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Juvenile Hormone Analogue, S-31183, Causes a High Level Induction of Presoldier Differentiation in the Japanese Damp-Wood Termite

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ABSTRACT—The effect of a juvenile hormone analogue, 2-[1-methyl-2(4-phenoxyphenoxy)ethoxy]pyridine (S-31183), on differentiation of the presoldier was tested with the Japanese damp-wood termite, *Hodotermopsis japonica*. Sixth and 7th instar larvae were reared on filter papers impregnated with various amounts (10–1000 μg/filter paper) of the analogue for 3 weeks. While presoldiers were induced, with some mortality, under all the conditions tested, the rate of induction was significantly higher (>90%) with lower mortality (<10%) than under the other conditions when 10 μg or 30 μg of S-31183 were administered to the 6th instar larvae. The length of the premolt interval of larval-presoldier molt was about 5.5 days, equal to that of larval-larval molt.

INTRODUCTION

The society of termites is characterized by polymorphism and polyethism, which, like other social insects, are based on the caste system. Termites usually consist of several castes: primary reproductives, supplementary reproductives, soldiers, and workers or pseudergates. In addition, replacement reproductives appear after the death of primary reproductives. The set of castes and scheme of postembryonic pathway producing castes vary with species.

The caste of the termite is not genetically, but epigenetically determined: the first instar larva can become any caste. It is well established that juvenile hormone (JH) is a key factor controlling the caste differentiation and regulation [12]. Yin and Gillot [23] proposed the following relationship between the titer of JH and the outcome of molts in a primitive termite, *Zootermopsis angusticollis* (Termopsidae). A high titer of JH in hemolymph at a specific stage leads to the formation of soldier via presoldier, and a low titer to the production of alata, which will shed the wings and become primary reproductive. An intermediate titer induces the molts without differentiation, or stationary molts.

While many studies have shown that the artificial increase of JH titer in larvae of termites by exposing them to exogenous JHs or their analogues (JHAs) causes an increase in the number of presoldier [5, 14], its production at the rate of 100% has never been observed. Luescher [9] explained this by the concept of competence in asserting that termite can respond to stimulus only at a specific period ("sensitive" or "competence" period) within the intermolt. This explanation was confirmed for some termites in several studies [11, 15, 18]. In the meantime, Lenz [8] emphasized the influence of nutrition and caste composition on the differentiation of soldiers. For example, the presence of soldiers in an experimental colony will tend to inhibit the differentiation of larvae to presoldiers. The actual JH titer in a termite is supposed to be influenced by these environmental factors. Lenz suggested that continuous contact

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and exchange of food among termites bring about the transfer of pheromones. Although Luescher [10] hypothesized the existence of pheromone which is involved in the termite caste regulation, no actual substance has been identified yet.

In spite of its decisive role, little is known about the mechanism of JH action in the caste regulation [17]. This is mainly due to the difficulty in performing biochemical and molecular biological analyses on termites. In order to overcome the difficulty, it is desirable to control the caste differentiation artificially. If larvae are successfully induced into presoldiers synchronously at a high rate, we will be able to obtain much important information about the physiological, biochemical, and molecular biological mechanism underlying the caste determination in termites.

The Japanese damp-wood termite, *Hodotermopsis japonica* Holmgren is a lower termite species whose differentiation of caste occurs at a late stage of development. Even the last (7th) instar larvae are capable of differentiation into either of nymphs, neotenics and soldiers. Soldiers also derive from either the 6th instar larvae or nymphs [13]. Since 2-[1-methyl-2(4-phenoxyphenoxy)ethoxy]pyridine (S-31183) displayed higher hormonal activity than that of a well-known JHA, methoprene on some insects [1, 22], it was expected that the JHA would also induce the differentiation of presoldiers of termite at a high rate. To determine the conditions that induce differentiation of larvae into presoldiers at the highest rate, we have examined the effects of the amount of S-31183, group size, instar of larvae, and locality of original colony on the presoldier production in *H. japonica*.

MATERIALS AND METHODS

Termites

Hodotermopsis have been known from Sichuang, Guizhou, Hunan, Guangxi, Guangdong, Zhejiang, Fujian, Jiangxi, Vietnam on the continent, and also from the Hainan and Taiwan islands. In Japan, *H. japonica* have been found from the Nansei archipelago and the Capes of Seta and Ashizuri [21]. Colonies of *H. japonica* were collected in the natural forest at Onoaida in the Yaku-shima island and at Takamoto in the Nakano-shima island of the Nansei archipelago, Japan. They were brought back to the laboratory and kept in plastic cases in an incubator at 25°C in the constant darkness.

Juvenile hormone analogue

2-[1-Methyl-(4-phenoxyphenoxy)ethoxy]pyridine, S-31183 was kindly offered by Sumitomo Chemical Company, Osaka, Japan. Technical grade (98.1%) of S-31183 was used in this study.

Treatments

Dark-colored 6th and 7th instar larvae were picked up from stock colonies, and groups of 10 or 20 individuals were placed in glass petri dishes (70 mm diam.) containing filter paper disks (approx. 33 cm^2). Generally, newly-molted termite larvae are light-colored and gradually become darker. Preliminary studies suggested that the light-colored larvae do not have the competence to differetiate into presoldiers under the influence of JHA. Six experimental groups, each consisting of either 10 or 20 6th instar larvae, were kept with filter paper disks containing 0 (control), 10, 30, 100, 300, and 1000 ug of S-31183, respectively. The disks had been uniformly impregnated with 150 μl of the acetone solutions of S-31183 and then air-dried, on which insects fed instead of wood. The same procedures were used for the experiments with the 7th instar larvae. Equal numbers of males and females were included in each experimental group. Insects were supplied with water daily and the treated filters were renewed weekly. All experimental groups were kept at 25°C in the constant darkness.

Experimental groups were observed daily for three weeks. Each experimental series was triplicated. Larvae from the stock colony collected in the Yaku-shima island were used in one series, and larvae from the Nakano-shima island were used in two other series. The data were evaluated by multiple-way of variance and Tukey's multiple comparison.

Determination of the length of premolt interval

At the premolt stage, larvae empty the hindgut

and become white, which is called "whitening". We observed the larvae every about 12 hours and labeled individuals which began to whiten with the date and time by attaching sticker on the back. In the following days, the number of newly-molted larvae and presoldiers were counted. We estimated the length of premolt interval of each individual based on the duration between the whitening and molting. Larvae which did not molt and died after whitening were counted in mortality, and not subjected to post-mortem examinations. Larvae which died a few days after molting were counted in both mortality and those molted.

RESULTS

It was obvious that S-31183 impregnated in filter paper disks caused superfluous production of presoldiers and some mortality of larvae (Table 1). In control group with no JHA, no presoldier was produced while a few larvae underwent larval-larval molt with little mortality. Significantly more presoldiers were produced with all the amounts tested (10–1000 μg) compared with the controls ($P<0.01$). Regardless of other factors, with 10 or 30 ug of S-31183, about 90% of larvae differentiated into presoldiers within 3 weeks from the start of experiment. The three higher doses (100, 300, 1,000 μg) led to significantly fewer ($P<0.01$) production of presoldiers than the above two. As shown in Fig. 1, with higher amounts of S-31183, the level of presoldier production attained the maximum on earlier days.

As the amount of S-31183 was increased, the mortality increased. The mortality of the larvae treated with 300 or 1,000 μg of S-31183 was significantly higher than with the lower dosages ($P<0.01$), which was incurred mainly as a result of incomplete ecdysis (Table 1). The dosage of JHA also effected the larval-larval molting ($f=9.37$, $P<0.01$). Throughout all the experimental groups except control, only a few larvae underwent larval-larval molt.

With the 6th and 7th instar larvae, results were significantly different on the rate of presoldier production ($f=15.34$, $P<0.01$) and mortality ($f=11.03$, $P<0.01$). In the 7th instar larvae, the rate of presoldier production was lower and mortality

Table 1. Differentiation and mortality of *H. japonica* exposed to various doses of S-31183 for 3 weeks

6th-instar larvae				7th-instar larvae			
JHA dose	% molted to		% mortality	JHA dose	% molted to		% mortality
	Larvae[a]	Presoldiers[a,b]			Larvae[a]	Presoldiers[a,b]	
Groups of 10 larvae				Groups of 10 larvae			
0	10.0±5.8	0.0± 0.0	6.7± 3.3 (0.0±0.0)	0	3.3±3.3	0.0± 0.0	6.7±3.3 (0.0±0.0)
10	0.0±0.0	96.7± 3.3	3.3± 3.3 (0.0±0.0)	10	6.7±3.3	90.0± 0.0	3.3±3.3 (0.0±0.0)
30	0.0±0.0	96.7± 3.3	3.3± 3.3 (0.0±0.0)	30	0.0±0.0	93.3± 3.3	6.7±3.3 (0.0±0.0)
100	00.0±0.0	83.3± 8.8	10.0± 5.8 (0.0±0.0)	100	0.0±0.0	70.0±10.0	13.3±8.8 (0.0±0.0)
300	3.3±3.3	76.7± 3.3	30.0± 5.8 (6.7±6.7)	300	0.0±0.0	63.3± 8.8	43.3±8.8 (13.3±3.3)
1000	0.0±0.0	56.7± 6.7	23.3± 8.8 (16.7±8.8)	1000	0.0±0.0	46.7± 8.8	40.0±0.0 (30.0±0.0)
Groups of 20 larvae				Groups of 20 larvae			
0	11.7±1.7	0.0± 0.0	5.0± 2.9 (0.0±0.0)	0	8.3±4.4	0.0± 0.0	3.3±1.7 (0.0±0.0)
10	3.3±3.3	93.3± 1.7	3.3± 1.7 (0.0±0.0)	10	0.0±0.0	90.0± 2.9	16.7±1.7 (0.0±0.0)
30	0.0±0.0	91.7± 1.7	3.3± 3.3 (0.0±0.0)	30	0.0±0.0	93.3± 1.7	3.3±1.7 (0.0±0.0)
100	1.7±1.7	85.0± 2.9	16.7± 3.3 (0.0±0.0)	100	0.0±0.0	63.3± 6.0	20.0±2.9 (0.0±0.0)
300	0.0±0.0	70.0±10.0	30.0±10.4 (11.7±6.7)	300	3.3±1.7	48.3± 6.0	35.0±2.9 (25.0±2.9)
1000	0.0±0.0	55.0±15.0	33.3±12.0 (25.0±7.6)	1000	0.0±0.0	40.0± 2.9	50.0±2.9 (35.0±7.6)

Values are means±SE of % initial 10 or 20 larvae of three replicates of experimental group. Values in parentheses are means±SE of mortality due to incomplete molt.

[a]: including individuals died after complete molting.

[b]: not including individuals died due to incomplete molts into presoldiers.

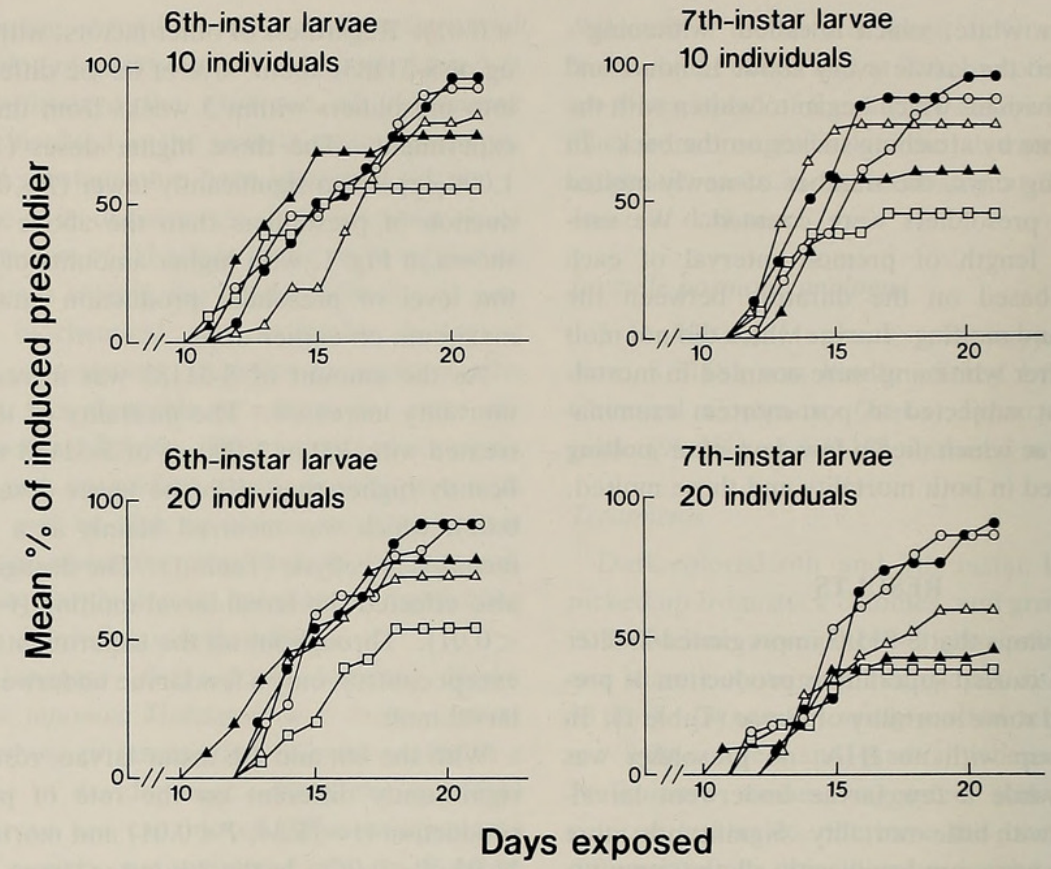

FIG. 1. Induced presoldiers in experimental groups exposed to various amounts of S-31183. Cumulative rates of induced presoldiers against initial number of larvae were plotted. Values represent means of triplicated experimental groups. Amount of S-31183 impregnated into each filter disk: ○, 10 μg; ●, 30 μg; △, 100 μg; ▲, 300 μg; □, 1,000 μg.

TABLE 2. Timing of premolt interval for various types of molt

Types of molt / Length of premolt stage (days)	6th-instar larvae to				7th-instar larvae to			
	Larvae		Presoldiers		Larvae		Presoldiers	
	Molting %	total	Molting %	total	Molting %	total	Molting %	total
4.5	2	4.7	8	6.9	3	5.9	5	4.6
5	9	20.9	25	21.6	7	13.7	14	13.0
5.5	25	58.1	71	61.1	35	68.6	66	61.1
6	6	14.0	10	8.6	4	7.8	21	19.4
7	0	0.0	1	0.9	1	2.0	0	0.0
Total	43	100.0	116	100.0	51	100.0	108	100.0
Mean (SD)	5.44 (0.39)		5.39 (0.40)		5.46 (0.42)		5.50 (0.38)	

was higher than in the 6th instar larvae (Table 1).

Group size and locality of original colony did not influence the rate of induction of presoldiers or mortality (Table 1). As shown in Table 2, there was no significant difference between the length of premolt interval of larval-larval molt and that of larval-presoldier molt with either the 6th or 7th instar larvae (Mann-Whitney's U-test, $P>0.05$). In both cases, the length of premolt interval was about 5.5 days with little variance irrespective of instar. However, the 6th instar larvae became presoldier earlier than the 7th instar larvae (Mann-

Whitney's U-test, $P<0.05$).

DISCUSSION

In a preliminary experiment, it was shown that the lower amounts of S-31183 (1 and 3 μg) caused just a few presoldier production accompanied with intercaste production. As shown in Table 1, with 10 and 30 μg of S-31183, the rate of presoldier production was over 90%. Moreover, the intercaste production, which has inevitably accompanied the presoldier production even under the most ideal conditions so far, was not observed in this study.

The successful induction of presoldier in this study will be due to several factors. Among others, one important factor is strong JH activity of S-31183. While S-31183 is structurally different from other well-known JHAs such as methoprene and hydroprene, it exerts a higher activity than others in some insects [1, 22]. In *Musca domestica,* its activity in inducing supernumerary larvae is 50 times higher than methoprene [1]. High chemical stability of S-31183 [22] probably accounts, in part, for the strong activity of this JHA.

In the meantime, it is likely that the successful induction of presoldier by S-31183 in *H. japonica* is not only due to the strong JH activity of the analogue, but also due to the high sensitivity of this species of termite to S-31183. Actually, while in *Coptotermes formosanus* S-31183 is not as effective as methoprene in inducing the presoldier differentiation [2], our preliminary experiments have suggested that in *H. japonica* the effects of the two JHAs are just the reverse. Su and Scheffrahn demonstrated also that *C. formosanus* and *Reticulitermes flavipes* show different sensitivity to S-31183 [20].

High rate of the presoldier induction in this study may be also due to our experimental procedures employed, in which we examined experimental groups composed entirely of larvae instead of natural colonies including soldiers, considering that some termite species with lower population density of soldier are more sensitive to JHAs [8]. In addition, relatively low population density of soldier in the natural colony of *H. japonica* (<8%) [13] may also account for its high sensitivity to S-31183.

As shown in Fig. 1, presoldier production was saturated earlier in the experimental groups treated with higher amounts (100, 300, 1,000 μg) of S-31183. This may indicate that when the concentration of JHA in the filter paper is higher, the level of JHA in the insect reaches the threshold earlier. Alternatively, this is a reflection of the difference of amount received by termites. Although, with these concentrations of S-31183, the rate of overall presoldier production was relatively low, this was not because of low induction to presoldier but because of high mortality under these conditions. Indeed, many of the termites that had survived under these conditions differentiated to presoldiers (Table 1). Toxic effects of excess amount of JHA were reported by many investigators [2, 4–7, 14, 15, 19, 20].

In the 6th instar larvae, the rate of presoldier production was higher and mortality was lower than in the 7th instar larvae. This suggests that the 6th instar larvae are more resistant to the toxic effect of S-31183 and have more potentials for differentiation to presoldiers.

Presoldiers induced by S-31183 appeared 11–21 days after the first treatment (Fig. 1). It has been known that the timing of molting depends on not only the titer of JH during the intermolt period, but also many other factors such as the titer of ecdysteroid [16]. We demonstrated that both the premolt interval of larval-larval molt and larval-presoldier molt are fixed on about 5.5 days (Table 2). In addition, when the larvae treated with S-31183 underwent larval-larval molt, it always took place during the first 9 days from the first treatment. In other words, all the larvae that whitened on day 6 or later did not become larvae but presoldiers after molting 5.5 days later. This enables us to predict the fate of larvae at intermolt stage, and analyze the events underlying molting to presoldier.

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