Inhibition of Oogenesis and Ovarian Ecdysteroid Synthesis by Azadirachtin in *Locusta migratoria migratorioides* (R. & F.)

Heinz Rembold and Klaus-Peter Sieber

Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München

Z. Naturforsch. **36 c**, 466–469 (1981); received November 10, 1980/February 23, 1981

Locusta migratoria, Azadirachta indica, Ecdysteroids, Azadirachtin, Ovary

The sterilizing effect of azadirachtin, an insect growth regulator from the neem tree, *Azadirachta indica* A. Juss, was tested with mature females of *Locusta migratoria*. After a single injection of 10 µg into females between 2 and 13 days after emergence, about 60% died during the following four days and all lost weight in the range of about 50%. If the compound was injected into animals of an age between 2 and 10 days, no maturation of the terminal oocytes could be observed. Injection at an age between 10 and 13 days after adult emergence resulted in ovaries which contained almost mature oocytes. Most of the treated locusts had no oviposition and only traces of ecdysteroids were present in their ovaries, as quantified by radioimmunoassay. The inhibition of oogenesis and of ovarian ecdysteroid synthesis by azadirachtin is discussed on the basis of its interference with the neuroendocrine control of hormone synthesis.

The various parts of the neem tree, *Azadirachta indica* A. Juss, have long been used for medicinal and plant protection purposes in Asia and Africa. Seed kernel extracts have recently been found to act as strong feeding inhibitor for *Locusta migratoria* and several other insect species [1, 2]. Defined neem compounds with such properties are meliantriol [3], azadirachtin [4–6], and salannin [7]. The treatment of insects with neem seed extracts also causes growth inhibition, malformation, mortality, and loss of fecundity [8–12]. Growth regulating activity of azadirachtin has been demonstrated on *Epilachna varivestis, Ephestia kuehniella* and *Apis mellifera* [13, 14]. For *Locusta migratoria* it could be demonstrated that a single injection of 10 µg azadirachtin into the adult female disrupts oogenesis. When the compound was applied at an early stage of egg development, oogenesis stopped after previtellogenesis. However, when injected after the start of vitellogenesis, only a few egg patches were laid. From the eggs only a few embryos developed to first instar and none came to a second moult. It was concluded that azadirachtin interferes with the endocrine regulation or synthesis of ecdysteroids in adult female locusts [15]. Ecdysone and other ecdysteroids are synthesized in the cells of the follicular epithelium at the end of oocyte maturation. Practically all the ovarian ecdysteroids are finally contained in the newly-laid eggs and control cuticulogenesis during early development of the embryo [16]. We have therefore studied the influence of azadirachtin on oogenesis and ovarian ecdysteroid pools in more detail and now report data which demonstrate the interference of this compound with both egg maturation and ecdysteroid synthesis.

Reprint requests to Prof. H. Rembold.

0341-0382/81/0500-0466 $ 01.00/0

Materials and Methods

Azadirachtin was isolated from neem seeds by extraction with methanol. Lipids were removed with hexane and the polar compounds were fractionated by open column silicic acid chromatography as previously described [14]. After preparative TLC, azadirachtin was purified by HPLC on an RP-18 (Merck) column.

Ecdysone was purchased from Serva (Heidelberg).

Locusta migratoria migratorioides (R. & F.) were grown at 40% r. h., 38 °C (12 h light) and 24 °C (12 h dark), respectively, and were kept under crowded conditions.

The females were individually marked immediately after adult moult with a number on Scotch tape; their weight was measured daily for a period of 25 days. Azadirachtin (10 µg/10 µl 90% ethanol) or 10 µl 90% ethanol was injected in the abdomen with a micro-applicator (ISCO). Insects between 2 and 13 days after adult moult were injected. State of egg maturation was expressed by the length of terminal oocytes (LTO).

Extraction of the ovaries. The organs were dissected 4–7 days after azadirachtin injection, length of the terminal oocytes (LTO) was measured, and the ovaries were then homogenized in 2–4 ml (ten times the organ fresh weight) methanol with a Potter homogenizer. The homogenate was centrifuged (6000 rpm), the precipitate washed twice with 1 ml of 90% methanol. Lipid was extracted from the methanol/water phase with dichlormethane and the alcohol concentrated in vacuo to a volume of 1 ml per ovary for measurement in the radioimmunoassay.

Radioimmunoassay (RIA). The procedure described by Spindler *et al.* [17] was followed. Tritiated ecdysone (80 mCi/mmole, NEN, Dreieichenhain) was used. Antiserum ICT-1 was generously donated by K. D. Spindler (Darmstadt). Aliquots of the methanol extract were dried under nitrogen, labelled ecdysone (4200 dpm) was added in 50 µl borate buffer, pH 8.4, and the mixtures were vigorously shaken. Then 50 µl antiserum was added, and the mixture was incubated for at least 3 h at room temperature. After cooling to 4 °C, 100 µl of saturated ammonium phosphate solution (4 °C) was added for precipitation of protein. After centrifugation, the supernatant was sucked off and the precipitate dissolved in 10 µl water plus 300 µl Unisolve (Koch-Light) and measured in a TriCarb scintillation counter (Packard). To test for an interference of azadirachtin with this assay, 0.5, 1 and 5 µg of the compound were added to labelled ecdysone under standard test conditions. No effect was observed.

Results and Discussion

1. Injection of azadirachtin 2–10 days after adult emergence

After a single injection of azadirachtin into mature female locusts between days 2 and 10 after emergence, follicle growth is inhibited (Fig. 1). In the control group growth starts after day 5 and ends with a length of the terminal oocytes (LTO) of 6.2 mm. Oviposition follows around day 15 after adult emergence. However, no substantial increase in the length of the terminal oocytes can be observed in the azadirachtin-treated group. The course of oogenesis was apparently stopped after azadirachtin injection. This is possibly a consequence of its interference with vitellogenin synthesis and/or incorporation into the oocytes either directly or indirectly through its endocrine control.

2. Injection of azadirachtin 10–13 days after adult emergence

Concentration of ecdysteroids in ovaries of control females increased near the end of vitellogenesis and reached its maximum within hours (Fig. 2). This

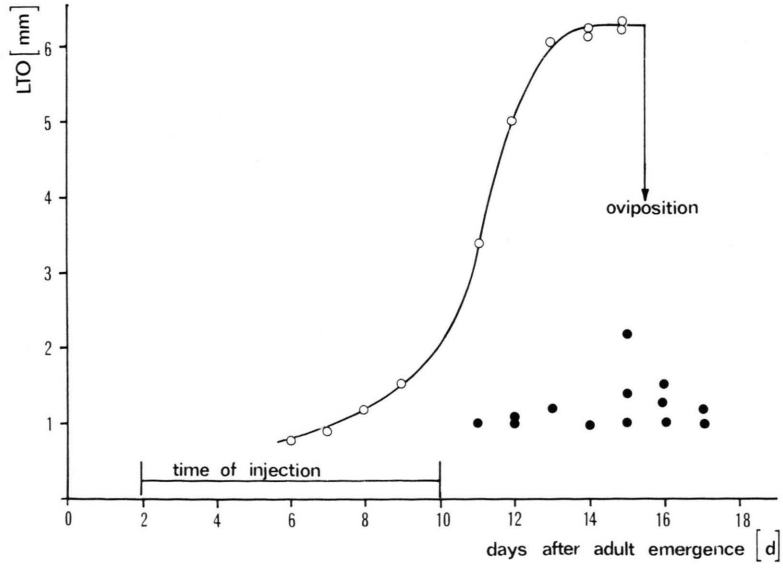

Fig. 1. Effect of a single azadirachtin injection (10 µg/individual) on the oogenesis of individual females of *L. migratoria.* Abscissa: course of ovarian development (in days from adult emergence); ordinate: oocyte development (in mm of terminal oocyte length, LTO). The animals were injected at an age between 2 and 10 days after emergence. –○–: Control animals (10 µl of 90% ethanol); ●: azadirachtin treated animals.

high rate of ecdysteroid synthesis has also been reported by other groups [18, 19]. When azadirachtin was injected at the end of oogenesis (days 10–13 after adult emergence), only small amounts of moulting hormone were found in the ovaries (Figs. 2, 3). Growth of the terminal oocytes was normal (Fig. 3). Azadirachtin reduced the total amount of ovarian ecdysteroids. Moreover, the ovaries of the azadirachtin-treated animals were smaller (average weight: 158 ± 87 mg) than those of controls (av. weight: 339 ± 79 mg) as the number of mature oocytes was reduced apparently due to complete resorption without any visible residue. The ecdysteroid concentration of the azadirachtin group amounted to 6 ± 4.35 ng/mg ovary ($n = 10$), whereas the control ovaries contained as much as 27.9 ± 15.6 ng/mg ($n = 10$).

3. Effect of injected azadirachtin on body weight and death rate

There is a steady weight gain in untreated animals (Fig. 4) due primarily to an increased protein production and incorporation into the developing oocytes [20]. In the ethanol control group, weight is reduced only during the first day after injection and then parallels the growth rate of the untreated group. The azadirachtin treated animals continuously lost weight. About 60% of them died within the first four days after injection, without an indication of acute toxicity.

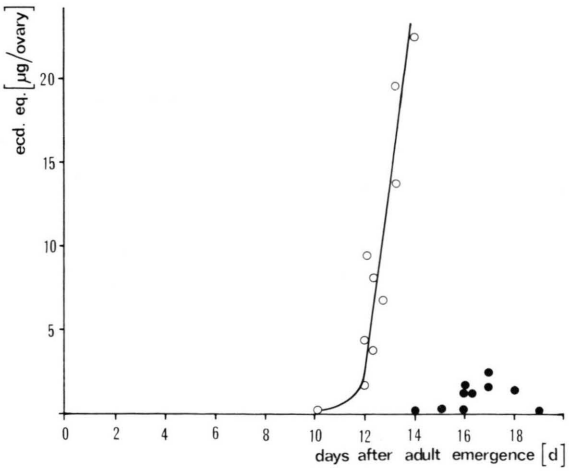

Fig. 2. Effect of a single azadirachtin injection (10 µg/individual) on the ovarian ecdysteroid content of *L. migratoria*. Abscissa: days from adult emergence; ordinate: amount of RIA ecdysteroid equivalents (ecd. eq.) per individual ovary. The animals were injected at an age between 10–13 days after emergence. –○–: Control animals (10 µl of 90% ethanol); ●: azadirachtin treated animals.

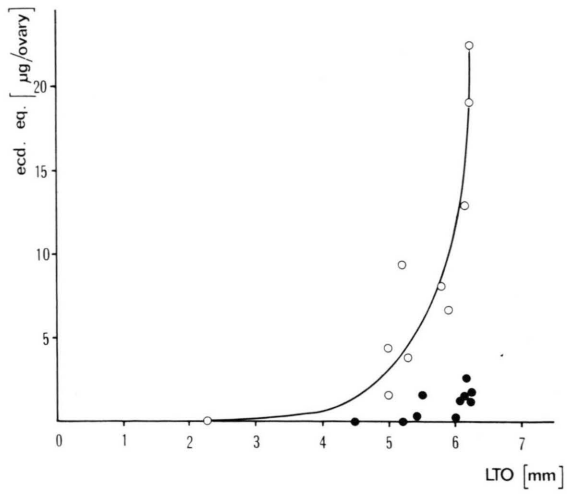

Fig. 3. Interrelation of ovarian development and ecdysteroid content (10 µg/individual). Abscissa: oocyte development (in mm of terminal oocyte length, LTO); ordinate: amount of RIA ecdysteroid equivalents (ecd. eq.) per individual ovary. The animals were injected at an age between 10–13 days after emergence. –○–: Control animals (10 µl of 90% ethanol); ●: azadirachtin treated animals.

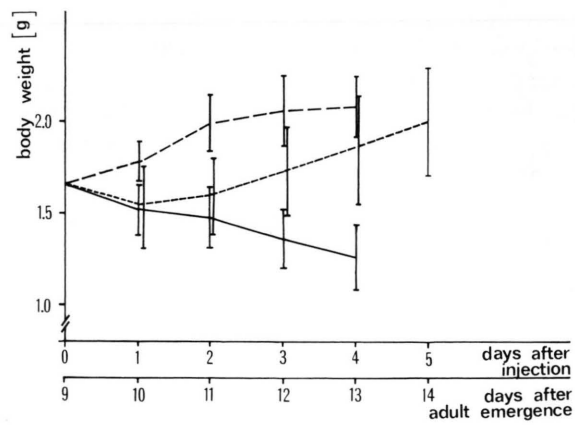

Fig. 4. Effect of azadirachtin on weight gain of mature female *L. migratoria*. On day 9 after emergence 7 animals were each injected with 10 µg azadirachtin (—), 9 control animals received the same volume of 10 µl 90% ethanol (- - - -), and 11 animals were untreated controls (– –).

Conclusions

Azadirachtin interferes with oogenesis at all stages. In the group injected 10–13 days after emergence,

none of the surviving females layed eggs, even though, the terminal oocytes were almost mature (LTO = 6.2 mm). Both oogenesis (Fig. 1) and ovarian ecdysteroid synthesis (Figs. 2, 3) are inhibited by a single injection of azadirachtin. This inhibition is not caused by interference with the synthesis of juvenile hormone, since ablation of the corpora allata after day 5 of adult life does not influence either oogenesis or oviposition [21]. Thus, ovarian ecdysteroid synthesis is probably under direct control of the brain hormone [18]. Chorion synthesis and oviposition cease when the cerebral neurosecretory cells of the pars intercerebralis are cauterized [22, 23]. Azadirachtin most probably reduces the total amount and concentration of ecdysteroids relative to the controls by acting upon the neuroendocrine system. This neuroendocrine interaction could also explain both the decrease in body weight and the inhibition of oviposition.

[1] T. L. Ladd, M. Jacobson, and C. R. Buriff, J. econ. Ent. **71**, 810–813 (1978).
[2] J. D. Warthen, USDA Sci. Educ. Agric. Rev. Man., ARM-NE-4 (1979).
[3] D. Lavie, M. K. Jain, and S. R. Shpan-Gabrielith, Chem. Communs. **1967**, 910–911.
[4] J. H. Butterworth and E. D. Morgan, Chem. Communs. **1968**, 23–24.
[5] J. H. Butterworth and E. D. Morgan, J. Insect Physiol. **17**, 969–977 (1971).
[6] P. R. Zanno, I. Miura, K. Nakanishi, and D. L. Elder, J. Am. Chem. Soc. **97**, 1975–1977 (1975).
[7] J. D. Warthen, E. C. Uebel, S. R. Dutky, W. R. Lusby, and H. Finegold, USDA Agri. Res. Results, ARR-NE-2 (1978).
[8] R. Steets, Z. ang. Ent. **77**, 306–312 (1975).
[9] R. Steets, Dissertat. Univ. Giessen, 1976.
[10] R. Steets, Z. ang. Ent. **82**, 169–176 (1976).
[11] R. Steets and H. Schmutterer, Z. PflKrankh. PflSchutz **82**, 176–179 (1975).
[12] H. Schmutterer and H. Rembold, Z. ang. Ent. **89**, 179–188 (1980).
[13] H. Rembold and H. Schmutterer, Abstracts, Int. Conf. on Regulation of Insect Develop. and Behav., Karpacz, Poland, June 23–28, 1980.
[14] H. Rembold, G. K. Sharma, Ch. Czoppelt, and H. Schmutterer, J. Plant Dis. Prot. **87**, 290–297 (1980).
[15] H. Rembold and K.-P. Sieber, Proc. 1st Neem Conference, Ringberg Castle, June 16–20, 1980 (in press).
[16] J. Hoffmann, Reprod. Nutr. Develop. **20**, 443–456 (1980).
[17] K. D. Spindler, C. Beckers, U. Gröschel-Stewart, and H. Emmerich, Z. Physiol. Chem. **359**, 1269–1275 (1978).
[18] M. Lagueux, M. Hirn, and J. A. Hoffmann, J. Insect Physiol. **23**, 109–119 (1977).
[19] F. Goltzene and A. Porte, Gen. Comp. Endocrin. **35**, 35–45 (1978).
[20] T. T. Chen, P. Couble, R. Abu-Hakima, and G. R. Wyatt, Develop. Biol. **69**, 59–72 (1979).
[21] L. Joly, F. Goltzene, and A. Porte, J. Insect Physiol. **24**, 187–193 (1978).
[22] A. R. McCaffery, J. Insect Physiol. **22**, 1081–1092 (1976).
[23] M. Charlet, F. Goltzene, and J. A. Hoffmann, J. Insect Physiol. **25**, 463–466 (1979).