be a reflection of the decreased synthesis of lymphokines and immunoglobulins as a result of immunosuppression, which is known to be prevalent in clinical and experimental filariasis especially in the chronic/patent stages [5-13]. The above postulate is supported by the observation of Novogrodsky et al. [1] of a direct correlation of γ-GT activity with synthesis and secretion of lymphokines/immunoglobulins from T and B-lymphocytes, respectively. Furthermore, the production of interleukin and interferon has been shown to be appreciably decreased in lymphocytes derived from microfilariaemic individuals [6]. It seems possible that the enzyme γ-GT in the host's immune system could very well be utilized as a marker of immunosuppression and/or immunostimulation. Further work on monitoring γ-GT in immunosuppressed and immunostimulated hosts is in progress, which should help to show whether this is indeed the case.

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Effects of a juvenile hormone mimetic, fenoxycarb, on post-embryonic development of the European corn borer, *Ostrinia nubilalis* Hbn.

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Summary. The effect of a juvenile hormone mimetic, fenoxycarb, Ro 13-5223, was tested on the larval instars of the European corn borer, *Ostrinia nubilalis*, by dipping or topical application. When larvae were treated in instars 2, 3 or 4, the duration of the fifth instar was modified. More permanent and fewer supernumerary larvae were obtained when treatment occurred in the early instars. This non-neurotoxic compound exhibited a strong dose-dependent juvenile hormone type of activity when it was applied to last instar larvae. Fenoxycarb prevented the onset of pupation and produced supernumerary larvae and intermediates. Permanent larvae were obtained if fenoxycarb was applied on day 0 or day 1 of the last instar. The use of such a JH mimetic in the understanding of endocrine control of diapause is discussed.
Key words. European corn borer; *Ostrinia nubilalis*; juvenile hormone mimetic; fenoxycarb; development; insect growth regulator.

Both juvenile hormone and 20-hydroxyecdysone are the key hormones regulating insect molting and metamorphosis [1]. In final instar larvae, the juvenile hormone titer falls to very low levels, allowing ecdysone release to initiate a metamorphic program. Applications of exogenous juvenile hormone during larval development may prolong various instars or delay and even prevent the onset of metamorphosis [2, 3].

Fenoxycarb is a non-terpenoid, non-neurotoxic carbamate exhibiting strong juvenile hormone activity [4]. It is thus classified as an insect growth regulator. It is now commonly used in orchard pest control [5] and tested in the protection of stored products [6]. It has been shown to interact with juvenile hormone esterase activity [7], to act on embryogenesis [8], and on the activity of the corpora allata [9].

Many studies have dealt with fenoxycarb, but few of them have dealt with laboratory experiments on development and molting in Lepidoptera.

The purpose of this study was to determine the effects of fenoxycarb on the larval development of the European corn borer, *Ostrinia nubilalis*, a major pest of maize throughout the world.

Materials and methods

European corn borer eggs were supplied by INRA (Institut National de la Recherche Agronomique, le Magneraud, 17700 Surgères, France). Larvae were reared on a semi-synthetic diet according to Poitout and Bues[10]. Rearing took place in plastic boxes in an incubator maintained at 25 °C with 75 % relative humidity and a 16L:8D photoperiod. These conditions lead to a development without diapause. The age of the larvae was checked according to Gelman and Hayes[11]. They were observed daily for ecdysis. Day of ecdysis corresponds to day 0 of the instar.

Fenoxycarb, Ro 13-5223 (ethyl 2-(4-phenoxy-phenoxy)ethylcarbamate) was obtained from Dr Maag Ltd, Dielsdorf, Switzerland, and used in technical form (95 % purity) in acetone.

Second, third and fourth instar larvae were dipped into various dilutions of fenoxycarb (0.04, 0.2, 1 and 5 µg/µl) on the day following ecdysis to test the effect of early application. They were then raised in individual plastic boxes (20 × 20 × 10 mm). The bigger size of the fifth instar larvae allowed us to perform topical application of 1 µl fenoxycarb solution in acetone to the dorsal integument of the larvae. A dose-response profile was obtained by applying fenoxycarb at three dilutions (0.1, 1 and 10 µg/µl) to day 0 larvae of the last instar. Importance of time of treatment was tested by application of 10 µg fenoxycarb on day 0 to day 8 last-instar larvae. Each larva received only one application. Larvae were then raised in plastic boxes (13 × 8 × 5 cm).

Two batches of larvae were used as control in each experiment; one received an acetone treatment, another was left untreated. More than 30 larvae were used for each experimental condition. After treatment, the larvae were checked daily to determine the time of molt and calculate the duration of each instar. Biological activity of fenoxycarb was determined according to the disturbance of normal morphogenesis. Insects were classified as a) permanent fifth instar larvae (which did not molt by day 30 after $^4/_5$ ecdysis), b) supernumerary larvae (which continued to feed and either molted to another supernumerary larval instar or to a moribund pupa), c) normal pupae and d) larval-pupal intermediates. These latter represent insects with both larval and pupal features; for example larvae with long antennae, partially sclerotized larvae, pupae in which some ventral parts of the integument were non-sclerotized... All these intermediates died without other transformation.

Results and discussion

Effects of fenoxycarb applied to early instars. Since control and acetone applications showed no difference, these results were pooled. Duration of third (second and third instar treated larvae) and fourth instar were not affected whatever the time of treatment and the dose of fenoxycarb applied (means of 3.3 and 4.5 days, respectively). The duration of the last instar (larvae producing pupae in less than 30 days after 4/5 ecdysis) increased with the fenoxycarb concentration whatever the instar of application. However, the fifth instar seemed to be longer when treatment occurred during instars 3 and 4. (17.7, 22 and 24 days with 5 µg fenoxycarb applied during instar 2, 3 and 4 respectively, as compared with 9.5 days for the control population). The duration of the pupal stage was very stable under all conditions (overall mean 11.1 days). Figure 1 shows the relative proportion of each stage. For a treatment at a given instar, proportions of the permanent fifth instar increased with the concentration of fenoxycarb. However, following treatment performed at instars 2–4, the proportion of permanent larvae decreased from 39 % to 15 % with the same 5-µg fenoxycarb treatment. Supernumerary sixth-instar larvae are only obtained with the highest concentrations 4–7 days after 4/5 ecdysis; their proportion increased from 9 % to 39 % with 5-µg treatments on instar 2–4. 30–40 % of sixth-instar larvae developed into the seventh instar. Exceptionally, 2 larvae molted into an eighth instar after a

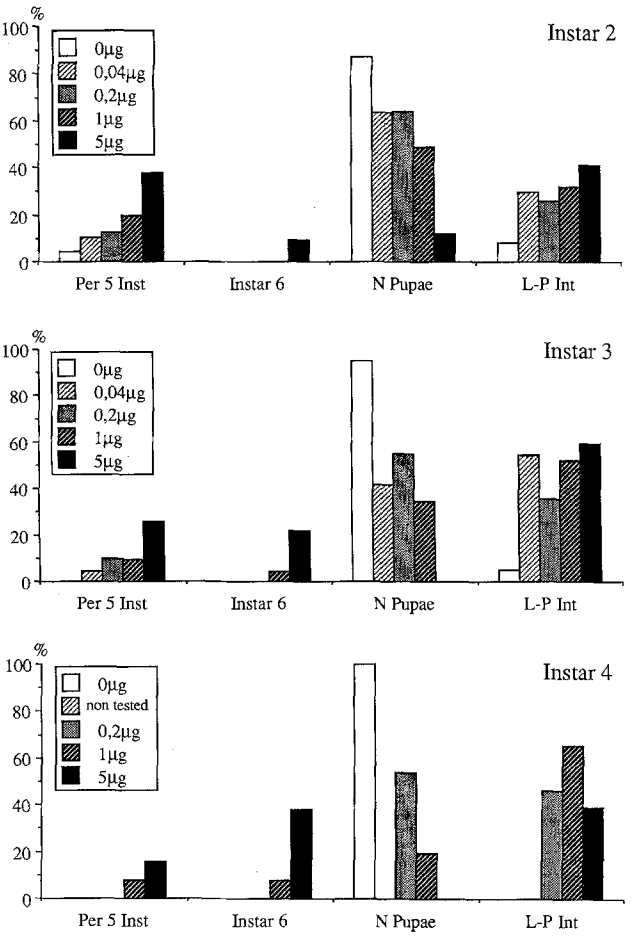

Figure 1. Mean percentages of permanent fifth larval instar (Per 5 Inst), supernumerary sixth instar (Instar 6), normal pupae (N Pupae) and larval-pupal intermediates (L-P Int) of *Ostrinia nubilalis*, after dipping into acetone solutions of fenoxycarb with 0 (control), 0.04, 0.2, 1 and 5 µg/µl. Applications were made at the beginning of instar 2, 3 or 4. No test was performed with fenoxycarb 0.04 on instar 4.

5-µg treatment during instar 4. Rates of occurrence of normal pupae decreased when the fenoxycarb concentration increased, and when the treatment took place later in the larval development. No normal pupae were obtained with 5 µg fenoxycarb during instars 3 and 4, and only 64 % pupae were obtained with 0.04 µg in instar 2. Whatever the instar of application, at least 25 % larval-pupal intermediates were obtained. Some intermediates were obtained from acetone treatment in instars 2 and 3. This could be accounted for by the small number of non-viable animals which occurs in any insect population.

Thus, fenoxycarb applied on early instars induced a strong perturbation of development.

Effects of fenoxycarb applied on last instar larvae. Three doses (0.1, 1 and 10 µg) were topically applied to day 0 last instar larvae (fig. 2). Mortality increased after 10 days post treatment in larvae treated with 1 or 10 µg. Although 50 % of the control population had pupated by day 10, more than 90 % of the treated larvae had not molted by that time. Fenoxycarb disturbed metamorphosis at all doses tested, showing the strong effect of the chemical. As expected, the higher the concentration, the stronger the effect was. Larvae treated with 10 and even 1 µg could not pupate properly and produced either supernumerary larvae or intermediates. Perfect supernumerary larvae could thus be obtained with fenoxycarb treatments. Those larvae fed, then pupated and finally died. As many as 30 % permanent larvae were produced with a single topical application of 10 µg fenoxycarb. The proportion of permanent larvae decreased after 10 days, when the rate of supernumerary larvae increased. The fenoxycarb delayed the molt, producing larvae which finally molted to supernumerary larvae instead of intermediates or pupae.

Topical application was performed on day 0 to day 8 of the last instar up to the pupal period (fig. 3). Toxicity was very high for day 2, day 3 and day 4 treatments, probably reflecting the changing of commitment to pupal molt. A 10 µg fenoxycarb application affects the pupal program throughout the entire last instar. Although perfect pupae

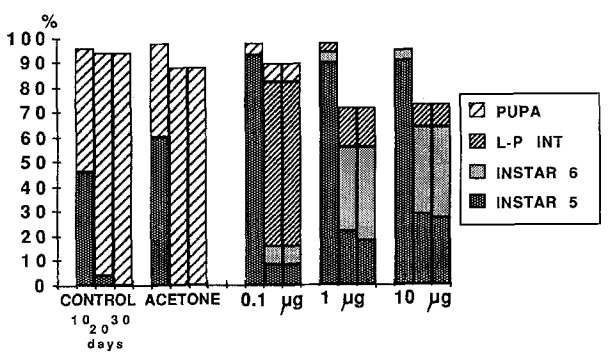

Figure 2. Effects of fenoxycarb treatment (0.1, 1 and 10 µg in topical applications) performed on day 0 of last instar larvae of *Ostrinia nubilalis*. For living borers only, the percentages of the different stages are shown at 10, 20 and 30 days post-treatment.

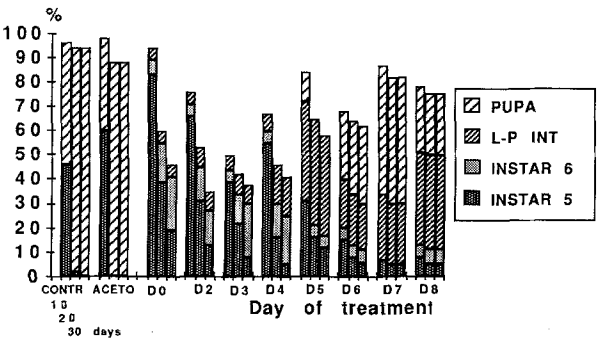

Figure 3. Effects of fenoxycarb treatment (10 µg in topical application) performed on day 0 to day 8 of the last instar larvae of *Ostrinia nubilalis*. For living borers only, the percentages of the different stages are shown at 10, 20 and 30 days post-treatment.

could be obtained when larvae were treated on day 8 (24 h after the gut purge), 40 % intermediates and a few superlarvae can still be obtained. Interestingly, more perfect pupae were produced when treatment occurred on day 7. Fenoxycarb applied on day 8 might have interacted with the ecdysone release at the end of the instar.

The sooner the treatment was performed in the instar, the higher was the percentage of permanent larvae obtained. The formation of permanent larvae might not be due to the persistence of fenoxycarb inside the borer since Mauchamp et al. [12] found that it was fully excreted at the time of gut purge in *Heliothis virescens*. If applied very early in the instar, it might have interacted with the PTTH-ecdysone axis before degradation. Among permanent larvae obtained by day 0 and day 2 treatments, most of them continued to feed by day 30. However a few of them purged their gut and survived a long time before they died or molted finally to a moribund larval-pupal intermediate. Dauer larvae have also been obtained by injection of a JH analog, methoprene, into day 3 fifth-instar larvae of *Bombyx mori* [2].

In the first half of the instar, perfect supernumerary larvae may be obtained, showing that pupal commitment might have occurred in the middle of the instar, as already shown by the high toxicity at this period. JH analogs have been applied to the European corn borer [13, 14]. They delayed the pupal molt and produced larval-pupal moribund intermediates. However, no supernumerary larvae were obtained in these early experiments using JH 1 or the JH analogs ZR-515 or ZR-512. Fenoxycarb treatments proved to affect normal morphogenesis of *Ostrinia nubilalis* to a great extent, even when performed during early development. This observation is contrary to ideas generally accepted for Lepidoptera [15]. Knowledge of physiological effects of fenoxycarb could help in an understanding of the endocrine control in the European corn borer of molting, metamorphosis and especially diapause, in which JH is supposed to play no role in induction, maintenance and termination [13, 14], but of which several aspects remain unclear. Moreover, these effects could be of great use for

a better understanding of interaction with parasitoids such as Tachinid flies, whose larval development is closely correlated with that of the host[16].

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Developmental changes of aldehyde dehydrogenase isozymes in human livers: Mitochondrial $ALDH_2$ isozyme is expressed in fetal livers

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Summary. Previous reports suggested that the major cytosolic aldehyde dehydrogenase ($ALDH_1$) was present in fetal and infant livers, but the major mitochondrial isozyme ($ALDH_2$) was absent or severely diminished. Re-examination by means of starch gel electrophoresis followed by enzyme activity staining, and by means of dot blot immuno-hybridization of liver samples with known genotypes of the $ALDH_2$ locus, indicated that both $ALDH_1$ and $ALDH_2$ genes are expressed in fetal and infant livers. In addition, $ALDH_4$ isozyme was also observed. The results imply that a fetus with the 'usual' homozygous $ALDH_2^1/ALDH_2^1$ genotype, but not one with the atypical $ALDH_2^1/ALDH_2^2$ or $ALDH_2^2/ALDH_2^2$, is capable of detoxifying acetaldehyde transferred from the mother.
Key words. Aldehyde dehydrogenase; developmental changes; gene expression.

Developmental changes in human alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase, EC 1.1.1.1, abbreviation ADH) and aldehyde dehydrogenase (aldehyde: NAD^+ oxidoreductase, EC 1.2.1.3, abbreviation ALDH) have previously been observed. At the embryonic and fetal stages, liver ADH activity is much lower than at the adult stage. Among the three non-allelic genes for the class I ADH isozymes, the ADH_1 gene (for the α subunit) is expressed during the early stages of embryonic development, and the expression of the ADH_2 gene (for the β subunit) follows. The ADH_3 gene (for the γ subunit) starts to be expressed in infants[1]. In parallel with the isozyme activities, the quantity and quality of the mRNA components for the individual subunits also change during development[2, 3].

Relatively little is known about the developmental changes of ALDH isozymes. It was reported that the activity of cytosolic isozyme ($ALDH_1$) was detected, while the activities of other ALDH isozymes, i.e. mitochondrial $ALDH_2$, $ALDH_3$ and $ALDH_4$, were undetectable in fetal and infant livers[4, 5]. Acetaldehyde is far more toxic than ethanol, and the mitochondrial $ALDH_2$, which has a low K_m value for acetaldehyde, is considered to play a major role in aldehyde detoxification. In order to understand the background of fetal alcoholic syndrome, developmental changes of alcohol metabolizing enzymes, particularly $ALDH_2$, need to be examined. We found that, contrary to the previous reports, both $ALDH_1$ and $ALDH_2$ activities are expressed even in early embryonic stages.

Materials and methods

Liver samples: Liver samples (all Japanese) were from fetuses obtained after stillbirths and therapeutic abortions, and from neonates and infants who died due to various complications. Six samples (25 weeks of gestation to 45 days after birth), which were used for the preliminary study, were from the Department of Pathology, Kitasato University Hospital, Japan. 37 samples (32 samples of 15–42 weeks of gestation, and 5 infants) were