Effects of the Juvenile Hormone Mimic Pyriproxyfen on Egg Development, Embryogenesis, Larval Development, and Metamorphosis in the Desert Locust *Schistocerca gregaria* (Orthoptera: Acrididae)

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ABSTRACT The juvenile hormone mimic pyriproxyfen disrupted embryogenesis when applied topically to eggs of the desert locust *Schistocerca gregaria* Forskål. Eggs treated on days 3–6 were inhibited at various stages of development, depending on dose and age. In particular, 0.001–0.01 μg blocked development of 3- and 4-d-old eggs at blastokinesis. Treatment of 7- to 11-d-old eggs was ineffective up to 10 μg. Insects that hatched successfully failed to display any postembryonic defects. Topical application of the mimic to females had a small ovicidal effect. The metamorphic molt was disrupted when the mimic was applied topically to 5th-instar *S. gregaria*. Insects retained characteristics of the 5th instar and in extreme cases supernumerary 5th instars were formed. Additional defects included essentially normal adults that were malformed and could not fly. Oral doses were considerably less effective. Application to 4th-instar nymphs did not produce supernumerary characteristics in postecdysial insects, though a large proportion of insects showed abnormalities when they reached adult, which in some cases prevented flight. Topical application of the mimic to 5th instar affected the length of the instar. The effects depended on dose and day of treatment

KEY WORDS *Schistocerca gregaria*, pyriproxyfen, locust, development

LOCUST SWARMS HAVE been effectively treated in the past with chemical insecticides (Brader 1988). However, currently available pesticides failed to give efficient control during the last plague (1987–1989) and there is widespread belief that alternative, more environmentally friendly forms of locust control should be developed (Lomer and Prior 1992). One alternative approach is the use of growth regulators like juvenile hormone mimics. Juvenile hormones are a small family of sesquiterpenoid hormones produced in the corpora allata of insects (Riddiford 1994). Juvenile hormone is first produced in the late embryo and appears to be important for normal dorsal closure, formation of the larval cuticle, and differentiation of the midgut. The corpora allata continue to produce juvenile hormone throughout larval life until the final instar. Intermolt juvenile hormone influences maintenance of larva-specific organs, behavior, and the production of the prothoracicotropic hormone. At the molt, juvenile hormone regulates metamorphosis. Juvenile hormone levels are low or undetectable early in the final instar. In the migratory locust *Locusta migratoria* L., small surges of the molting hormone ecdysone, which occur in the absence of juvenile hormone, initiate metamorphic changes in the epidermis. When the main surge of ecdysone occurs a new adult cuticle is produced (Riddiford 1995). Application of natural juvenile hormones or juvenile hormone mimics at appropriate times can result in disruption of normal development. Effects include interference with metamorphosis, induction of sterility in female insects and the disruption of embryogenesis (Retnakaran et al. 1985, Menn et al. 1989).

The object of this research was to investigate the effects of a comparatively recent juvenile hormone mimic, pyriproxyfen, with enhanced UV stability and persistence, on egg development, embryogenesis, larval development, and metamorphosis in the desert locust *Schistocerca gregaria* Forskål.

Materials and Methods

Schistocerca gregaria was reared in conditions similar to those described by Hunter-Jones (1966). Stock and experimental insects were fed wheat seedlings and bran with a 1% dried yeast supplement.

Treatment of Eggs. Eggs were treated with the Sumitomo juvenile hormone mimic Pyriproxyfen

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(technical grade, 99.5%) (Sumitomo, Chuo-ku, Osaka, Japan) under conditions similar to those described by Injeyan et al. (1979). Eggs obtained from pods laid 3–6 h previously were dispersed and washed in distilled water and placed in petri dishes lined with sterile muslin wetted with 3 ml of sterile 1% vol:vol antibiotic–antimycotic solution containing penicillin (10,000 u), streptomycin (10 mg), and amphotericin (25 μg). The eggs were incubated in the dark at 34°C. After the appropriate incubation period the eggs were prepared for treatment by placing them, with hydropyle uppermost, in a plastic mesh with holes of sufficient size to hold the eggs in an upright position. The mimic was then applied to the hydropyle in 1 μl of acetone using a Drummond microcap capillary tube. The eggs were then immediately transferred into a sterile petri dish lined with autoclaved muslin cloth wetted with antibiotic-antimycotic solution and placed back in the incubator. Eggs that did not hatch after 13 d of incubation were treated with 3% sodium hypochlorite solution for 1 min to clear the chorion and then rinsed in distilled water. Eggs were then preserved in Bouin solution. Subsequently, the eggs were examined under a dissecting microscope and embryo development scored using the system described by Bentley et al. (1979).

Treatment of Adult Females. *Isolated Individuals.* Female locusts were removed from the stock cages at a time estimated to be immediately before oviposition of the 1st egg pod (confirmation was achieved when the event occurred). Females were placed individually in cylindrical nylon gauze cages (17 cm diameter by 40 cm long) with 2 mature males. A tube with damp sand was positioned in the base of the cage for the female to deposit the egg pod. The date of deposition of the 1st pod was recorded and the pod was removed. The female was then dosed with the mimic after the 1st egg pod was recorded. Topical doses in ethanol were applied through 10-μl microcaps to the anterior sternum. Suspensions for injection were prepared by dissolving the mimic in 50 μl of ethanol followed by dispersion in 950 μl of locust ringer solution (Maddrell and Klunsuwan 1973). The ensuing egg pods were collected and incubated in situ in the metal tubes for 14 d at 34°C. Percentage of hatch was recorded, unhatched eggs were removed, and the percentage of development of embryos was recorded as described above.

Crowded Individuals. Females in caged populations of maturing adults were marked in felt tip pen with a number. Egg layers were identified by filming with a Hitachi CCTV Video Camera fitted with a zoom lense. The camera was fixed in such a way as to focus on the egg laying tube filled with moist sand. Recording was done over 24-h periods using a Panasonic video recorder. Having established the time that individual females laid their 1st egg pods, the insects were then treated with the juvenile hormone mimic 2 or 3 d before they laid their 2nd pod. Because often >1 female laid eggs at the same time it was difficult to match egg pods to females. Hence only females that laid eggs singly were used in the experiment.

Topical Application. Fourth- and 5th-instar nymphs were treated topically on the 1st abdominal sternite with pyriproxyfen in either acetone or rape seed oil (Sigma, St. Louis, MO) using a 10-μl microcap. Control insects were treated with solvent only. Unless otherwise stated insects were held in cylindrical nylon gauze cages (33.5 cm high and 10 cm diameter, 8 insects per cage) during the course of the experiment. Insects were examined after the final molt and scored on an arbitrary scale according to morphology, as follows: 0, normal adult; 1, slight yellow coloration of the thorax; 2, distal part of the wings bent; 3, wing rotation incomplete; 4, wing rotation half complete; 5, wing rotation half complete, some yellow/black pigmentation (larval coloration); 6, wing rotation half complete, pronotum shows some larval characteristics (black/yellow pigment, shape); 7, wing rotation half complete, larval pronotum; 8, wings failed to rotate, larval pronotum; 9, wings failed to rotate, larval pronotum, overall larval pigmentation; 10, supernumerary 5th or 5th that died in molt. 7 d after the molt insects were thrown into the air to ascertain whether they could fly.

Oral Application to Nymphs. Two cages (55.5 by 38 cm) were set up with 100 newly molted 4th-instar hoppers. A daily feed of treated wheat seedling was prepared for 1 cage of insects by dipping 3 g of fresh cut wheat in 100 ppm of pyriproxyfen dissolved in ethanol. The seedlings were air dried after treatment to remove the solvent. The other cage of insects was given wheat seedlings treated with ethanol. Insects were fed with the treated seedlings and untreated bran until the final ecdysis when they were examined for morphological abnormalities using the scale described above. Some of the insects were kept as adults and egg production and hatch rate were recorded.

Statistics. Probit analysis of dose response data was carried out using the probitan procedure in Genstat (Payne et al. 1993). Three models were fitted to the data; single line for all data, separate parallel lines for each treatment and separate lines with different slopes. An analysis of deviance was used to determine goodness-of-fit. Analysis of variance (ANOVA) and regression analysis (accumulated ANOVA), where appropriate, were carried out using Genstat (Payne et al. 1993).

Results

Treatment of Eggs. The juvenile hormone mimic inhibited embryonic development of the locust. The effectiveness depended on the dose and the timing of application (Table 1). The probitan procedure in the Genstat library was used to calculate the dose of pyriproxyfen at which 50% of the eggs failed to hatch ($=ED_{50}$). Data from day 4 were excluded

Table 1. Effect on embryonic development (mean ± SEM) of topical application of juvenile hormone mimic to the egg

Age of embryo, d	Dose of juvenile hormone mimic (μg/egg)	*n*	% hatch	% reaching 1st instar	% development[a] *unhatched embryos* (*n*)
3	0	32	87.5	87.5	
	0.001	36	30.5	2.7	64.8 ± 1.6 (22)
	0.01	25	8.0	8.0	46.1 ± 0.7 (18)
	0.1	34	0	0	45.1 ± 0.3 (19)
	1.0	34	5.8	5.8	46.4 ± 0.7 (28)
	10.0	35	2.8	2.8	46.3 ± 0.8 (28)
4	0	29	100	100	
	0.01	19	0	0	49.1 ± 1.1 (19)
	0.1	26	0	0	45.0 ± 0.2 (26)
	1.0	28	0	0	45.0 (28)
	10.0	29	0	0	45.0 ± 0.3 (29)
5	0	29	93.1	93.1	
	0.001	22	77.3	0	69.0 ± 4.1 (5)
	0.01	31	3.2	3.2	54.0 ± 0.5 (21)
	0.1	31	3.2	3.2	54.3 ± 0.5 (29)
	1.0	29	0	0	54.7 ± 0.8 (22)
	10.0	29	0	0	57.7 ± 1.1 (24)
6	0	27	92.6	88.9	
	0.001	40	67.5	40.0	73.9 ± 1.0 (14)
	0.01	22	9.1	4.5	61.0 ± 0.7 (15)
	0.1	9	0	0	61.7 ± 2.2 (9)
	1.0	23	0	0	64.7 ± 2.3 (16)
	10.0	10	0	0	53.3 ± 1.1 (9)
7	0	45	86.6	77.7	—
	0.1	44	79.5	75.0	—
	1.0	45	88.9	88.9	—
	10.0	46	80.4	73.9	—

n, Number of eggs.
[a] Development according to Bentley et al. (1978). —, No insects in this category.

from this analysis because there was no dose response. Three models were fitted to the data: a single line for all the data, separate parallel lines, and separate lines with different slopes. An analysis of deviance was carried out to determine which model fitted the data best (see Table 2). Although there was significant deviance from all 3 models, the ED_{50} for each age of embryo was calculated using the separate single line model because this model gave significantly improved fit over that achieved with the other 2 models ($\chi^2 = 28.83$, df = 5, $P < 0.001$).

Eggs treated on days 3–6 were inhibited by doses of juvenile hormone mimic as low as 0.00 1μg. Indeed, ED_{50} against 3-d-old embryos was just 0.04 ng (Table 2). However, treatment of 7- to 11-d-old eggs was ineffective up to 10 μg. An application of 0.01 μg at day 3 after oviposition stopped development at blastokinesis (45% level of development, which in control eggs occured on day 4) of 90% of the embryos. The lower dose of 0.001 μg resulted in 31% hatching, but only 2.7% reached 1st instar (the rest did not shed the vermiform cuticle) (Table 1). Examination of the unhatched embryos showed that they had successfully undergone blastokinesis but died during subsequent development. Application of 0.01 μg of the mimic at day 4 blocked development of 100% of the eggs, again at blastokinesis. Development of 5- and 6-d-old eggs was blocked at 54 and 61%, respectively, using 0.01 μg of juvenile hormone mimic. A dose of 0.001 μg resulted in 77% hatch of eggs treated on day 5 but vermiform larvae were unable to shed the embryonic cuticle. Forty percent of day 6 embryos dosed with 0.001 μg were blocked at a similar stage, but 40% were able to reach the 1st instar. Embryos treated after 7 d with a dose of up to 10 μg (data not shown) were not visibly affected by the juvenile hormone mimic. Thus, the period of sensitive to external juvenile hormone mimic application is just before and just after blastokinesis (corresponding to embryonic development of 40–60%). Insects that had successfully developed to the 1st instar did not show any postembryonic defects in response to juvenile hormone mimic. The hoppers developed to adult and reproduced normally. Only 2 insects out of > 200 hatching from eggs dosed with juvenile hormone mimic displayed green coloration characteristic of solitary locust adults.

Table 2. ED_{50} of pyriproxyfen in preventing egg hatch calculated using the separate lines model

Age of embryo at dosing, d	ED_{50}, ng	95% CL	Slope	SE	Deviance	df
3	0.04	0.12	0.4	0.11	7.87	3
5	2.3	1.56	1.69	0.33	7.76	3
6	2.1	1.1	1.91	0.46	0.01	3

Treatment of Adult Females. Tobe and Pratt (1975) established a link between juvenile hormone and ovarian maturation in *S. gregaria* with a peak of juvenile hormone synthesis at the onset of the pre-

Table 3. Effect of topically applied juvenile hormone mimic on egg production and development in females kept individually (mean ± SEM)

Time of application (days before 2nd pod)	Egg pod	No. insects	Eggs/pod	% hatch	% 1st instar
1	2	10	30.0 ± 7.5	80.7 ± 8.5	78.7 ± 9.8
	3	8	32.1 ± 3.7	96.8 ± 3.5	96.8 ± 3.5
	4	6	31.3 ± 1.6	95.9 ± 2.6	95.9 ± 2.6
2	2	3	28.5 ± 0.5	23.3 ± 0.4	23.3 ± 0.4
	3	1	17.0	100	100
	4	4	35.0	100	100
3	2	2	36.5	50.0	50.0
	3	1	19.0	100	100
	4	1	37.0	100	100
4	2	4	30.5 ± 3.0	90.8 ± 5.7	71.2 ± 0.2
	3	3	35.0 ± 3.1	100	68.5 ± 0.3
	4	2	24.0	100	100
5	2	2	38.0	100	100
	3	2	39.0	100	100
	4	2	33.5	100	100
6	2	2	27.5	92.6	65.8
	3	1	37.0	100	100
7	2	2	25.5	100	100
	3	1	27.0	100	100
Control	2	7	30.7 ± 6.6	94.6 ± 2.0	94.6 ± 2.0
	3	6	34.3 ± 2.8	100	100

Experimentals were topically applied with 10 μg of juvenile hormone mimic in 10 μl of acetone. Controls were treated with 10 μl of acetone 3–5 d before oviposition.

vitellogenic period. Previtellogenesis occurred on days 5–6 in the females of our stock colony and vitellogenesis occured on days 7–8. Application of the juvenile hormone mimic was timed with respect to the gonadotrophic cycle of individual insects by caging single females with 2 males, but within sight and odor (close proximity) to other caged females. Females housed in this way immediately after the adult molt laid their 1st egg pod 31 ± 2.2 d (n = 6) after ecdysis. In comparison, insects placed together in small cages after copulation laid the 1st egg pod 20.2 ± 0.8 d days after ecdysis (n = 16). It was clear from these provisional experiments that the timing of the 1st oviposition is difficult to predict in single females. Therefore, the mimic was applied at different times to females during the 2nd gonadotrophic cycle, using the 1st oviposition to time events. Although development of eggs laid by dosed females was affected to varying extents (Table 3), there was no discernible pattern. Some of the insects dosed on days 1, 2, 3, 4, or 6 before the 2nd pod was oviposited produced egg pods with lower hatch rates. There was no carryover into the 3rd and 4th cycles because eggs from the 3rd and 4th pods gave a similar percentage of hatch to the controls.

The major problem with the experiment described above was that insects kept in small numbers (1 female and 2 males) developed slowly and asynchronously. Therefore, in a separate experiment, video equipment was used to follow the effect of juvenile hormone mimic on females kept in high-density populations. Ten micrograms of mimic applied to females either 2 or 3 d before oviposition of the 2nd egg pod caused a reduction in subsequent egg hatch (Table 4). Development of embryos in eggs that did not hatch was arrested at 48% development (approximately at blastokinesis). However, there was little evidence for carryover to the 3rd gonadotrophic cycle.

Injection of juvenile hormone in Ringer solution into females caged individually with 2 males had a greater effect (Table 5) than either juvenile hormone mimic applied topically to individually housed females (Table 3) or to females retained in dense populations (Table 4). Only 27.6% of eggs

Table 4. Effect of topically applied juvenile hormone mimic on egg production and development in females kept crowded (mean ± SEM)

Time of application (days before 2nd pod)	Egg pod	n	Dose, μg	Eggs/pod	% hatch	% 1st instar	% development of failed embryos
2	2	3	10	27 ± 2.7	69 ± 0.2	69 ± 0.2	48 ± 4
	3	2		26	100	88 ± 3	90 ± 3
3	2	3	10	32 ± 4.4	61 ± 0.2	39 ± 0.3	48 ± 4.2
	3	2		30 ± 2	100	100	—
2	2	2	0	35 ± 1	100	100	—
	3	2	0	30 ± 1	100	100	—
3	2	2	0	24 ± 1	100	96	90
	3	2	0	22 ± 1	100	100	—

Juvenile hormone mimic was applied in 10μl of acetone. n, Number of females. —, No insects in this category.

Table 5. Effect of injected juvenile hormone on egg production and development in females kept individually (mean ± SEM)

Time of application (days before 2nd pod)	Egg pod	No. insects	Eggs/pod	% hatch	% 1st instar
1	2	3	23.3 ± 2.7	74.6 ± 13.0	74.6 ± 13.0
	3	2	31.0	83.8	83.8
2	2	2	27.5	27.6	9.2
	3	1	33.0	97.0	97.0
3	2	3	26.7 ± 7.2	55.7 ± 20.2	25.0 ± 28.9
	3	3	26.3 ± 5.4	94.0 ± 2.9	88.9 ± 7.4
	4	3	20.3 ± 3.1	98.5 ± 1.4	98.5 ± 1.4

Insects were injected with 10 μg of juvenile hormone mimic in 50 μl of Ringer solution.

hatched from 2nd egg pods laid 2 d after treatment with the mimic.

Topical Application of Pyriproxyfen to 5th Instars. The principal effect of the juvenile hormone mimic was that insects retained characteristics of the 5th instar when they ecdysed to adult (Table 6). In extreme cases, supernumerary 5th instars were formed. The effects ranged from slight chromotropicity (yellow pigmentation on a hind leg) to adultoids with complete larval pigmentation, larval pronotum, and failure of the wing buds to rotate and develop. The insects were scored on a scale of 0–10, where 0 denoted a normal adult and 10 denoted a supernumerary 5th or 5th instar that failed to ecdyse to adult. Failure to ecdyse was also recorded independently of the score. Additional defects included essentially normal adults that were malformed in some way as well as malformed adults that could not fly (Table 6).

The mimic caused retention of 5th-instar characteristics, failure to ecdyse, malformed adults, and adults unable to fly when applied to 1-, 3- and 5-d-old 5th instars. In general, the older the insects on application, the greater the effect. Three models were fitted to the data; a single line for all data, separate parallel lines, separate lines with different slopes. The best fit in each case was the last model. This was used to calculate the dose required to give a 50% maximum score (ED_{50}) for each characteristic (Table 7). An analysis of deviance was used to determine goodness-of-fit. In each case, apart from failure to ecdyse among insects dosed at 1 and 3 d, the chi-square was not significant, indicating an adequate fit of the model.

When ecdysis was incomplete, failure to shed the cuticle occurred at any point in ecdysial behavior. Death in these cases was probably caused by dehydration following splits in the weakened new cuticle and starvation of insects trapped in the remains of the old cutilcle. The malformations of the adults included retention of larval coloration, incomplete wing rotation, soft wings, soft cuticle, as well as twisted femur and tibia. Inability of the adults to fly was related to the deformation of the wings. There was a significant dose reponse for the effect of pyriproxyfen on flight capability when insects were dosed at 1, 3, and 5 d.

Pyriproxyfen also had an effect on the duration of the 5th instar (see Tables 6 and 8) Analysis of the results for each dose separately revealed a consistent pattern; dosed day 1 insects tended to have significantly longer stadia than day 3 or day 5 insects. Regression analysis of the results revealed also significant interactions between age at application and dose on stadium length ($F = 11.23$, df = 8, $P < 0.001$). In particular, increasing the

Table 6. Treatment of 5th-instar *S. gregaria* with pyriproxyfen in rape seed oil

Age, d	Dose, μg	*N*	Score[a] (mean ± SEM)	% failure to ecdyse	% malformed adults	% adults unable to fly	% normal adults	Duration of 5th instar, d[b]
1	0	25	0.1 ± 0.1	0	4.0	4.0	96.0	8.9 ± 0.3a
	0.01	17	1.6 ± 0.8	11.7	17.6	17.6	70.7	9.9 ± 0.2a
	0.1	17	1.2 ± 0.6	5.9	23.5	11.8	70.6	9.8 ± 0.4a
	1.0	19	7.9 ± 0.6	52.6	47.4	94.7	0	11.9 ± 0.3a
	10.0	30	8.7 ± 0.2	13.3	86.7	100.0	0	8.3 ± 0.3a
3	0	24	1.7 ± 0.8	16.7	0	16.7	83.3	8.7 ± 0.2a
	0.01	14	3.7 ± 1.3	35.7	14.2	35.7	50.1	8.8 ± 0.5b
	0.1	22	2.9 ± 0.9	22.7	18.2	31.8	59.1	8.6 ± 0.3b
	1.0	25	9.2 ± 0.4	70.8	29.2	96.0	0	10.2 ± 0.5b
	10.0	21	8.9 ± 0.3	38.1	61.9	100	0	11 ± 0.4b
5	0	20	2.1 ± 0.9	15.0	15.0	15.0	70	9.0 ± 0.4a
	0.01	21	3.6 ± 0.9	28.5	28.5	38.1	43.0	8.5 ± 0.4b
	0.1	21	6.2 ± 1.1	57.1	9.5	57.1	33.4	8.8 ± 0.3b
	1.0	14	7.3 ± 1.2	71.4	14.3	71.4	14.3	7.9 ± 0.3c
	10.0	17	9.2 ± 0.5	88.2	12.8	94.1	0	9.0 ± 0a

Values followed by different letters are significantly different ($P < 0.05$).

[a] Degree of retention of 5th-instar characteristics, score awarded using the scale outlined in the *Materials and Methods*.

[b] Similar doses between ages have been compared using ANOVA and the Fisher pairwise comparison.

Table 7. Treatment of 5th-instar *S. gregaria* with pyriproxyfen dissolved in rape seed oil: ED_{50} dose for various characteristics using the model separate lines with different slopes

Characteristic	Age in days at dosing	ED_{50}, μg	95% CL	Slope ± SEM	Residual deviance	Significance of χ^2	df
Retention of 5th-instar characteristics	1	0.40	1.0	8.02 ± 1.80	3.52	NS	2
	3	0.26	0.57	7.75 ± 2.28	4.1	NS	2
	5	0.15	0.38	4.55 ± 1.95	0.22	NS	2
% failure to ecdyse	1	1.79	2.23	1.29 ± 0.25	13.74	S	2
	3	7.7	32.1	0.31 ± 0.19	9.53	S	2
	5	0.18	0.3	0.65 ± 0.19	0.42	NS	2
% malformed adults	1	0.83	0.9	0.85 ± 0.21	1.77	NS	2
	3	5.55	10.6	0.49 ± 0.15	1.57	NS	2
% adults unable to fly	1	0.36	0.37	3.53 ± 1.77	2.18	NS	2
	3	0.27	0.23	2.87 ± 0.94	1.73	NS	2
	5	0.11	0.09	0.65 ± 0.20	0.48	NS	2

Significance of the residual deviance was determined by using the value in chi-square tables with 2 df. A level of probability of <0.05 was taken as significant. S, significant. NS, not significant.

dose of juvenile hormone mimic from 0.1 to 1.0 μg caused a lengthening of the stadium in day 1 dosed insects. A similar increase in stadium occurred with day 3 dosed insects but in this case 1 and 10 μg were effective. Application to day 5 insects had no effect.

Single oral doses (ingestion of 2-cm strip of wheat seedling dosed with pyriproxyfen in ethanol or oil) were less effective regardless of the formulation and never achieved scores >2.2 whatever the dose or age at treatment (C.V., unpublished data). Insects fed daily on wheat seedling dipped in 100 ppm juvenile hormone mimic from day 1 fourth instar until ecdysis to adult had a substantial effect on development, score 5.4 ± 0.4, percentage of malformed adults 95.8, percentage that failed ecdysis 4.1, percentage unable to fly 95.8.

Interestingly, some malformed adults/supernumerary nymphs with a score of 9/10 were able to reproduce though egg hatch was reduced (percentage of hatch 69.7 ± 10.7, $N = 17$),

Table 8. ANOVA of the length of the 5th stadium of insects dosed with pyriproxyfen at 1, 3, and 5 d after ecdysis to 5th instar

Dose of pyriproxyfen, μg	df	*F*	*P*	Significance
0	56	0.32	0.729	NS
0.01	50	6.18	0.004	S
0.01	50	4.42	0.017	S
1.0	56	15.66	<0.001	S
10.0	56	21.63	<0.001	S

Analysis performed for 4 doses and the controls. S, signficant bx;1 ($P < 0.05$).
NS, not significant ($P > 0.05$).

Topical Application of Pyriproxyfen to 4th Instars. Pyriproxyfen disrupted effectively the molting of 5th-instar locusts. However, it would be more effective in the field if earlier molts were also affected and so we looked also at the effect of the juvenile hormone mimic on 4th-instar insects. In contrast to the effects on 5th instars, application of the juvenile hormone mimic to 4th-instar nymphs did not produce supernumerary characteristics in the postecdysial insects. A small number of the insects treated at 1 d old failed to ecdyse successfully either at the 5th ecdysis or at the adult ecdysis (Table 8). A larger proportion of insects showed abnormalities when they achieved adult stage, which in some cases prevented flight. Application of pyriproxyfen to older 4th-instar insects had a greater effect on the 5th ecdysis, indeed 100% of 5-d-old 4th instars treated with 1.0 μg failed to

Table 9. Treatment of 4th-instar *S. gregaria* with pyriproxyfen dissolved in rape seed oil

Age, d	Dose, μg	*N*	% failure to ecdyse to 5th instar	% failure to ecdyse to adult	% malformed adults	% malformed adults unable to fly	% normal adults
1	0	20	0	10.0	0	0	90.0
	0.1	12	8.2	10.5	46	21.0	35.3
	1.0	9	11.1	12.5	62.5	37.5	13.9
	10	10	20.0	0	62.5	25.0	17.5
3	0	20	0	10	0	0	90
	0.1	17	94.1	0	5.9	0	0
	1.0	10	0	0	10	0	90
	10	20	95.0	5	0	0	0
5	0	9	11.1	0	0	0	89
	0.1	18	89	0	0	0	11
	1.0	8	100	0	0	0	0
	10	20	95	0	5	5	0

complete the 5th ecdysis. However, there was no dose response.

Discussion

Application of the juvenile hormone mimic between 3 and 6 days after oviposition had a marked effect on embryogenesis of *S. gregaria.* Application before and on the day of blastokinesis (day 4) blocked development at this stage, whereas application on days 5 and 6 blocked development at a slightly later stage. Older eggs (>6 d after oviposition) were considerably less sensitive to the mimic.

Novak (1969) reported that embryonic development in *S. gregaria* could be inhibited by juvenile hormone analogues at any stage of development, but the effectiveness decreased with increasing age of the eggs. Sbrenna-Micrarelli (1977) obtained also a range of abnormalities by incubating *Schistocerca* eggs on blotting paper impregnated with 100 μg/ml farnesyl methyl ether solution.

Consistent with the current work, Riddiford (1972) found that treatment of *Pyrrhocoris apterus* L. and *Hyalophora cecropia* L. eggs with a juvenile hormone mimic soon after oviposition resulted in a developmental block at blastokinesis. However, eggs of *Thermobia domestica* Packard (Rohdendorf and Sehnal 1973) became progressively less sensitive to juvenile hormone mimics after germ disc formation, whereas *Schistocerca* eggs retained significant susceptibility until day 6. Contrary to the results here, Injeyan et al. (1979) reported the effectiveness of a juvenile hormone mimic or juvenile hormone III on disrupting embryogenesis in *Schistocerca* from days 3 to 9 with the greater effect being from days 7 to 9. The reason for the difference between their findings and ours is not obvious.

Reduced appendage length of embryos with development arrested at postblastokinesis was noted, also disruption of embryonic development by the juvenile hormone mimic was accompanied by an increase in pigmentation of the embryo. Similar observations were made by Novak (1969) and Injeyan et al. (1979). The latter suggested that the pigmentation was a result of the morphological role of juvenile hormone on cuticle formation.

Previous studies on other insects have shown that application of juvenile hormone mimics to females can disrupt also egg development (e.g., pyriproxyfen versus the tsetse fly *Glossina morsitans* Westwood [Langley et al. 1988]). A strong chemosterility effect was not seen in this study. The enhanced effect of an injected dose (cf., topical application) may indicate that the formulation fails to deliver the mimic to the site of action. Certainly dosing both males and females in crowded populations by feeding throughout the 1st oviposition cycle on wheat seedling sprayed with 100 ppm mimic in rape seed oil did not appear to affect the reproductive ability of the insects (C.V., unpublished data).

These results show that this juvenile hormone mimic has significant ovicidal activity. However, this mode of action is of limited value for practical locust control. Egg deposition sites are hard to find and may extend unseen over many square kilometers. Attempts to control egg development would probably be more successful by treating the parents directly. Thus, a formulation that improved penetrability of the mimic may be of use in locust control. The strong larvicidal effects of this juvenile hormone mimic (reported elsewhere, see below) will probably prove more useful as part of an integrated pest management strategy for locusts.

Pyriproxyfen had a marked effect on the adult molt when applied during the first 5 d of the 5th instar, the extent of the response did not vary with age at dosing. De Kort and Koopmanschap (1991) found similar symptoms in *L. migratoria* treated topically or by injection with low doses of pyriproxyfen during the last instar. Juvenile hormone from *H. cecropia* L. injected in peanut oil at a dose of 5–20 μg/g into *L. migratoria* produced similar symptoms (Joly and Meyer 1970). However, injections late in the instar caused the production of green adults (solitarization). This was not observed in the current work, possibly because applications were made past day 5 (length of the instar in the control insects was ≈9 d). De Kort and Koopmanschap (1991) achieved green adults among *L. migratoria* treated with pyriproxyfen, but it is not clear at what stage in the 5th instar that application was made.

Roussel and Perron (1974) produced morphogenetic effects in *S. gregaria* by injecting juvenile hormone mimic into young 5th instars. Fagoonee (1979) saw symptoms of solitarization in *S. gregaria* treated on days 5–6 of the 5th instar and developmental abnormalities by using a high injected (300 μg/g) and topical (75 μg) doses of the juvenile hormone mimic methoprene. Injected doses of 5–50 μg/g insect juvenile hormone I, II and to a lesser extent III also caused developmental defects in *L. migratoria* (Roussel 1977). However, unlike the present work there was a peak of activity in the first 40 h of the 5th instar

There was some evidence that pyriproxyfen may prolong the 5th instar if applied in the first 3 d. Similar effects have been found previously with desert locusts using the juvenile hormone mimic hydroprene (El-Refai et al. 1974) and methoprene (Fagoonee 1979). The difference between the effects of pyriproxyfen on metamorphosis in the desert locust and other mimics is one of degree. Significant morphogenetic activity was seen with 0.1 μg topical dose. Impairment of flight activity occurred in adults treated as 5th instars with as little as 0.01 μg of pyriproxyfen. This is an important observation because reduced flight capability would reduce the impact of locust swarms. Pyriproxyfen has a similarly powerful activity in stimulating vitellogenin production in adult *L. migratoria* (Edwards et al. 1993).

Oral application was much less effective than topical application. A high daily dose throughout the 4th and 5th instars produced similar effects to a single topical dose applied early in the 5th instar. However, single oral doses of up to 10 μg had little effect.

In contrast to the effects on 5th instars, pyriproxyfen did not appear to affect development of 4th instar *Schistocerca*. This is to be expected because early instars are not usually directly affected by juvenile hormone mimics because of the high titres of endogenous juvenile hormone (Retnakaran et al. 1978). However, low doses of mimic applied to day 3 and day 5 insects prevented ecdysis. Others have noted detrimental effects of juvenile hormone mimics on early instars (Fagoonee 1979, Mauchamp et al. 1989). Application of methoprene to 2nd-instar *S. gregaria* caused a slight but significant extension of the following 3 instars (Fagoonee 1979). Fourth-instar *Heliothis virescens* F. responded to the juvenile hormone mimic fenoxycarb by becoming dauer larvae in the last larval stage (Mauchamp et al. 1989). Whatever the cause of ecdysial failure in the 4th-instar locusts, this effect, which occurs at low dose, would add considerably to the value of pyriproxyfen for locust control. It would be interesting to see whether ecdysial failure occurs in earlier instars in response to pyriproxyfen.

In conclusion, low doses of the juvenile hormone mimic pyriproxyfen have deterimental effects on the development of 4th- and 5th-instar *S. gregaria*. These effects range from the inability of treated insects to fly, to adultoids with reduced reproductive potential, to death during failed ecdysis. Therefore, this compound could take its place alongside selective insecticides, such as chitin synthesis inhibitors, fungal pathogens, and semiochemicals in an integrated control program against locusts.

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