination in termites. Individual development of larvae, workers and nymphs is controlled by hemolymph titre of JH [2]. Its critical threshold is required for development of a soldier. External applications of JH or JHA to termite groups induced an increased and precocious formation of soldiers [3]. Such effect may cause a disruption of social homeostasis, followed by exhaustion of workers, starvation of dependent castes, and can result in colony death.

Recently, a new series of juvenogens (JHA) have been synthesized at our Institute [4]. They proved to be potential termite control agents in laboratory as well as in field trials [5].

2. PURPOSE

To examine the effects of juvenogens evoking increased metamorphosis of workers to soldiers in termite colonies, a methodology has been developed for laboratory trials simulating natural conditions, that allows the use of a single selected termite out of the whole experimental set for gaining information about both the juvenoid metabolism and metamorphosis. This normalized setup obviates the conventional costly, laborious and time-consuming processing of the whole experimental group. The applicability of results obtained from the analyzed individual for representing the events taking place in the whole system has been assessed by presenting their final form by radiography at the end of the experiment, prior to their use for the evaluation.

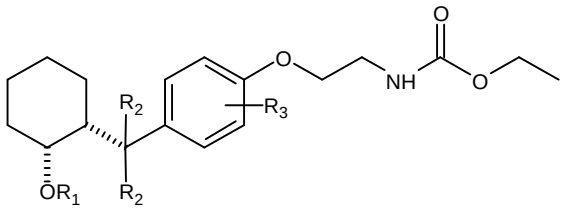
The principles of the underlying normalization described here can find broader application in other laboratory setups in biological and medical research and aid in increasing the effectivity and decreasing the costs.

3. METHODS

3.1 Selected species and biologically active juvenogen

Two following model species (Isoptera: Rhinotermitidae) were used: *Reticulitermes santonensis* (the colony was collected in 2005 at Oléron Island in Southern France) and *Prorhinotermes simplex* (collected in 1964 at Piñardel Rio, Cuba). Both species belong to the smallest termites. The first species, recognised to be introduced species *Reticulitermes flavipes* from the United States [6], has been established to be a model species within European Union for testing the timber resistance against wood pest insects.

Ethyl */cis/-/N/*-{2-{4-{[2-(butanoyl)oxycyclohexyl] methyl}phenoxy}ethyl} carbamate labeled with tritium in the benzene ring (305 GBq/mmol) was used as a model juvenogen (Fig.1).



R_1 = $COCH_2CH_2CH_3$, R_2 = H, R_3 = ^3H [305 GBq/mmol]

Fig.1. The radiolabeled juvenogen used in the study.

3.2 Model laboratory system.

Group of 40 workers was established from a colony containing several thousands of individuals. The group was held in a Petri dish (60 mm in diameter) on fine quartz sand (5 ml) saturated with distilled water (2 ml) in complete dark at 28 °C. A spruce wood block (30 x 10 x 2 mm) is offered to the termites as the only source of food. It is treated with 0.4 ml of acetone solution of 92.5 kBq/ml radiolabeled juvenogen (total amount 0.05 mg/ml) used for caste differentiation. For the group of juvenogens prepared in our laboratory, this concentration proved in numerous previous

tests with several termite species to induce the best response in terms of high differentiation and minimal mortality. The experiment was terminated and the caste composition was scored 14 days after its establishment, when the maximum response to the regulators of caste differentiation is known to occur in Rhinotermitidae species.

The number of termites in an experimental group is established in order to provide the individual with a sufficient social environment. The minimal number of individuals necessary for a long viability of a group without a significant mortality is species-specific. As confirmed by many experimental breedings of Rhinotermitidae, 40 individuals per group is the experimental standard for an experiment lasting a few weeks.

3.3 Treatment of individuals.

The workers of the fourth, fifth and sixth instar were chosen to participate in the experiments. These instars are known to be active in fulfilling work task and retain the full array of developmental possibilities. The anatomic, behavioral and ontogenetic characteristics of the three instars are comparable, they differ only slightly in size.

Before entering the experiment the comparable individuals were checked under a binocular microscope for optimal fitness, they were photographed and individually weighed. Scales with μg precision are used for weighing and the activity of the individuals is lowered by low temperature before weighing.

After termination of the experiment, a representative soldier as well as one remaining worker was visualized by X-ray transmission radiography.

3.4 X-ray transmission radiography.

The common methods of insect imaging, i.e. optical microscopy, electron microscopy (both transmission and scanning), and fluorescence microscopy provide detailed information about external and internal anatomy. However, these methods suffer from two major disadvantages: 1. The sample preparation is usually laborious, it takes a long time and is often expensive for higher quantities of samples; 2. The samples after imaging cannot be used for other purposes such as subsequent study or conservation. Moreover, an ideal approach would allow also observation of living animals to study physiological, biomechanical or ontogenetic aspects. Among such methods X-ray [7-9] and NMR [10] methods, often processed in a tomographic mode to allow 3D images, are the most promising for visualization of insects instantly and without damaging [7] or to analyse the physiological processes in a living insect body [8].

The imaging of termites is particularly difficult due to their non-pigmented and poorly sclerotized cuticle that makes it difficult to observe the anatomic structures with an optimal contrast. Moreover, they are vulnerable to damage when they are manipulated or treated during sample preparation.

The experimental setup consisted of a microfocus X-ray tube with a tungsten anode (Hamamatsu L8601 with focal spot size of 5μm at 40kV and 100μA), sample holder and a pixel detector Medipix2 with a 300μm thick silicon sensor as described in [11]. The response of each individual detector pixel was calibrated for different absorber thicknesses to suppress the beam hardening effect [12]. The termite to be imaged is cooled to ensure a stable position during exposure: *Reticulitermes santonensis* to ca. 2^{o}C and *Prorhinotermes simplex to* ca. 8^{o}C. Altering the distance between the cold termite and the detector the geometric magnification was adjusted to a level of 20 (source to detector distance was 70cm). Radiograms with exposure time of 5s are displayed in Fig.2. The results demonstrate the high spatial resolution and high contrast. No radiation damage can be assumed to occur for the calculated dose lower than 1 mGy.

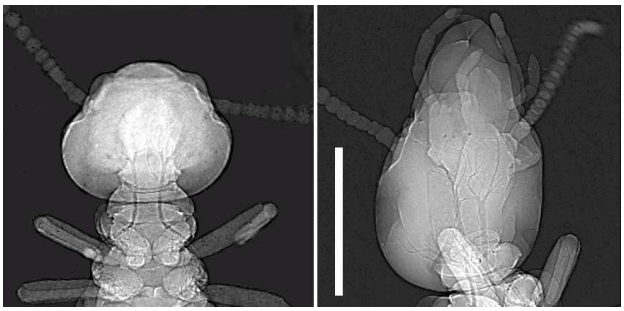


Fig.2. The termite head after experiment termination (the white abscissa equal 1 mm): left – worker; right – soldier.

3.5 Radioactivity measurement

A representative worker and a representative soldier, morphologically defined by X-ray transmission radiography, were checked for an average filling of the digestive system by being immersed in a drop of water on a depression slide, covered with coverslip and photographed by a digital camera attached to a microscope. This imaging documents at the same time that no initial stages of metamorphosis have as yet occurred in the worker. Prior to radioactivity measurement the termite was washed with 200 μl reagent grade chloroform for about 5s to remove surface contamination arriving from wood or excrements. For radioactivity measurements documenting the course of juvenogen metabolism in both representative individuals the termite body was placed into a scintillation vial and covered by 1 ml of Tissue Solubilizer (NCS-II, Amersham). After 5 days, 10 ml of a liquid scintillator Rotiszint eco plus (Roth, Karlsruhe) was added and the radioactivity was measured in tritium channel of a liquid scintillation spectrometer Beckman LS-6500 with a standard deviation lower than ± 1%.

For alternative evaluation of the radio-HPLC the representative worker or soldier was placed in a closure-equipped micro test tube, slit, overlaid with 0.1 ml HPLC-purity methanol and the test tube was closed. 50 μl of the solution was used for radio-HPLC.

The radio-HPLC analyses were performed using a Waters (USA) HPLC instrument equipped with an on-line Waters 490E programmable multi-wavelength UV detector and a Beckman (USA) 171 radiometric flow-through detector [12]. The analyses were performed on a stainless steel analytical column (125 x 4 mm i.d.), packed with Supersphere 100 RP-18 endcapped, particle size 4 um, protected by a guard column (4x4 mm i.d.) with LiChrosphere 100 RP-18, particle size 5 um (Merck, FRG).

Acetonitrile (grade for HPLC analysis,Merck) and double-distilled water were used as the mobile phases in a linear gradient programme: 20 min linear gradient 30-70% of acetonitrile, then 5 min linear gradient 70-98% of acetonitrile, followed by an isocratic regime with starting conditions for 20 min. The flow rate used was 0.8 ml/min under continuous degassing with helium. The UV detector wavelength was set at 230 nm, 0.05 AUFS.

4. RESULTS AND DISCUSSION

The total radioactivity of each representative individual (worker or soldier) was measured (Table 1). This value gives the proportion of the mixture of the applied juvenogen and its radiolabeled metabolites at the end of the experiment. The values allow us to determine the ratio between the radioactivity of the worker and the soldier used in the experiment and their ratios to the input radioactivity of 37 kBq.

Table 1. Comparison of radioactivity [Bq] after experiment termination determined by evaluating individuals selected out of the experimental set and by using the whole set.

	Selected individuals	Experimental group	
		Number of individuals	The measured mean
Worker	14.2 ± 0.1	11	14.0 ± 0.1
Soldier	8.0 ± 0.05	23	8.1 ± 0.05

The radioactivity values obtained for the individuals were compared with mean values obtained by processing the whole experimental group (Table 1). In the initial group, 4 workers perished during the experiment. The remaining 34 individuals (without the representative worker and soldier) included 11 workers and 23 soldiers (juvenilizing effect 60 %). These comparisons showed the applicability of selected individuals for basic evaluation of the different fate of the juvenogen in the worker and in the soldier.

The radio-HPLC conditions were defined for further analysis, which involved the determination of the composition of the mixture of radioactive substances in the worker and the soldier, using a higher input radioactivity. Precision expressed by means of the coefficient of variation CV (%) from four experiments ranged between 1.8 and 3.4%. Precision of measured retention times was in the range of 0.3-2.6 CV (%) based on six measurement within one day. The linear response of the radiometric detector was verified by means of calibration curves of standard solutions of the tritiated juvenogen in the range of 150Bq – 100 kBq ($r = 0.996$). The absolute detection limit of the radio-HPLC analysis defined by signal–to–noise ratio of 3, was assigned for the juvenogen standard in the range of 100 – 150Bq (3 pmol).

Therefore, it can be concluded that these conditions of radio-HPLC make possible precise and sensitive detection. The detailed analysis of compositions of radiolabeled metabolites in the soldier and in the worker, representing a further independent step of analysis of the normalized system, is in progress.

5. CONCLUSIONS

Conditions under which the use of an individual out of the whole experimental set can reliably be used for evaluating the proportion of an applied substance and/or its metabolites in the organism at the end of an experiment performed on a biological test system have been determined and verified. The principles of the underlying normalization can find broader application in increasing the effectivity and decreasing the costs in other laboratory setups in biological and medical research.

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