

THE ACTION OF JUVENILE HORMONE ANALOGUES ON CASTE DEVELOPMENT IN ZOOTERMOPSIS (ISOPTERA).

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It was shown earlier that juvenile hormone extracts as well as the juvenile hormone analogue, farnesyl methyl ether, inhibit the development of replacement reproductives in Kaloterme flavicollis and that the synthetic juvenile hormone (Cecropia-hormone 1) could induce soldier development when injected or fed to larvae of the same species. From histological evidence it was also concluded that the development of the winged imago must depend on the juvenile hormone titre and that juvenile hormone therefore is somehow involved in all steps of caste differentiation (Lüscher 1972). We have now investigated the influence of various doses of juvenile hormone analogues on caste development in Zootermopsis angusticollis and Z. nevadensis, using groups of older larvae (pseudergates) and last instar nymphs. It was also of interest to use larvae of known age within their moulting interval in order to find out at what stage they are most susceptible to becoming soldiers.

Since juvenile hormone was available only in very limited amounts, we have used the analogues farnesyl methyl ester and ZR 512 of Zoëcon Corporation. These were fed on filter paper or applied as vapours. The experimental groups of termites were kept in plastic boxes of 6 cm. diameter. In order to supply the substances as vapours they were diluted with acetone or ether which was allowed to evaporate, either in a glass cover of the box, or in a glass dish, which was placed on the bottom of the box and covered with wire gauze. When the solvent had evaporated, the termites were introduced into the container. The termites were observed daily and moulted individuals were marked with nitro-cellulose paint.

When orphaned groups of larvae of Z. nevadensis were exposed for 2 weeks to high doses of ZR 512 vapour, obtained from 0.4 to 40 mg. of the chemical, the frequency of moults in the experimental colonies was similar to that in the controls during the first 10 days. By the end of 30 days, however, up to 80% of the experimental termites had moulted, in contrast to about 20% in the control groups. The high number of moults in the experimental groups was due to presoldier moults (60-70%) while in the controls no presoldiers were produced. The outburst of presoldier formation occurred between 18 to 28 days after the onset of the treatment. After 50 days, 30-75% of the presoldiers had moulted into soldiers, but the number of unmoulted larvae remained the same, while in control colonies moulting continued at the same rate throughout the experiment.

It is of interest that, even after treatment with the highest doses

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of ZR 512, the rate of presoldier formation never reached 100%. Instead about 20% of the larvae remained unmoulted after 50 days. In order to see if the larvae are at all times competent to react to juvenile hormone with presoldier formation we have performed the following experiment. Several groups of orphaned larvae of Z. angusticollis were observed daily for 100 days and all moulted individuals were marked. Then farnesyl methyl ester was applied as vapour at a dose of 50  $\mu$ l, lasting for 3 weeks. The larvae which had moulted up to 40 days previously did not moult again, but with increasing age within the moulting interval, the proportion of presoldier moults increased and reached a maximum of 37.5% in those which had not moulted in the previous 100 day period. This means that there is, during the moulting interval, not only a changing competence for replacement reproductive formation as has been shown in Kaloterme flavicollis (Lüscher 1952), but also for soldier formation. While replacement reproductive competence is highest at the beginning of the moulting interval, soldier competence is highest at its end. This confirms the recent findings of Springhetti (1972) that the soldier competence period in Kaloterme flavicollis lasts approximately from the 45th to the 60th day of the moulting interval. We can now assume that the unmoulted individuals in our first experiment were larvae which had undergone a moult shortly before applying the hormone analogue vapour and which were therefore not competent to become soldiers.

The second experiment was carried out after the outburst of soldier formation was over, and it was noted that no further moults occurred. Some of the unmoulted larvae were fixed 100 days after the farnesyl methyl ester treatment in order to investigate the condition of the prothoracic gland. This gland consists, in Zootermopsis, of two strands of cells in the prothorax. In most cases the strands are fused for a short distance near their anterior end. Behind this fusion they run more or less parallel posteriorly. In order to estimate the volume of the glands we measured the cross sectional area of the strands behind the fusion, in at least 12 serial sections. In 8 treated larvae this area was found to be  $120 \pm 16 \mu^2$ , while in 8 control larvae it was as large as  $314 \pm 31 \mu^2$ . In old replacement reproductives, in which the prothoracic gland is said to be completely degenerate, the strands have a cross section area of  $63 \pm 7 \mu^2$ . The prothoracic glands of the treated larvae can not therefore be considered degenerate, but rather they are rudimentary as in soldiers and workers of higher termites, which in most cases do not moult again (Kaiser 1956). Some of our treated larvae are still alive after 3 years, without having moulted. We have therefore produced something which is physiologically similar to a worker caste through the action of a juvenile hormone analogue. This may be an indication that juvenile hormone, in higher termites, is also involved in the determination of workers. The degeneration of the prothoracic gland probably occurs soon after hormone treatment since following treatment with 0.4 mg of ZR 512 vapour, 25% of the termites had partly degenerated glands within 15 days. The remaining

having rather large glands, were probably differentiating into presoldiers.

Small quantities of ZR 512 (0.04 mg) did not stimulate presoldier formation but significantly inhibited moulting during the 50 day period of the experiment. By the end of this period only 10% of the experimentals had moulted in comparison to 30% moults in the controls. In addition, the moults of the experimentals gave rise to larvae (stationary moult) and nymphs (progressive moult) only, while in the control groups replacement reproductives also developed. After 50 days, however, the rate of moulting in the experimental colonies increased again and replacement reproductive moults were then also observed. These results indicate that juvenile hormone in small quantities retards moulting and prevents the formation of replacement reproductives, while it induces presoldier development or a partial breakdown of the prothoracic gland, when it is present in larger amounts.

The influence of juvenile hormone analogues on progressive and regressive development was studied in homogeneous groups of last instar nymphs. If such groups of Z. nevadensis were exposed to doses of between 0.02 mg. and 0.4 mg. of ZR 512 as vapour, for a period of 14 days, the lowest dose led to moult inhibition. With the increase of the dose, there was first an increasing number of pseudo-imagoes (intermediates between imago and nymph) then of stationary moults, then of regressive moults and finally of presoldier moults. In all these cases the moulting rate was increased, i. e. the moults occurred before they would normally have taken place as imaginal moults. Very similar results were obtained by feeding homogeneous groups of Z. angusticollis with doses of between 0.03 and 3.0  $\mu$ l of farnesyl methyl ester. Thus juvenile hormone analogues inhibit imaginal development as they do in other insects. In addition they can also lead to regressive moults and in higher concentrations to presoldier moults. It can be assumed that juvenile hormone itself should have the same effects. It is noteworthy that last stage nymphs easily develop into presoldiers with wing pads when treated with juvenile hormone analogues, although 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 presoldiers does not normally occur.

In conclusion, it may be stated that juvenile hormone analogues in low doses inhibit moulting. In slightly higher doses they prevent the development of replacement reproductives and alates. An even higher dose is necessary for regressive development and the highest doses tested led to presoldiers and to workerlike forms. Our study offers additional evidence for the dependence of the development of the different castes of the primitive, and possibly also of the higher termites, on the juvenile hormone titre during the moulting intervals of larvae and nymphs (Lüscher 1972).

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