Morphological and Physiological Changes in *Bovicola limbata* (Mallophaga: Trichodectidae) Treated Topically with a Juvenile Hormone Analogue[1]

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ABSTRACT

Mixed isomers of the synthetic compound juveth (ethyl 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate), an analogue of the juvenile hormone of insects, caused morphological and physiological changes in the Angora-goat biting louse, *Bovicola limbata* (Gervais), when they were applied topically. Some lice molted prematurely after being treated as 0- to 1-day-old 3rd instars with 0.01, 0.1, or 0.5% juveth, and some molted a 4th or 5th time. Also, nymphal characteristics were retained, so true 4th-, 5th-, and 6th-stage nymphs or nymphal-adult-intermediates resulted. In addition, sexually nonfunctional 5th-stage pseudoadults developed, and fecundity and survival of 4th-stage nymphs were depressed.

Wigglesworth (1936) was the first to demonstrate that atypical morphogenetic change can occur in insects because of an imbalance of the juvenile hormone. When he implanted the corpora allata (which he determined to be the source of the juvenile hormone) of 3rd- or 4th-stage nymphs of *Rhodnius prolixus* (Stål) in the abdomens of 5th-stage nymphs, he observed supernumerary molts. Williams (1956) extracted a substance from male cecropia moths, *Hyalophora* (as *Platysamia) cecropia* (L.), that reproduced the effects of the juvenile hormone secreted by the living corpus allatum. Roller et al. (1967) identified the juvenile hormone as methyl 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate. A synthetic juvenile hormone has shown insecticidal and ovicidal effects (Vinson and Williams 1967) on the body louse, *Pediculus humanus humanus* L. (as *P. h.* var. *corporis*) (Anoplura).

The present paper reports morphological and physiological changes in the Angora-goat biting louse, *Bovicola limbata* (Gervais), treated topically in what is normally the penultimate (3rd) stage with a juvenile hormone analogue consisting of mixed isomers of ethyl 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate, herein assigned the name of juveth.

MATERIALS AND METHODS

During the present test, the lice were maintained in 0.5-dr glass shell vials, and fresh diet was provided at least every 7 days. The rearing procedures, diet, temperature, and relative humidity were the same as those described by Hopkins and Chamberlain (1969). They also described the laboratory biology, in which normally there are 3 nymphal instars and a sexually mature 4th instar.

Lice, whose ages as 3rd instars were 0–1, 3–4, or 5–6 days (hereafter referred to as young, intermediate, and old 3rds, respectively), were placed on a glass microscope slide and treated topically with ¼ μliter of an acetone solution of juveth applied with a micrometer-actuated ¼-ml syringe. The ¼ μliter of solution saturated each louse, and some of the juveth remained on the glass slide after evaporation of the solvent. The young 3rds were tested twice with several concentrations of juveth, each test being made with a separate sample of juveth. The intermediate 3rds (10 ♂ and 10 ♀/treatment) and the old 3rds (5 ♂ and 5 ♀/treatment) were tested only once. Acetone-treated (¼ μliter/louse) lice and untreated lice served as controls. All lice were observed daily, and any deviations in the development of treated lice from that of the controls were recorded. Also, mortality was recorded daily, and the viability of eggs collected from inter se crosses of the treatment groups was determined.

RESULTS

Molting, 3rd- instar mortality, and morphogenetic effects observed in the 2 tests with young 3rd-stage nymphs were very similar so the records have been combined. However, the fecundity and survival of 4th instars are reported separately because the results sometimes differed, probably because of slight differences in the activity of the 2 samples of hormone.

Premature Molts and 3rd-Instar Mortality.—The numbers of young 3rds treated, the numbers that molted to the 4th and 5th instars, the mortality, and the average length of the 3rd stage are shown in Table 1. Duncan's multiple range test (the 5% level

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Table 1.—Effect of juveth on the length of the 3rd stage, survival of 3rd instars, and number that molted to 5th instars after topical treatments were applied to young 3rd instars of *B. limbata*.

% concn juveth	No. in test		No. molted to 4th instar		Avg no. days in 3rd stage		% mortality during 3rd stage		No. molted to 5th instar	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0.0001	5	5	5	5	6.0	6.0	0	0	0	0
.001	15	14	15	14	5.1	5.5	0	0	0	0
.01	16	13	16	10	5.1	5.5	0	23	1	0
.1	16	12	16	9	4.8	5.1	0	25	11	7
.5	15	15	12	6	4.9	5.2	20	60	7	1
None	16	14	16	14	5.9	5.9	0	0	0	0
Acetone only	30	35	29	35	5.7	5.8	4	0	0	0

of confidence) showed that the time to molting for both males and females at all concentrations of juveth down to and including 0.001% was significantly less than that of the controls. Appreciable mortality occurred among males treated with 0.5% of juveth and among females treated with 0.01% or above.

Generally, the molting patterns of the intermediate 3rds did not differ from those of the controls; males and females treated with the highest concentration exhibited the same molting pattern as the untreated controls. Also, there was no mortality among the males, but the females were affected; only 5 survived treatment with 0.5% and only 7 the treatment with 0.1%; survival of females given the other treatments was 90% or above.

Generally, the molting patterns among the old 3rds did not differ, and because 5- to 6-day-old 3rd instars were selected for testing, any molts before the 6th day were precluded. There was no mortality among these 3rd-stage lice.

Supernumerary Molts.—The old 3rds and the intermediate 3rds did not molt more than the normal 3 times. However, supernumerary molts occurred when young 3rds were treated (Table 1). A large number molted to a 5th instar, and at both 0.1 and 0.5% a male molted to a 6th instar.

Morphogenetic Change.—All lice treated with 0.01, 0.1, and 0.5% of juveth were morphologically atypical as 4th or supernumerary instars based on the degree of melanization of the abdominal tergites and sternites

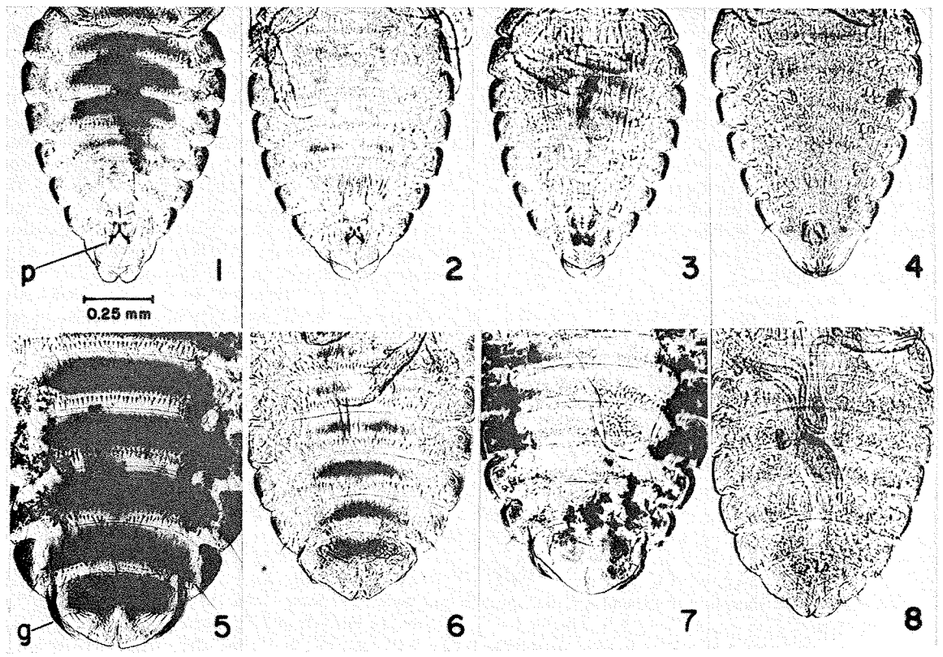

FIG. 1–8.—*B. limbata* phenotypes. Abdomens (venters) of 4th instars: 1, type I ♂; 2, type II ♂; 3, type III ♂; 4, type V ♂; 5, type I ♀; 6, type II ♀; 7, type III ♀; 8, type V ♀ (see descriptions in text) (p, paramere; g, gonapophysis).

Table 2.—Phenotypes observed among 4th and 5th instars of *B. limbata* treated with juveth as young 3rd instars.

% concn juveth	No. treated		No. of indicated phenotype[a]							
			I		II		III		V	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
4th instars										
0.001	10	10	0	1	9	6	0	1	0	0
.01	10	10	0	0	0	0	7	9	1	0
.1	10	10	0	0	0	0	0	0	10	7
.5	10	10	0	0	0	0	0	0	7	3
None	10	10	10	10						
Acetone only	25	30	24	30						
5th instars										
.1			1	1	0	3	0	1	1	0
.5			0	1	2	0	0	0	0	0

[a] Phenotype descriptions are in text.

and the presence or absence of observable (in situ) parameres in the males and the presence or absence of gonapophyses in the females. With these characteristics, several phenotypes were distinguished and numbered as follows: I—typical melanization, typical appendages; II—degree of melanization less than typical but observable, appendages typical; III—melanization absent, appendages typical or rudimentary; IV—melanization typical, appendages absent or rudimentary; V—melanization and appendages absent. Type I 4th instars were adult, but the type I supernumeraries were pseudoadults. Types II, III, and IV were classified as nymphal-adult-intermediates, and type V was classified as nymphal. Classification of specimens was delayed until 48 hr postmolt because melanization is not distinct in young specimens. Type I, II, III, and V are shown in Fig. 1–8. Type IV is not illustrated because only 1 type IV 5th-stage male was observed in these tests.

The phenotypes were described after the records of the 1st test were complete. In that test, the young 3rds treated with 0.1 or 0.5% juveth were type V (nymphal) in the 4th stage; also, those treated with 0.001 and 0.01% were either type II or III (nymphal-adult-intermediates), those treated with 0.0001% were type I, and all treated as intermediates or as old 3rds were type I.

The types obtained in the 2nd test are shown in Table 2; all those treated with 0.1 or 0.5% of juveth were type V in the 4th stage. Also, we measured the temple width, that is, the distance between the posterior portions of the genae. This characteristic is of considerable importance in separating species and instars among the Mallophaga. The measurements in Table 3 show that the temple width of the 5th-stage lice was considerably greater than that of the 4th-stage lice. The temple width of the 6th-stage male resulting from treatment with 0.1% in the 2nd test was 0.417 mm.

Fecundity.—All lice treated as young 3rds with 0.1 and 0.5% of juveth in both tests were nymphal in the 4th stage and therefore produced no eggs. However, viable eggs were collected from inter se crosses of 4th instars resulting from treatment with 0.01% in the 1st test and from the treatment with 0.001% in the 2nd test. None of the 5th instars was a sexually functional adult. When a type I 5th-instar male was placed with 4 normal females for 12 days, 26 eggs were collected, but none hatched; when a type II 5th-instar male was placed with 3 normal females for 17 days, 34 eggs were collected, but none hatched. The receptaculum seminis of 5 of these females was then examined for sperm, and none was found to be inseminated. Six 5th-instar females (2 of type I, 3 of type II, and 1 of type III) were also observed for 113 female-days; no eggs were deposited.

All inter se crosses of 4th instars from lice treated as intermediate or old 3rds produced viable eggs except those resulting from treatment of the intermediates with 0.5% of juveth; 5 of these females lived an average 10 days each and deposited no eggs.

Survival.—The survival of 4th instars in these tests was assessed as a percentage based on the life days attained (the sum of the numbers of days all lived) and the potential life days (length of record × starting numbers).

Table 3.—Temple widths of some 4th and 5th instars of *B. limbata* after topical treatments with juveth as young 3rd instars.

% concn juveth	Sex	No. lice	Width (mm) of temple	
			Avg	Range
4th instars				
0.1	♂	10	0.378	0.375–0.392
.1	♀	7	.501	.500– .508
.5	♂	7	.373	.367– .375
.5	♀	3	.477	.466– .483
None	♂	10	.367	.341– .375
None	♀	10	.497	.483– .508
5th instars				
.1	♂	3	.408	.391– .417
.1	♀	5	.566	.541– .583
.5	♂	3	.403	.392– .417
.5	♀	1	.541	

In both tests, all young 3rds treated with 0.1 or 0.5% of juveth were nymphal as 4th instars and either molted or died within 7 days; their survival was not calculated. In the 1st test, insufficient numbers were treated with 0.01 or 0.001% to obtain trends of survival; however, in the 2nd test, at least 10 ♂ and 9 ♀ from each treatment were observed for 20 days. The 0.001% treatment had no effect on survival but the 0.01% treatment reduced that of the males to 49% and that of the females to 27% compared with the 90 and 82%, respectively, for the control males and females.

With intermediate 3rds, the survival of the 4th-stage males over the first 23 days was not affected by any treatment (the percent was 94 for treatment with 0.5%). However, the survival of the 4th-stage females over the same period was reduced by some treatments: the percents were 78 for 0.0001%, 92 for 0.001%, 66 for 0.01%, 61 for 0.1%, 49 for 0.5%, 97 for the acetone controls, and 93 for the untreated controls.

With old 3rds, the survival of the 4th-stage males over the first 24 days was not reduced by any treatment (the percent survival was 100 for treatment with 0.5%). However, the females were affected: the percents were 83 for 0.0001%, 100 for 0.001%, 96 for 0.01%, 79 for 0.1%, 65 for 0.5%, 88 for the acetone controls, and 92 for the untreated controls.

Because limited numbers of the lice existed as types I, II, or III in the 5th stage, the average life of 5th instars was not established. However, the numbers of days various phenotypes lived were recorded. A type I male survived 1 day; 2 ♀ of this type survived 12 and 17 days. With type II's the survival was 1, 8, and 21 days for males and 19, 22, and 27 for females, and 1 type III female lived 16 days.

DISCUSSION

The experiments show that juveth caused marked effects on the physiology of *B. limbata*. Young 3rd instars were most susceptible. Although the treatments were topical, the possibility exists that there was some fumigant action. Also, some material was probably ingested because of the nature of the rearing procedure: immediately after the lice were treated, they were placed in vials containing sufficient food to maintain them for several days. This diet was relatively bulky (about the consistency of corn meal), and because the lice were active, they probably transferred some of the juveth to the surface of the food particles. Some effects of synthetic hormonal treatment of diet are reported elsewhere (Chamberlain and Hopkins 1970).

If hormone-mimicking agents are to be used in insect control, then the stage and physiological age at which the insect receives the hormone mimic are of utmost importance. If this critical time is missed, there may well be no effect on the succeeding generation; if the timing is correct, complete control may result.

The juveth caused readily observable effects on the metamorphic and the reproductive processes of *B. limbata,* but other physiological functions appeared to be normal.

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