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THE INFLUENCE OF THE JUVENILE HORMONE ANALOGUE ZR 512 (ZOECON) ON CASTE DEVELOPMENT IN *ZOOTERMOPSIS NEVADENSIS* (HAGEN) (ISOPTERA)

By KIZITO WANYONYI (*)

Division of Animal Physiology, Zoological Institute, University of Berne, Engehaldenstrasse 6, CH-3012 Bern, Switzerland.

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SUMMARY

The juvenile hormone analogue, ethyl 3,7,11-trimethyl-dodeca-2,4-dienoate (ZR 512), can affect all the steps of caste development in the termite, *Zootermopsis nevadensis.*

The formation of replacement reproductives in orphaned groups of larvae and nymphs, and that of alates in groups of last instar nymphs, was inhibited by the exposure of these groups to the vapour of low doses of the juvenile hormone analogue. With intermediate doses of the analogue on groups of last instar nymphs, mainly nymph-imago intermediate forms as well as stationary moults were obtained. High doses of the JH analogue induced regressive and presoldier development in groups of last instar nymphs, and presoldier development in groups of larvae.

It was also demonstrated that ZR 512 affects the prothoracic glands of the experimental larvae.

It is concluded that caste development in *Z. nevadensis* depends on the juvenile hormone titer during the moulting interval of the larvae and nymphs.

ZUSAMMENFASSUNG

Der Einfluss des Juvenilhormon-Analogons ZR 512 (Zoecon) auf die Kastenbildung bei Zootermopsis nevadensis (Hagen) (Isoptera).

Das Juvenilhormon-Analogon, Aethyl-3,7,11-Trimethyl-Dodeca-2,4-Dienoat (ZR 512) kann alle Schritte der Kastenbildung bei der Termite *Zootermopsis nevadensis* beeinflussen.

Die Bildung von Ersatzgeschlechtstieren in verwaisten Gruppen von Larven und Nymphen und die Entwicklung von Geflügelten in Gruppen von Nymphen des letzten

(*) International Center of Insect Physiology and Ecology (I.C.I.P.E.), P.O. Box 30772, Nairobi, Kenya.

Stadiums wurden durch niedrige Dosen von ZR 512-Gas gehemmt. Bei Behandlung von Gruppen von Nymphen des letzten Stadiums mit mittleren Dosen des Juvenilhormon-Analogons entstanden hauptsächlich Nymphen-Imago-Zwischenformen oder nach stationären Häutungen wieder Nymphen. Hohe Dosen von ZR 512 bewirkten regressive Häutungen und Vorsoldatenentwicklung in Gruppen von Nymphen des letzten Stadiums und in einem sehr hohen Prozentsatz Vorsoldatenbildung in Gruppen von Larven.

Es konnte auch gezeigt werden, dass ZR 512 die Prothorakaldrüsen der behandelten Larven beeinflusst und auch ihre Degeneration herbeiführen kann.

Aus den Ergebnissen lässt sich ableiten, dass die Differenzierung der Kasten bei *Zootermopsis nevadensis* vom Juvenilhormon-Titer während des Häutungsintervalls der Larven und Nymphen abhängt.

INTRODUCTION

The post-embryonic development of termites is characterized by a complex polymorphism. From the same immature individuals, different adult castes may arise which, in the lower termites *Zootermopsis* (Hagen) and *Kalotermes* (Fabr.), are distinguishable into winged imagos (alates), replacement reproductives and soldiers. Older larvae (pseudergates) and nymphs (1) often undergo stationary moults while nymphs are also capable of regressive development. Definitions of termite castes and schemes of post-embryonic development in a number of genera have been given by MILLER (1969), NOIROT (1969) and LÜSCHER (1974 *a*).

The newly hatched larvae of *Zootermopsis* and other lower termites so far investigated are equipotent, their castes being the expression of social and environmental factors (MILLER, 1969). Increasing evidence indicates that the development of one or the other caste in *Kalotermes flavicollis* (Fabr.) is dependent on the juvenile hormone titer during the moulting interval of the larvae and nymphs (LÜSCHER and SPRINGHETTI, 1960; LEBRUN, 1964, 1967; LÜSCHER, 1960, 1963, 1972 and 1974 *a*). It has also been reported that juvenile hormone and juvenile hormone analogues induce the development of superfluous soldiers in *Reticulitermes lucifugus santonensis* (HRDY and KRECEK, 1972). Corresponding information on other termites, however, is fragmentary or lacking.

It has been of interest, therefore, to study the effect of varying doses of the juvenile hormone analogue, ZR 512, supplied as a vapour, on caste development in *Z. nevadensis*. From histological studies, I have further demonstrated that the juvenile hormone analogue has different effects on the prothoracic glands of the experimental larvae probably depending on the competence of these larvae to become soldiers.

(1) In this paper the term « larvae » is used for stages without wing pads and the term « nymphs » for stages with wing pads, following the proposal of LÜSCHER (1960) which was based on the French and German terminology and which for termites is now generally accepted (MILLER, 1969).

MATERIALS AND METHODS

Termites, collected in Del Monte Park near Pacific Grove, California, were maintained as stock colonies under laboratory conditions (for details, see BECKER, 1965 and 1969).

The juvenile hormone analogue used was ZR 512 or ethyl 3,7,11-trimethyldodeca-2,4-dienoate, kindly supplied by Zoëcon Corporation, Palo Alto, California. The termites under investigation were kept in petri dishes measuring 6 cm in diameter at 20-22 °C and supplied with adequate wood and moistened filter paper. In order to supply the juvenile hormone analogue as vapour, the substance was diluted with ether which was allowed to evaporate in a glass cover of the dish. In each case, a control, in which the pure solvent was applied and allowed to evaporate, was performed. Daily observations were made and moulted termites marked with quick drying nitro-cellulose paint while they were under carbon dioxide narcosis. Measurements were made with the use of an ocular micrometer.

The size of the prothoracic glands was determined from serial cross sections of 8 μ thickness stained with haemalaun, erythrosin and Prenant, and mounted in Dipex or Eukitt (BURCK, 1969). The cross-sectional area (mean value of the posterior strands of the gland in at least 12 sections) was taken as an index of its size.

The χ^2-test and the t-test were used for the statistical analysis of the results.

RESULTS

The effect of ZR 512 on the development of older larvae (pseudergates).

When homogeneous groups of older larvae of *Z. nevadensis* were exposed to the vapour of 40, 4.0, 0.4 and 0.04 mg of ZR 512 for 14 days, marked differences in the total number of moults from that of corresponding control groups, were noted by the end of 30 days from start of the experiment. In the groups exposed to 40-0.4 mg of the juvenile hormone analogue, about 77 % of the initial number of larvae had moulted during this period. In contrast, only 23 % of the control larvae and 6.8 % of the experimental groups exposed to 0.04 mg of the juvenile hormone analogue moulted during the same period. Details of these results are given in figure 1. The differences are statistically significant as indicated by $p < 0.01$ when the groups exposed to 0.04 mg of ZR 512 are compared with the controls, and $p < 0.0005$ when the groups exposed to 0.4-40 mg of ZR 512 are also compared with the same controls.

Examination of the moults and of the time when ecdysis occurred revealed that the large number of moults induced in the groups exposed to 0.4 mg or more of juvenile hormone analogue was due to presoldiers appearing in 20-30 days from the start of the experiment. Most of these presoldiers later moulted into soldiers. No replacement reproductives were produced in these groups. Within the control groups, moulting occurred at about the same rate during the 50 day observation period and replacement reproductives were

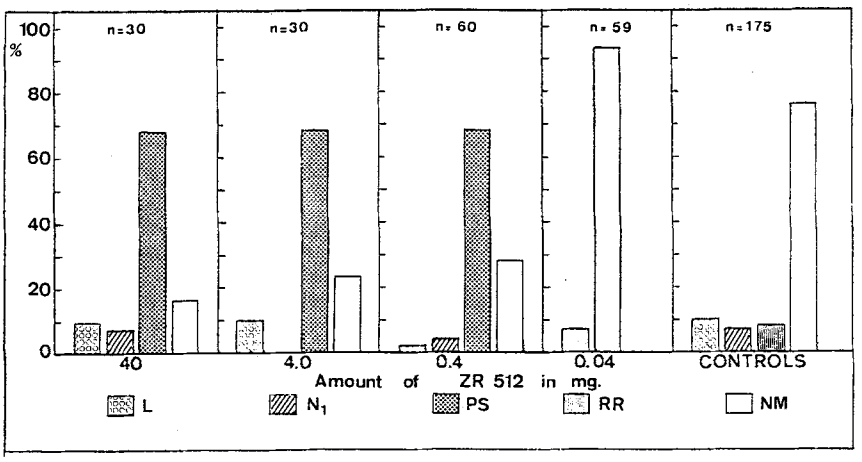

FIG. 1. — *Total moults within 30 days of larvae treated with ZR 512 (% of surviving).* Groups of 30 individuals were used for experiments and controls. *L*, larvae; *n*, number of surviving larvae (in most cases this is the same as the initial number of larvae at the start of the experiment; the death rate was extremely low during these investigations); *RR*, replacement reproductives; *PS*, presoldiers; *NM*, unmoulted larvae.

ABB. 1. — *Die während 30 Tagen erfolgten Häutungen von Larven, die mit ZR 512 behandelt wurden (in % der Ueberlebenden).* Für jedes Experiment wurden 30 Tiere verwendet. *L*, Larven; *n*, Anzahl der überlebenden Larven (entspricht meist der Zahl der eingesetzten Larven, da die Mortalität sehr gering war); *RR*, Ersatzgeschlechtstiere; *PS*, Vorsoldaten; *NM*, nichtgehäutete Larven.

produced. With 0.04 mg of the juvenile hormone analogue, moulting of the larvae was inhibited and replacement reproductives appeared only towards the end of the 50 day observation period. Details of these results are presented in figures 2 and 3.

The effect of ZR 512 on larval prothoracic glands.

Even with the use of the highest doses of the juvenile hormone analogue, 100 % presoldier development did not occur. Instead about 20 % of the experimental larvae always remained unmoulted after the 50 day observation period. From this observation it was suspected that the JH analogue might have differing effects on the prothoracic glands of the exposed larvae. In order to verify this, homogeneous groups of pseudergates of *Z. nevadensis* were exposed to 0.4 mg of ZR 512 for 14 days and fixed the following day in alcoholic Bouin's solution. The size of their prothoracic glands as well as that of corresponding controls was determined, the results of which are given in figure 4.

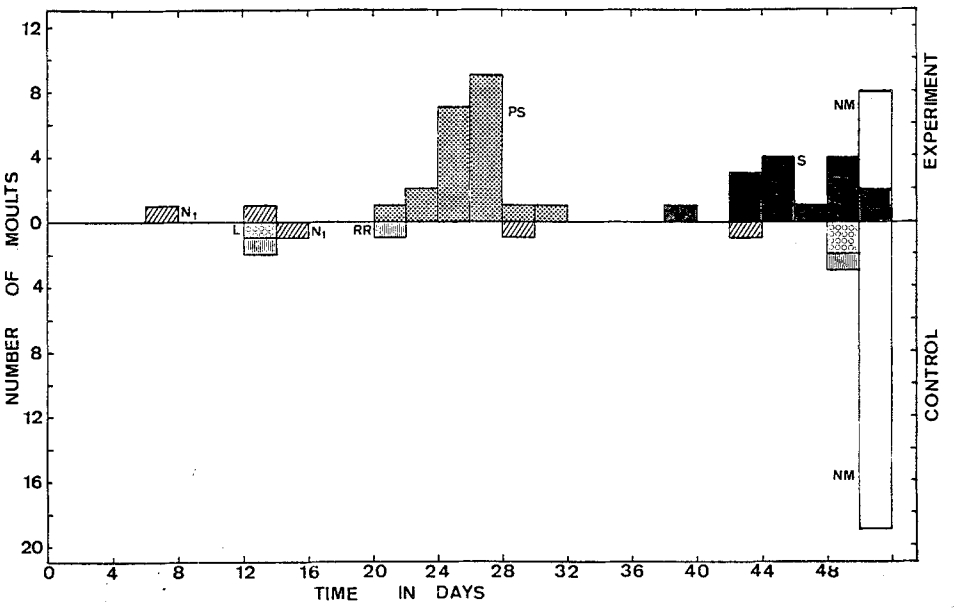

FIG. 2. — *Effect of 0.4 mg of ZR 512 on larval development.* 30 pseudergates, all from the same stock colony, were used in the experimental and corresponding control group. *S*, soldiers. For other abbreviations, see figure 1.

ABB. 2. — *Wirkung von 0.4 mg ZR 512 auf die Entwicklung von Larven (Pseudergaten).* Für Versuch und Kontrolle wurden je 30 Pseudergaten der gleichen Stammkolonie verwendet. *S*, Soldaten. Andere Abkürzungen, siehe Abbildung 1.

The normal prothoracic gland cross-sectional area of the control larvae is between 250-600 μ^2. In most of the experimental larvae, however, the cross-sectional area of the prothoracic glands in above 1 000 μ^2 or below 100 μ^2. In *Z. angusticollis* the cross-sectional area of the prothoracic glands in replacement reproductives is 63 ± 7 μ^2 (2) in contrast to the value of 314 ± 31 μ^2 for the larval (pseudergate) glands (LÜSCHER, unpublished data). The JH analogue may therefore cause the enlargement of the prothoracic glands in those larvae which later moult (into presoldiers), or alternatively cause their reduction, making them comparable to those of replacement reproductives in which they are known to be degenerated (GILLOTT and YIN, 1972).

(2) The replacement reproductives used for these measurements were all older than 34 days.

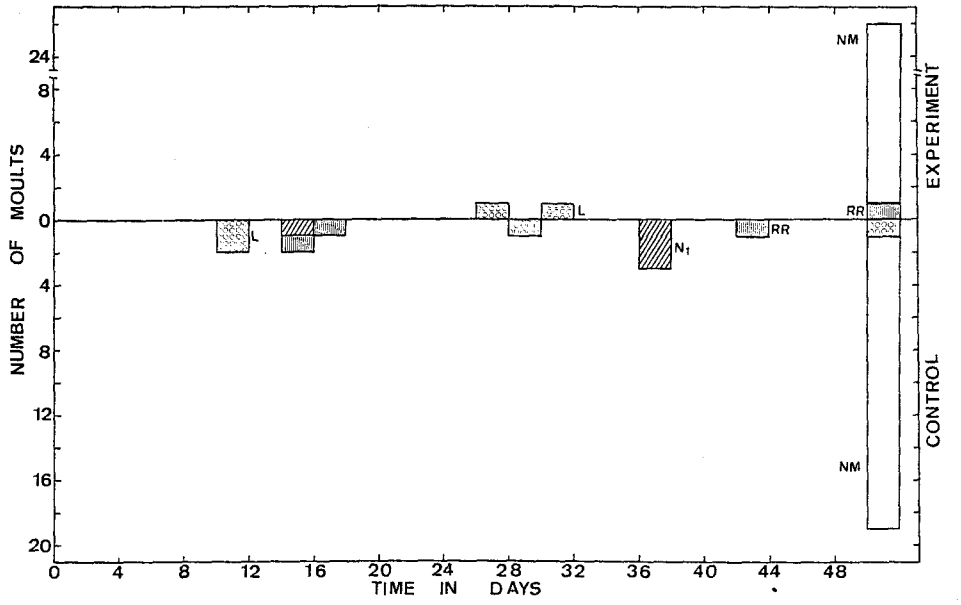

FIG. 3. — *Effect of 0.04 mg of ZR 512 on larval development.* 30 pseudergates, all from the same stock colony as those used in the results presented in figure 2 were used in the experimental and corresponding control group. For abbreviations, see figure 1 and 2.

ABB. 3. — *Wirkung von 0.04 mg ZR 512 auf die Entwicklung von Larven (Pseudergaten).* Je 30 Pseudergaten der gleichen Stammkolonie, die auch für den in Abbildung 2 dargestellten Versuch verwendet wurde, dienten als Versuch und Kontrolle. Abkürzungen siehe Abbildungen 1 und 2.

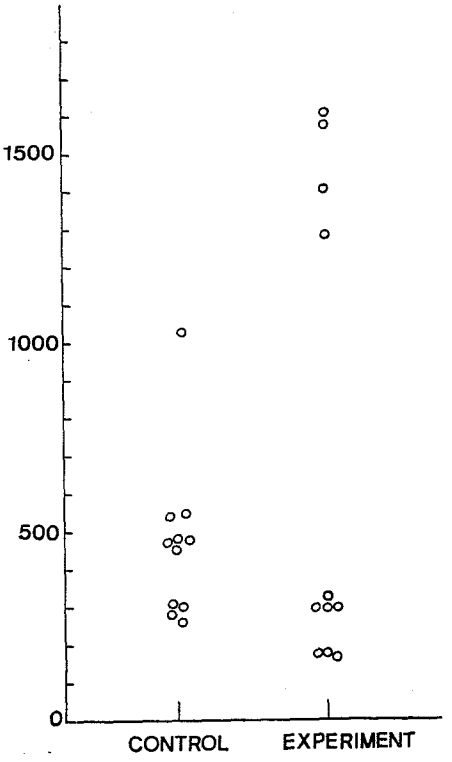

FIG. 4. — *Effect of 0.4 mg of ZR 512 on larval prothoracic glands 15 days from the start of the experiment.* Ordinate : cross-sectional area of prothoracic glands in μ^2.

ABB. 4. — *Wirkung von 0.4 mg ZR 512 auf die larvalen Prothorakaldrüsen 15 Tage nach Versuchsbeginn.* Ordinate : Querschnittsfläche der Prothorakaldrüsen in μ^2.

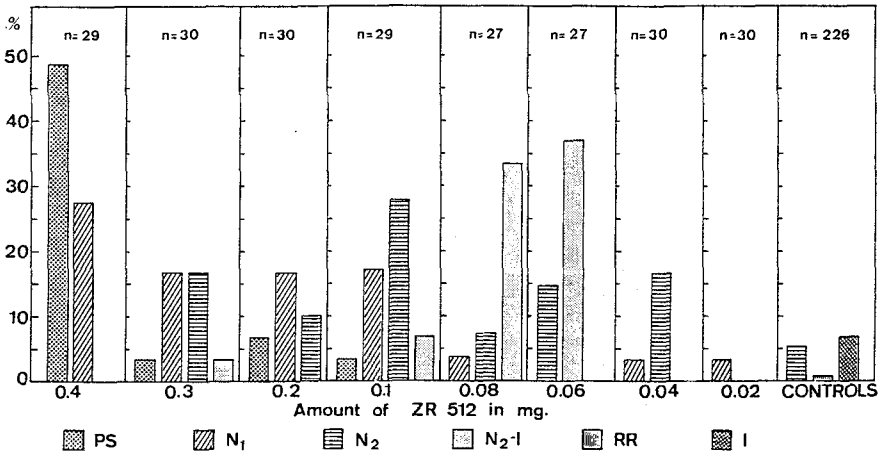

FIG. 5. — *Developmental alternatives of last instar nymphs occurring under the influence of ZR 512. S,* soldier; N_1 first instar nymph; N_2, last (or second) instar nymph; N_2-*I,* nymph-imago intermediate; *I,* imago of alate (for comparison only).

ABB. 5. — *Die Entwicklung von Nymphen des letzten Stadiums unter dem Einfluss von ZR 512. S,* Soldat; N_1, Nymphe des ersten Stadiums; N_2, Nymphe des zweiten oder letzten Stadiums; N_2-*I,* Nymphe-Imago Zwischenkaste; *I,* geflügelte Imago (nur zum Vergleich).

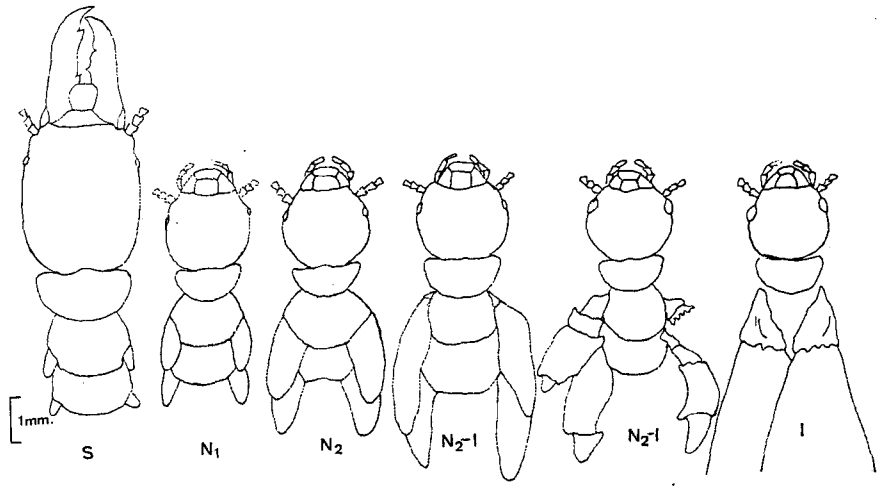

FIG. 6. — *Total moults within 50 days of last instar nymphs treated with ZR 512 (% of surviving). PS,* presoldier; *RR,* replacement reproductive; *n,* number of surviving last instar nymphs (this is nearly the same as the initial number of nymphs, the death rate having been extremely low); for other abbreviations, see figure 5. 30 individuals were used for each experiment, 8 times 30 for the controls.

ABB. 6. — *Die innerhalb von 50 Tagen erfolgten Häutungen von Nymphen des letzten Stadiums, die mit ZR 512-Gas behandelt wurden (in % der Ueberlebenden). PS,* Vorsoldat; *RR,* Ersatzgeschlechtstier; *n,* Zahl der überlebenden Nymphen (meist gleich der Zahl der eingesetzten Nymphen, da die Mortalität gering war); andere Abkürzungen siehe Abbildung 5. Alle Gruppen enthielten 30 Individuen.

The effect of ZR 512 on the development of last instar nymphs.

Homogeneous groups of last instar nymphs of *Z. nevadensis* from stock colonies in which alates were forming, were exposed to 0.4-0,02 mg of ZR 512 for 14 days and their subsequent development observed for 50 days from the start of the investigation. The developmental alternatives of a last instar nymph are illustrated in figure 5. The correlation between these developmental alternatives and the dose of ZR 512 used are given in figure 6.

Thus with the high dose of 0.4 mg of ZR 512, presoldier and regressive development (giving rise to first instar nymphs) was induced. With intermediate doses ranging from 0.3-0.04 mg there was a substantial number of stationary moults as well as progressive moults giving rise to nymph-imago intermediate forms. The lowest amount of ZR 512 had an inhibitory effect on the moulting of the nymphs, comparable to that already observed when larvae of the same species were exposed to 0.04 mg of the juvenile hormone analogue.

DISCUSSION

Imaginal or alate development in *Z. nevadensis* occurred only in the absence of the juvenile hormone analogue, that is, in the control groups of last instar nymphs. Thus imaginal development in the termite is comparable to that in other hemimetabolous insects. The same applies to replacement reproductive development which however differs from imaginal metamorphosis in that it can arise, not only from last instar nymphs as in the latter case, but also from younger nymphs and larvae.

It is interesting that the lowest doses of the juvenile hormone analogue have an inhibitory effect on the moulting of homogeneous groups of larvae and nymphs. Only towards the end of the observation period or later, almost a month after the transfer of the termites into clean juvenile hormone analogue free dishes did the number of moults increase and replacement reproductives develop in these groups. Thus the effect of low doses of the juvenile hormone analogue compares very well with the known inhibition that functional reproductives exert on larvae and nymphs in this and other termites (MILLER, 1969; LÜSCHER, 1974 *a*).

It has also been demonstrated that excess juvenile hormone I and the juvenile hormone analogue geranyl phenyl ether (R-20458, Stauffer Compound), have a similar moulting inhibition when continuously supplied to the larvae of the cockroach *Blattella germanica* (MASNER and HANGARTNER, 1973).

With intermediate doses of ZR 512 on last instar nymphs of *Z. nevadensis*

mainly progressive development giving rise to nymph-imago intermediate forms and stationary moults occurred. It is noteworthy that intermediate, stationary and progressive moults occurred at a much higher rate in the experimental groups than in the corresponding controls. This indicates that, as in induced soldier development, the induction of regressive development also stimulates moults, that is, it must bring about an activation of the prothoracic glands.

High doses of the juvenile hormone analogue caused a large number of pre-soldier moults. The juvenile hormone analogue also in this case leads to an induced moult and therefore has a stimulating effect on the prothoracic glands. However, the opposite effect, a reduction of these glands, can also result from the same treatment, probably depending on the competence for soldier development which is low at the beginning and high towards the end of the moulting interval (*Kalotermes flavicollis,* SPRINGHETTI, 1972; *Zootermopsis angusticollis,* LÜSCHER, 1974 *b*). A similar response of the prothoracic glands has been observed when pupae of the Saturniid *Hyalophora cecropia* were injected with β-ecdysone. The activity of the prothoracic glands was profoundly stimulated in non-developing pupae but drastically decreased in those which had just initiated pharate adult development (SIEW and GILBERT, 1971).

It is also noteworthy that last instar nymphs like larvae and nymphs of the first instar, readily develop into soldiers when treated with high doses of ZR 512. These soldiers, in contrast to those developing from larvae and first instar nymphs, usually possess wing pads. According to the scheme of development which MILLER (1969) based on the findings of CASTLE, LIGHT and WEESNER, the transformation of last instar nymphs into soldiers does not readily occur in *Zoostermopsis* although it is pointed out that instances of brachypterous soldiers have been recorded. The addition of this developmental step in the scheme of LÜSCHER (1974 *a*), therefore, is considered justified. Since last instar nymphs of *Zootermopsis* also readily undergo regressive development and moult into nymphs of the first instar, it is suggested that this step too should be included in the scheme of development of *Zootermopsis.*

Apart from prothoracic gland effects, the juvenile hormone analogue ZR 512 can affect all the steps of caste formation in *Z. nevadensis.* These studies therefore offer additional evidence for the dependence of the development of different casts in the lower and possibly higher termites on the juvenile hormone titer during the moulting interval of larvae and nymphs, as postulated by LÜSCHER (1972).

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