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ORIGINAL ARTICLE

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Behavioral and electrophysiological investigation on taste response of the termite *Zootermopsis nevadensis* to wood extractives

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Abstract Termite feeding behavior and the chemoreception of plant extracts were evaluated to investigate the water extracts from akamatsu (*Pinus densiflora*), neem (*Azadirachta indica*), and their equivalent mixture using pseudoergates of *Zootermopsis nevadensis*. In behavioral assays, termite preference was akamatsu > akamatsu plus neem > neem. Electrophysiological recordings from the taste hairs on labial palps showed vigorous impulse discharge to akamatsu extract but much lower response to neem extract. The response to akamatsu plus neem was mostly the same as that to neem alone, suggesting the neem extracts inhibited the responses to akamatsu extracts. In the present article, we discuss the correlations between the feeding behaviors and the responses at their taste cells to these different extracts.

Key words Termite · Taste response · Electrophysiology · Feeding behavior · Wood extractives

Introduction

Termites are harmful pests to wooden structures around the world. Recently, there has been a growing interest in natural compounds such as plant extractives as alternatives to synthetic termiticides. For example, essensial oils of hiba (*Thujopsis dolabrata* Sieb. et Zucc. var. hondae Makino)[1] and neem (*Azadirachta indica* A. Juss.) extracts[2] are sold as natural termite control agents. Against this backdrop, it is important to elucidate the relation between the chemical senses of termites and their food selection in nature.

Termites form societies and usually live in low-light conditions in nests built in soil or wood. Thus, both mechanical (temperature, pressure, etc.) and chemical factors (olfactory and taste) are indispensable for them to detect and select food, and recognize nestmates or enemies. It is necessary to study the taste receptors that respond to food to understand the taste sensory mechanism of food selection in termites; however, little work has been done on the neural system of termite taste organs.[3]

A dampwood termite, *Zootermopsis nevadensis*, which originated in North America, is now established in an area of the Kansai region in Japan.[4] Their preferred habitat is fallen pinewoods (akamatsu; *Pinus densiflora* Sieb. et Zucc).

In order to investigate the feeding habits of *Z. nevadensis*, we observed their feeding behavior and the taste response to water extracts from akamatsu, neem, and the equivalent mixture of akamatsu plus neem. Considering the behavioral and electrophysiological inhibitory effects of neem extract on the responses to akamatsu, the elucidation of this taste-suppression mechanism could lead to a new way of controlling termites.

Materials and methods

Termites

A colony of *Zootermopsis nevadensis* (Hagen) was collected in the field at Kawanishi in Hyogo Prefecture. Only pseudoergates were used for behavioral and electrophysiological bioassays.

Structural observation

Scanning electron microscopy (JSM-6700F, Jeol) was used to observe the mouthpart of the heads of the pseudoergates.

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The samples were cleaned in distilled water, dehydrated through an ethanol series of 30, 50, 70, 80, 85, 90, 95, 99, 100%, and then transferred into ethanol/*t*-butyl alcohol (1/1) followed by 100% *t*-butyl alcohol. After cooling in a refrigerator, the samples were dried under vacuum and kept in a jar containing silica gel until observation.

Observation of feeding behavior

Feeding behaviors of termites in a plastic petri dish that were fed pieces of Japanese red pine (akamatsu: *Pinus densiflora* Sieb. et Zucc) sapwood were observed using a binocular microscope (SMZ 1500, Nikon) and a digital microscope (VH-7000, Keyence) equipped with a CCD camera.

Preparation of test solutions

Wood meal (20 g) of both air-dried akamatsu and neem (*Azadirachta indica* A. Juss) were extracted with 40 ml of distilled water for over 6 h. After filtering, half of each solution was concentrated to one half of its volume by lyophilization, and then mixed to obtain an equivalent mixture. Akamatsu, neem, and their equivalent mixtures (akamatsu plus neem) were all prepared as 10 mM NaCl solutions so as they contained electrolyte in the electrophysiological experiments. A solution of 200 mM NaCl was also prepared for the electrophysiological study.

Behavioral test

A cellulosic disc (diameter: 24 mm; Whatman) was quartered each quarter was placed on the bottom of a plastic petri dish (diameter 5 cm; height 1 cm) (see Fig. 1). Two diagonal pieces were treated with 35 ml of a test solution containing 0.2 mg/ml of a blue food color (brilliant blue FCF, Wako) per piece, while others were treated with a colorless test solution (Fig. 1). A pseudoergate was allowed to feed on the discs for 3 h with three replicates per test area, because the gut of a pseudoergate became stained within 3 h of starting the feeding test. Based on the results, the test period was set to 3 h to prevent the termites eating their feces. Three pseudoergates from the same test area were subjected to homogenization in 1.2 ml of 50% EtOH. After centrifugation, the absorbance at 630 nm of each was measured. On the basis of the relative intensity of absorbance, each absorbance index (AI) in the test of sample X versus sample Y, for example, was calculated by the following equation:

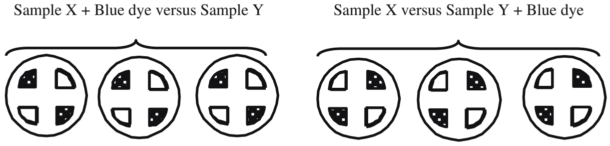

Fig. 1. Behavioral bioassay methods using blue dye

$$\mathrm{AI}(\mathrm{X})(\%) = \left(\mathrm{Abs}(\mathrm{X})/\left[\mathrm{Abs}(\mathrm{X}) + \mathrm{Abs}(\mathrm{Y})\right]\right) \times 100,$$

$$\mathrm{AI}(\mathrm{Y})(\%) = \left(\mathrm{Abs}(\mathrm{Y})/\left[\mathrm{Abs}(\mathrm{X}) + \mathrm{Abs}(\mathrm{Y})\right]\right) \times 100,$$

where Abs (X) and Abs (Y) represent the absorbance of the solutions of sample X and sample Y, respectively.

Electrophysiological technique

A modified tip-recording technique[5] was adopted for the electrophysiological tests. The head of a pseudergate (Fig. 2) was severed from the rest of the body, and a reference platinum electrode was fitted into the opening. The tip of the chemosensillum on the labial palp (Figs. 2, 3) was touched with a glass capillary (outer diameter 1.0 mm; inner diameter 0.6 mm) containing a test solution with a platinum wire working electrode. The signals through the preamplifier were further amplified and processed with a computer-based signal processing system (Synthech, The Netherlands).

Results and discussion

Movements of mouthpart

Matsuoka et al.[6] differentiated the feeding behavior of four species of termites into the following three types: (1) pulling, (2) cutting, and (3) scraping. Our observation of the feeding behavior of *Zootermopsis nevadensis* showed that they move mandible and maxillary palps alternately so as to carry wood fragments into their mouths effectively, whereas labial palps were used to hold the wood fragments they cut off. Furthermore, the termites did not swallow all wood

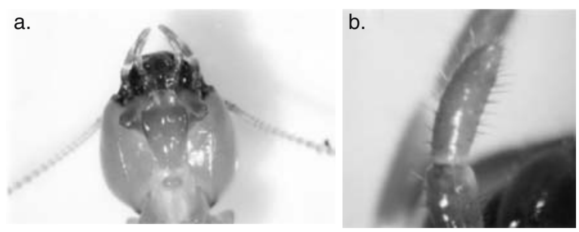

Fig. 2a,b. Structural observation of termite head. **a** Head on the abdominal side; **b** magnification of the labial palp

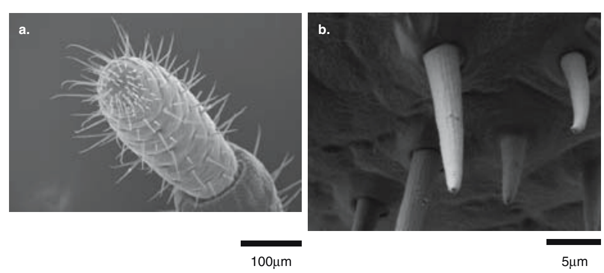

Fig. 3a,b. Scanning electron microscope image of the labial palp. **a** Labial palp; **b** short chemosensilla on the tip of the labial palp fragments that they cut off, but often put them onto the wall or bottom of the petri dish.

These observations indicated that termites consume more food substrates than they actually intake and digest, although the cue for swallowing food is still unknown. Recently, termite salivary secretion has been gathering interest for its various roles.[7,8] Yamaoka[9] and Watanabe et al.[10] reported that the workers of *Reticulitermes speratus* (Kolbe) secrete saliva containing carboxymethyl cellulase (CM-cellulase, endo β-1,4-glucanase) from their labial glands to degrade cellulosic food. In *Reticulitermes santonensis* and *Schedorhinotermes lamanians*, it works as a phagostimulating pheromone.[11] When *R. santonensis* or *S. lamanians* worker termites put the salivary secretion onto the food, food with the secretion causes an aggregation effect as well as a feeding stimulant. Further study will be needed to elucidate the cue for the salivary secretion. Reinhard and Kaib[12] reported that many kinds of termite species possess the phagostimulating signal in their salivary secretions. The phagostimulating signals might be a taste response to cellulosic food and/or a mechanosensitive response to the textures of food surfaces.

Feeding preference in behavioral test

As demonstrated by the observation of feeding behavior, there was a difference between the mass loss of food substrates and their intake. Here we investigated the feeding preference at the intake by comparing (1) akamatsu versus 10 mM NaCl, (2) neem versus 10 mM NaCl, (3) neem versus akamatsu, (4) neem versus akamatsu plus neem, (5) akamatsu versus akamatsu plus neem, or (6) 10 mM NaCl versus 10 mM NaCl (control), either of which was stained-blue. The whole gut of a pseudoergate was isolated after 3 h of feeding to evaluate intake by termites.

Neem intake was significantly less (Fig. 4, $P < 0.05$) than 10 mM NaCl, akamatsu, or neem plus akamatsu. The results suggest that neem suppressed feeding activity and akamatsu tended to stimulate it. Akamatsu was also preferred to neem plus akamatsu, providing more evidence that neem extracts had an inhibitory effect on feeding.

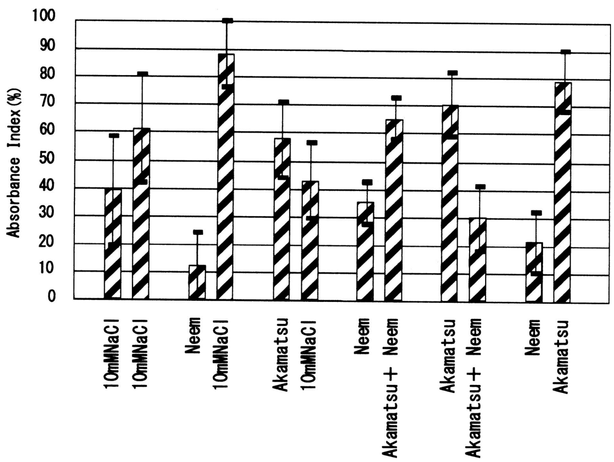

Fig. 4. Feeding preference in behavioral bioassay ($P < 0.05$)

Electrophysiological results of feeding preference

Pairs of maxillary and labial palps of the pseudoergates of *Z. nevadensis* are shown in Fig. 2a. On the tips of these palps, there were many short sensilla surrounded by long ones, whereas most of the sensilla were long on the side of the palps (Fig. 3a). We stimulated and recorded responses from the short sensilla on the tips of labial palps in Fig. 3b. Electrophysiological responses were obtained with each test solution in the order of akamatsu, neem, akamatsu plus neem, 10 mM NaCl, and akamatsu again.

From the short sensilla on the tips of labial palps, no response to the 10 mM NaCl was observed, but responses to both akamatsu and neem were observed. They may have been taste sensilla to akamatsu, where a highly frequent and less adaptative impulse pattern was observed, in contrast to the rapidly adaptative response pattern to neem. The response to the akamatsu plus neem mixture looked the same as that to neem alone, suggesting that the neem extracts inhibited the responses to akamatsu extracts. It was not apparent whether this inhibition was reversible. Hence, we checked the response to akamatsu after stimulating with

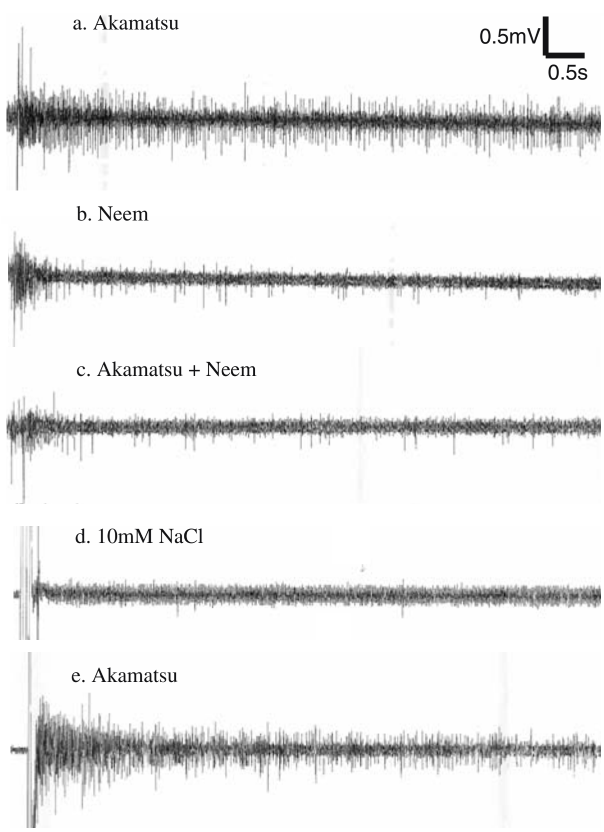

Fig. 5a–e. Electrophysiological responses to various stimulants. **a** Akamatsu, **b** neem, **c** akamatsu + neem, **d** 10 mM NaCl, **e** akamatsu. All stimulants were dissolved in 10 mM NaCl solutions and these stimulants were subjected in the order **a–e**

akamatsu plus neem from the same sensilla at 3-min intervals. As a result, the same shapes of impulses were obtained as those of the last stimulation with akamatsu (Fig. 5). Consequently, the inhibitory effect of neem on the response to akamatsu was found to be reversible.

As shown in the results of behavioral and electrophysiological assays, termites were considered to continue feeding, as long as the taste cells were continuously stimulated with akamatsu, but stopped feeding when they ceased responding; namely, termite feeding behavior was heavily related to the responses at the taste cells in the sensilla on their labial palps.

Conclusions

Structural and behavioral observations of termites indicated that the maxillary and labial palps on their mouthparts are important for their feeding behaviors. Termites prefer feeding on akamatsu, which induces a constant response in taste cells, whereas they avoid feeding on neem, which impresses response in taste cells. The components in these extracts that cause such differences in taste cells should be investigated in future. The elucidation of the taste-suppression mechanism as shown by neem might lead to the developement of termite control agents from natural sources.

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