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Effects of sublethal dose exposure to Dieldrin on *Rhodnius prolixus* (Hemiptera: Reduviidae) under laboratory conditions

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Summary

The effects of a sublethal dose of Dieldrin (7.2×10^{-5} g/day) were evaluated on oxygen consumption, survivorship, moulting and reproduction of *Rhodnius prolixus*. No difference was found in oxygen consumption between treated and control male insects. Survivorship proved to be very much affected by the sublethal exposure, but this was strongly dependent on the feeding stage of the individuals. Moulting times were prolonged by 2 to 3 days depending on the instar, but the number of individuals that succeeded in moulting was not affected. Total number of eggs laid by an average female was not affected, but the treated females laid fewer eggs in the first three weeks after feeding, and more eggs in the following weeks, than the untreated females. Possible physiological mechanisms that explain our results are related to the proteolytic activity of the midgut, to an interference with the hormonal system, to an insecticide hormoligasis effect, and to an interference with the ovarian follicles development. In terms of vector control these effects provide an alternative way of repopulating a sprayed house.

Key words: *Rhodnius prolixus*; Dieldrin; sublethal dose; Hemiptera; Reduviidae; Chagas' disease; vector biology; respirometry.

Introduction

The study of sublethal effects of insecticides is ecologically important because of the uncertainty involved in predicting these effects within and near sprayed areas (Moriarty, 1969). Of the few studies that deal with sublethal effects, none refers to *Rhodnius prolixus*.

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In Venezuela, where *Rhodnius prolixus* is the main vector of Chagas' disease, Dieldrin has been used since 1953 in the control of this species. The potency and persistence of this insecticide depends upon the type of surface sprayed and its evaporation rate (in Venezuela, 0.016 g/m²/day, thus 40 times more volatile than DDT but 13 times less than Lindane or BHC).

Incomplete spraying and/or prevalence of *Rhodnius prolixus* foci in house locations inaccessible to the insecticide suggest that this vector may be exposed to sublethal concentration of insecticide. In some insects sublethal doses of Dieldrin have been shown to affect longevity and reproduction (Chauthani and Adkinsson, 1966; Moriarty, 1968; Hodjat, 1971); this paper explores the possibility of a similar effect in *Rhodnius prolixus*. The effects on moulting and O₂ consumption were also evaluated. Since feeding condition has been shown to affect tolerance to insecticides in *Musca domestica* and *Aedes aegypti* (Gordon, 1961), in *Aglais urticae* (Moriarty, 1968), and in the triatomine *Triatoma infestans* (Hack, 1966), some experiments were designed to evaluate the effect of the feeding condition of *R. prolixus* on its tolerance to sublethal doses of Dieldrin.

Materials and methods

The *Rhodnius prolixus* population was obtained originally from the Ministry of the Health and Social Aid of Venezuela, and has been kept at the Center of Ecology for about 15 years; this population has never been exposed to insecticides.

The bugs were reared with the technique described by Gomez-Núñez (1964), and kept in a climatic room at 28° ± 1° C and 60% RH; the light regime was 13 h day and 11 h dark. The insects were fed on chickens for 1 h, once every week, unless an experimental treatment indicated differently.

The insecticide was obtained from the World Health Organization as Whatman No. 1 filter paper impregnated with Dieldrin in various concentrations. The sublethal doses were administered by placing filter paper with 0.4% Dieldrin in the bottom of 3.8-liter jars. These impregnated papers were inside a small plastic box covered by metal and nylon mesh, 5–6 cm apart from the insects, so that they were not able to touch the paper, but were exposed to the vapors. These jars were kept in a room, that had never been exposed to insecticide, and with the same climatic conditions as the rearing room.

Respirometry was conducted exclusively on male individuals that were selected after moulting into the adult stage and kept without food for 13 days. They were fed once, and only individuals that had a full meal were used. The distention of the pleural region of the abdomen, as suggested by Goodchild (1955), was used as evidence of feeding, and corroborated with an aftermeal body weight of about 160 mg. These insects were divided randomly into three groups: one received the insecticide treatment immediately after feeding, a second group one week after feeding, and a third group was kept as a control.

Oxygen consumption was measured in a Gilson differential respirometer at 28° C using 15 ml chambers without shaking. Measurements were made for 3 h, after a first initial hour to allow stabilization of the respirometer. The central compartment of each chamber had 0.2 ml of 20% KOH with a small piece of filter paper to increase the surface area for CO₂ absorption; the lateral branch had 0.2 ml of 5% H₂SO₄ for ammonia absorption. To avoid contact with KOH the insects were placed in the chamber wrapped in a small nylon mesh bag. For every measurement a new insect was used. The amount of oxygen consumed was calculated with the simplified formula proposed by Umbriet et al. (1964), and consumption was expressed in units of $\mu\text{l O}_2/\text{hour}/\text{individual}$, as suggested by Cirano and Zeledón (1964).

Table 1. Summary of the experimental design

Effect on	Application of sublethal Dieldrin*	No. of replicates	No. of individuals per replicate	Type of insect	Measurement or count	Time interval between measurement or count
Respirometry	immediately and one week after feeding	11**	1	adult males	$\mu\text{IO}_2/\text{h}$ weight (mg)	5-6 days
Moulting	immediately after feeding	3	100	nymphal instars 1. 2. 3. 4. 5	No. of moulted individuals No. of dead individuals	daily
Survival	8. 15 and 22 h after feeding	2	30	adult males	No. of dead individuals	daily
Oviposition and hatching	immediately after feeding	2	21 pairs	adult males and females	No. of dead individuals No. of eggs laid % eggs hatched	weekly

* For every treatment replicate there was always a control replicate.

** A new group was used for every measurement.

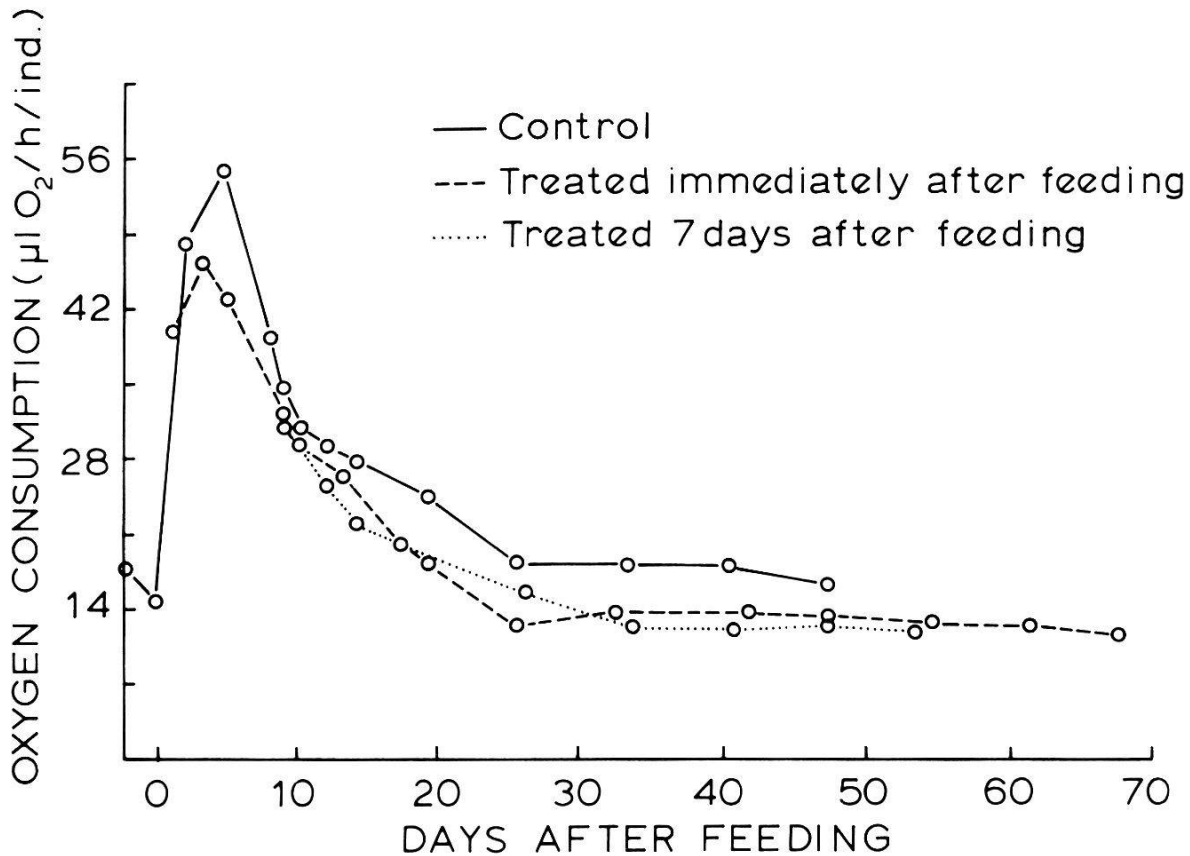


Fig. 1. Effect of a sublethal dose of Dieldrin on oxygen consumption of *R. prolixus* adult males.

The effect on the moulting process was evaluated by applying the sublethal doses of Dieldrin immediately after the blood meal. First instar nymphs were kept without food for 5 days before the blood meal was offered; all other instars were kept for 10 days before the blood meal. For every instar six groups were treated with insecticide, and the other three served as controls. Moulded and dead insects were counted daily.

Adult males were also used to evaluate the effect of insecticide on survival under different conditions. Four groups were used: control, and insects treated 8, 15, and 22 days after feeding. All tests were replicated twice. The jars were inspected daily and all dead individuals counted.

The effect of sublethal doses of Dieldrin on oviposition and fertility was evaluated with 21 pairs of adult males and females. Insecticide treatment was applied immediately after a verified full blood meal. Both treatment and controls were duplicated. The number of dead individuals and the number of eggs laid were checked weekly. Eggs were collected, labeled, and kept apart until hatching to check hatching success.

Table I summarizes the experimental design.

Results

Respirometry

Oxygen consumption and weight loss are shown in Figs. 1 and 2. There is a peak in oxygen consumption approximately 5 days after feeding, both in the control group and in the group treated immediately after the blood meal. Treated insects appear to consume less oxygen, loose weight less rapidly, and live a shorter time (Fig. 4) than control insects. However, the respiration rates and

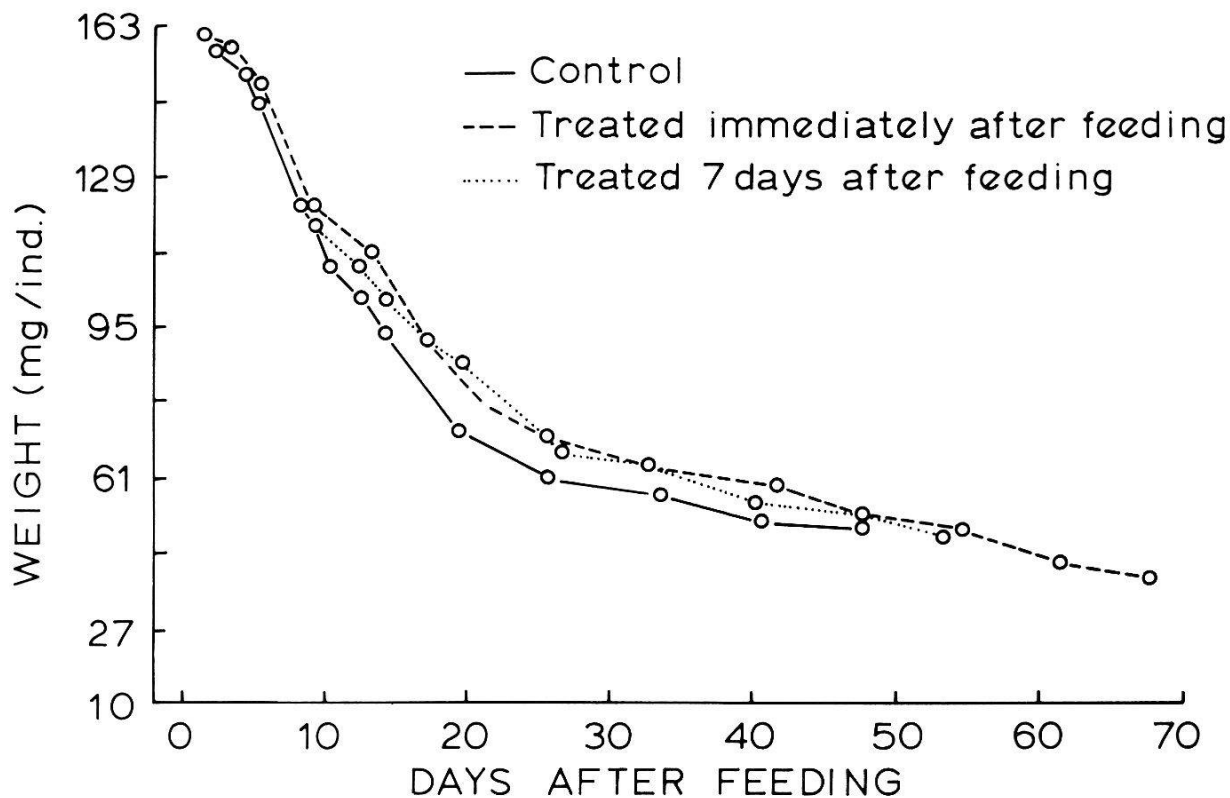


Fig. 2. Weight change of *R. prolixus* adult males treated with a sublethal dose of Dieldrin.

weights were not significantly different over time when checked with the Mann-Whitney U-test.

Moulting

Fig. 3 shows the frequency of moulting for control and treated groups for all stages; the values plotted are averages of three replicates of 100 individuals each. Moulting is delayed in treated insects. In both groups there is a marked tail to the right, usually more pronounced in the treated group. The Kolmogorov-Smirnov Dmax statistic was calculated, showing that all sets departed significantly from a normal distribution (Table 2). When compared by the Kruskal-Wallis test, the moulting time differences between the control and treated groups proved to be statistically significant at the 5% level.

The number of individuals used for calculating the mean moulting time of Table 2 also represents the number of bugs that successfully moulted out of the original 300 individuals. Although the treated group shows consistently a smaller number of successful moulting, when checked with the G-test the differences proved not to be statistically significant.

Survival

When the application of the sublethal dose started immediately or 8 days after the blood meal, survival was increased with respect to the control group (Fig. 4); on the other hand, if treatment was applied 15 or 22 days after feeding,

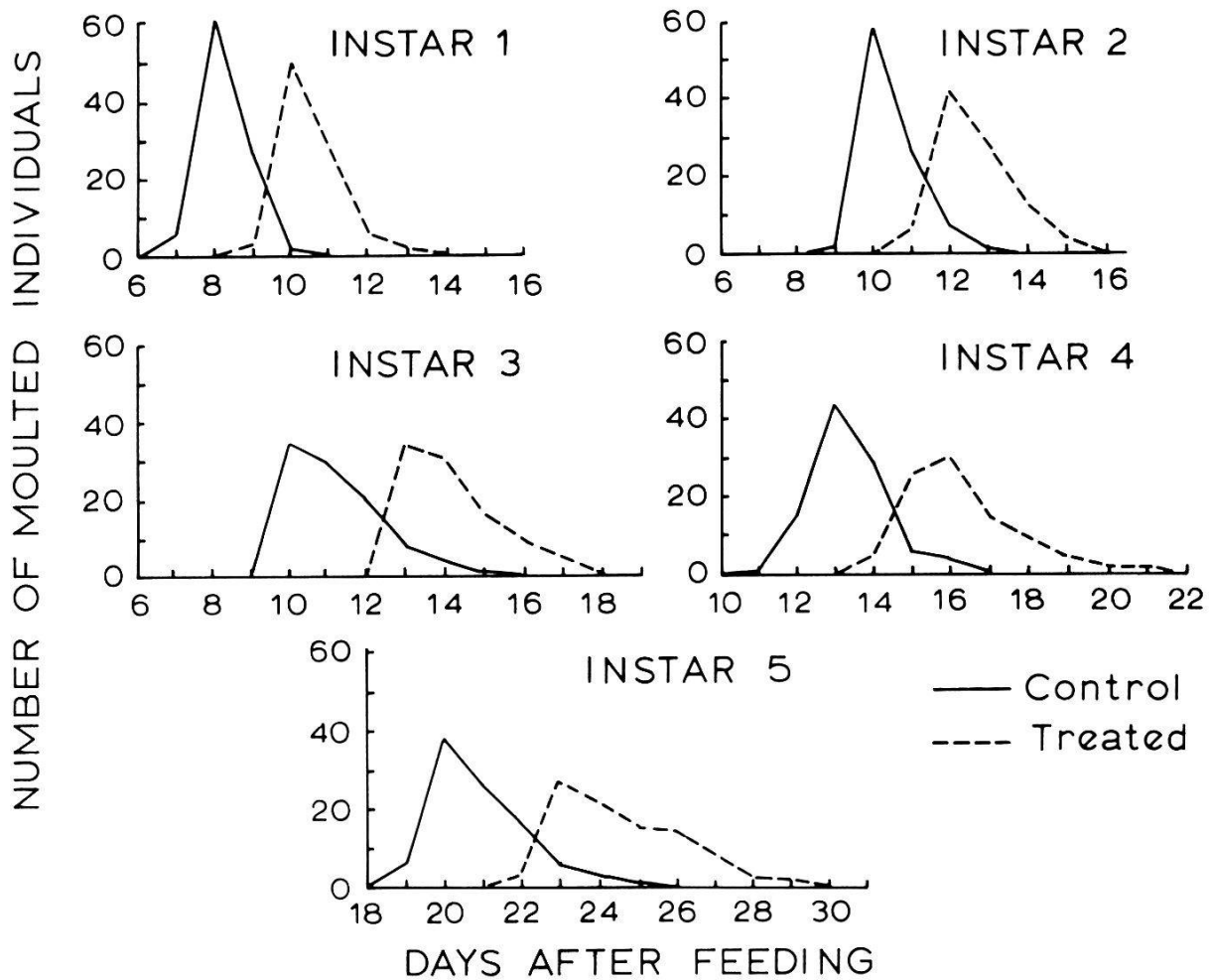


Fig. 3. Effect of a sublethal dose of Dieldrin on the moulting of *R. prolixus* nymphs.

Table 2. Moulting time in days after feeding, for the control group and the group treated with a sublethal dose of Dieldrin

Instar	Treated			Control		
	Mean	SD	N	Mean	SD	N
1	10.27	0.84	275	8.27	0.64	290
2	12.66	0.96	285	10.47	0.74	290
3	14.17	1.18	287	11.22	1.27	294
4	16.28	1.46	274	13.39	1.03	291
5	24.63	1.65	290	20.94	1.25	294

survival decreased. The interaction between feeding condition and the sublethal exposure is very strong; in Fig. 5 it is presented as changes in life expectancy at the day of feeding. Fig. 6 shows the survival curves of treated insects that were fed weekly, and their controls. Application of the Mann-Whitney U-test to the survival results of Fig. 4 showed that, with the exception of insects

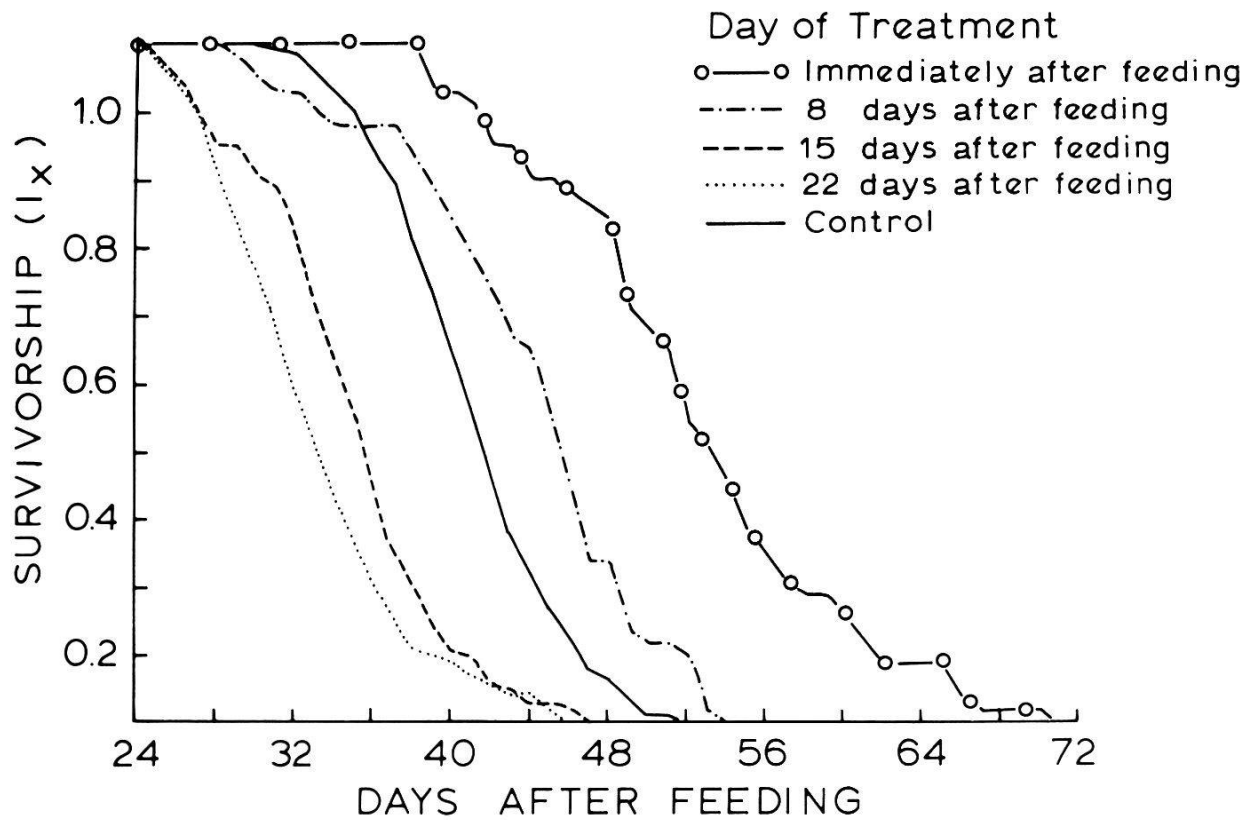


Fig. 4. Effect of a sublethal dose of Dieldrin on the survivorship of *R. prolixus* adult males under various feeding regimes.

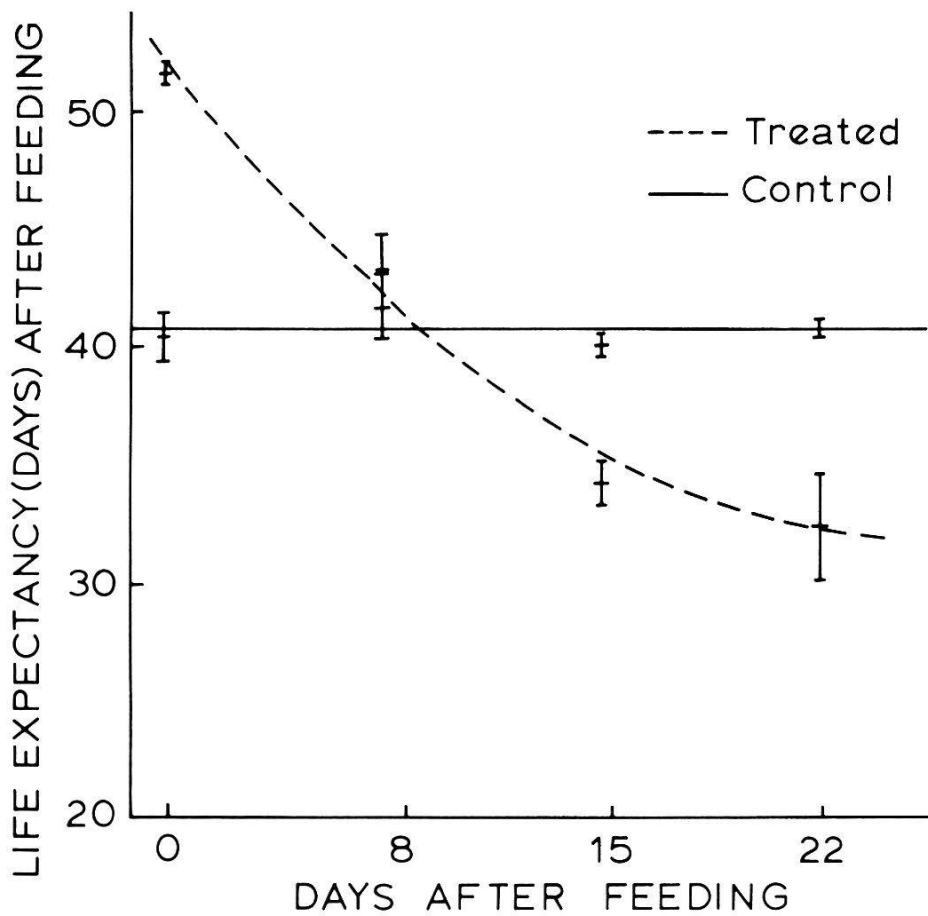


Fig. 5. Life expectancy in days of *R. prolixus* adult males treated with a sublethal dose of Dieldrin.

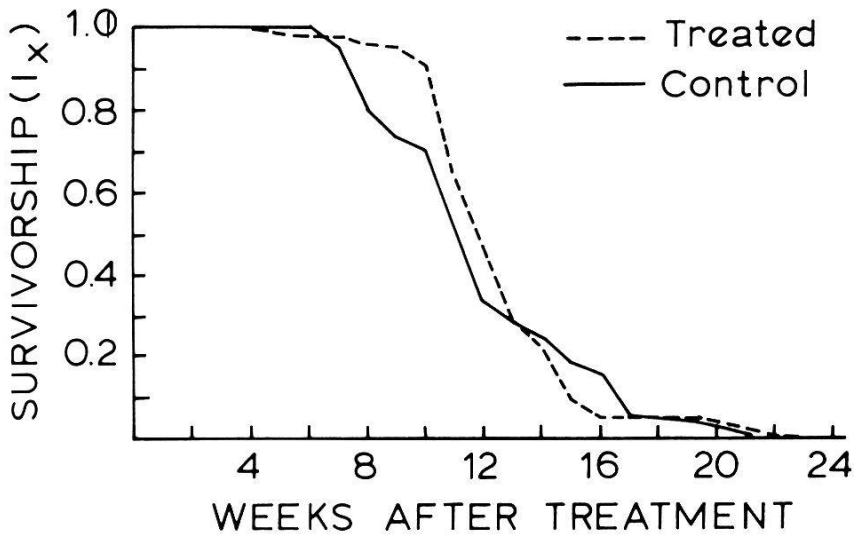


Fig. 6. Survivorship of *R. prolixus* adult males fed weekly and treated with a sublethal dose of Dieldrin.

treated 8 days after feeding and those treated immediately after the blood meal and then fed weekly, all differences from the controls were statistically significant.

Reproduction

Age specific fecundity (Fig. 7) shows there is a change in the fecundity schedule of the treated group, with a smaller number of eggs being laid at the peak of reproductive time, but a larger number in the weeks of declining laying activity. Using the Mann-Whitney U-test, the differences shown in Fig. 7 proved to be statistically significant at the 5% level, but the total number of eggs laid per female per lifetime were similar: 1002 and 1102 for the control group, and 934 and 943 for the treated group, for each replicate respectively.

As the survival of the females was also known, the net reproductive rate could be calculated using the formula:

$$R_0 = \sum_{x=\alpha}^w l_x m_x,$$

where x = age in weeks, l_x = proportion of females surviving to age x , m_x = number of female eggs laid per female aged x per week, α = age of first reproduction, and w = age of last reproduction. The mean and standard deviation of R_0 were 22.35 ± 0.15 and 25.05 ± 1.70 , for the treated and control groups respectively. Thus, the females exposed to sublethal doses of Dieldrin would be replaced, on the average, by 22 females in a period of one generation, compared with 25 for the control females. Assuming a normal distribution, this difference proved to be not significant by the Student t-test.

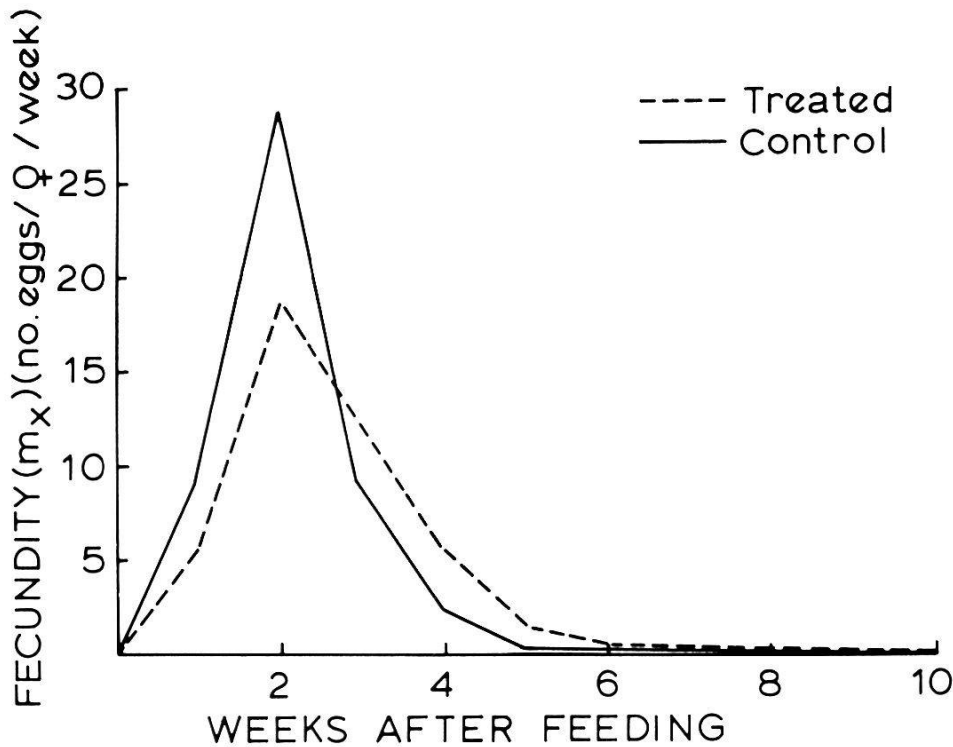


Fig. 7. Weekly fecundity of *R. prolixus* females fed once and treated with a sublethal dose of Dieldrin.

Hatching success was almost identical in the treated group (91.7%) and the control group (92.5%).

Discussion

The interpretation of these results should be considered in the light of the extremely low sublethal dose used in the experiments. A 45 cm² filter paper impregnated with 4% Dieldrin is equivalent to a spraying dose of 1.5 g/m² (González Valdivieso et al., 1971). Our concentration of 0.4% is equivalent to a spraying dose of 0.15 g/m². Considering Dieldrin's evaporation rate 16×10^{-3} g/m²/day (Busvine, 1970), and the surface area of the filter paper, we were exposing the insects to Dieldrin at a rate of approximately 7.2×10^{-5} g/day. The following discussion of the results takes this low exposure into account.

The oxygen consumption measured does not represent a basal metabolism because immobilization in the mesh bag cannot guarantee complete rest; Kleister and Buck (1964) call this condition standard metabolism, although Cirano and Zeledón (1964) claimed that this set-up may very well act as a stimulus because of the insect's effort to escape. Although there is no significant difference between treatment and control, our results indicate a tendency for lower metabolism in the treated group, while Spiller (1966) has shown that *R. prolixus* treated with DDT increases its metabolic rate. Whether this difference is due to the type of insecticide used or to the dosage level is an open question. The initial

metabolic rate increase observed 2 to 5 days after feeding was found both in treated and control groups and is probably related to a completely different mechanism, such as the high proteolytic activity of the mid-gut, as postulated by Okasha (1970).

However, if there is some mechanism for reducing the metabolic rate under sublethal insecticide exposures, this has great importance for vector control. This species has a large fasting capacity: on the average several months, and as an extreme record 7 months (Carcavallo, pers. comm.). Therefore, a lowering of metabolic rate may allow the insects to remain in protected spots for a longer period before seeking a new meal, possibly giving the toxin time to disappear.

This last point is also related to the delayed moulting effect found in the treated group. This was the most significant effect of the sublethal exposure to Dieldrin and is probably a consequence of insufficient oxygen for the increased protein synthesis associated with moulting, which demands a high level of oxygen (Zwicky and Wigglesworth, 1956). However, Okasha (1968) believes that a reduced oxygen level may also interfere with the endocrine moulting system either by inhibiting the secretion of the moulting hormone by the prothoracic gland directly, or indirectly by inhibiting the secretion of the cerebral hormone by the *corpus allatum*. Unfortunately, in our experimental design nymphs were not used for respirometry, so the idea that the differences in moulting times are due to reduced metabolic rates can only be considered a hypothesis. A longer moulting time also has implications for vector control, since the surviving population will have a lower growth rate.

The effect of sublethal exposures to Dieldrin on survivorship showed a very strong interaction with feeding condition. Two plausible mechanisms may explain it. First, we can suggest that the reduced metabolic rate of the insects after a long fasting period makes them inefficient in getting rid of the toxic materials, while recently fed insects and insects fed only 8 days before, having a much higher metabolic rate, can do it more efficiently. An alternative explanation is based on the insecticide hormoligasis effect as postulated by Luckey (1968). The high level of lipids and lipoproteins present in the haemolymph of recently fed insects may be used for storing the toxic molecules. As time since last feeding increases, the amount of lipids and lipoproteins in the haemolymph diminishes and the toxic effect is felt more strongly and more immediately.

The sublethal exposure to Dieldrin affected reproduction through the fecundity schedule and not through the total number of eggs. This may be due to changes in longevity, since females treated immediately after feeding had higher survivorship. The smaller number of eggs laid by the treated females during the first two weeks after feeding could be due to a reduction in metabolism, or to interference in the development of the ovarian follicles (Okasha, 1970).

Our overall conclusion is that sublethal exposure to Dieldrin may induce changes of kind and magnitude in domestic populations of *R. prolixus* that may very well explain the ability of this species to repopulate sprayed houses. In other words, passive and active immigration, as a domestic recolonization mechanism occurring in Venezuela (Gamboa Cuadrado, 1973a, b), may be only one of the ways of repopulating a house, with the effects of sublethal doses of insecticide presented here being another.

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