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# Laboratory evaluation of the acute and chronic toxicity of diflubenzuron against IV instar Mediterranean locust *Dociostaurus maroccanus* (Thunberg)

## ABSTRACT

The lethal effects of the benzoylphenyl urea diflubenzuron were assessed under laboratory conditions against one day old fourth (IV) *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae: Gomphocerinae) instars. Following exposure by ingestion of a single dose applied to short pieces of wheat seedlings, nymphs were monitored for two moults until the adult stage. The obtained acute response gave an $LD_{50}$ of 1.47 µg a.i./nymph. The male and female nymphs response to diflubenzuron did not show statistical significance but there was a narrow tolerance range within the female treated population. Analysis of the relative mortality in classes at individual dose rates showed that mortality occurring in the moult to V instar increased directly related to diflubenzuron dose rate. The duration of IV instar was significantly higher following treatments with all diflubenzuron doses, whereas the duration of the V instar did not show any clear differences between treated and control nymphs. The main sublethal effects on these adult females was a reduction of the number eggs per egg-pods, which could have major impacts on the evolution of *D. maroccanus* outbreaks and their management.

Key words: Moroccan locust, locust control, lethal effects, sublethal effects, reproduction.

## INTRODUCTION

*Dociostaurus maroccanus* poses a continual threat to agriculture and grazing in Spain and other Mediterranean countries (Latchininsky, 1998; Vàzquez-Lesmes and Santiago-Alvarez, 1993) and must be controlled to prevent the outbreak of high-density migratory gregarious populations that develop in well-defined breeding areas. *Dociostaurus maroccanus* outbreaks have been prevented by using conventional broad-spectrum insecticides such as organochlorines at first and organophosphates later (Latchininsky and Launois-Luong, 1992; Dominguez Garcia-Tejero, 1993) but there is widespread belief that alternative, more environmentally friendly forms of control should be developed (Lomer and Prior, 1992).

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One alternative approach is the use of Insect Growth Regulators (IGR) such as benzoylphenyl ureas, that interfere with the formation of insect cuticle by inhibiting chitin synthesis and deposition in the cuticle (GROSSCURT, 1978). Diflubenzuron is a highly persistent benzoylphenyl urea that has limited impact on non-target invertebrate populations and it is known not to affect vertebrates (WEILAND *et al.,* 2002). It may therefore be a candidate for replacement of organophosphates in *D. maroccanus* control. In fact, diflubenzuron has been used by the "barrier spraying" technique against gregarious hoppers bands of *D. maroccanus* in Morocco (BOUAICHI *et al.*, 1994) and by blanket or full-cover spraying in the breeding areas of *D. maroccanus* in Southern Spain (ARIAS-GIRALDA and JIMÉNEZ-VINUELAS, 1996). Nevertheless, the toxicity of this compound against the Mediterranean or Moroccan locust has not been studied in detailed laboratory tests which aimed to select the correct dose for field applications and to compare its toxicity with other benzoylphenyl ureas. Furthermore, the sublethal effects of diflubenzuron in *D. maroccanus* are still unknown.

The present investigation was carried out to study the toxicity of diflubenzuron against *D. maroccanus* IV instar nymphs and monitor lethal and sublethal effects.

## MATERIAL AND METHODS

The *D. maroccanus* nymphs used in this assay came from a stock colony maintained under controlled conditions L13: D11 photoperiod, 26º±4ºC T and 40-60% R.H. Wooden cages (50x50x50 cm) were used to maintain populations of nymphs, with a 60 W bulb inside that supplied extra heat during the light period and the locusts were fed with dry wheat bran and wheat (*Triticum* sp.) seedlings (SANTIAGO-ALVAREZ and QUESADA-MORAGA, 1999; QUESADA-MORAGA and SANTIAGO-ALVAREZ, 2001).

The insecticide evaluated was Dimilin 25W (WP-25), a wettable powder containing 250 g diflubenzuron per kg, supplied by Uniroyal Chemical B.V., The Netherlands.

Newly moulted IV instar nymphs 0-24 h old at the start of treatment were placed in individual plastic cups (40 mm; 20 mm depth) and were fed wheat leaf pieces (1.5-2 cm) treated by means of a microapplicator with 3 ml of product suspension. Consequently, a range of different formulation concentrations was prepared in distilled water 0.13, 0.66, 3.32 and 16.6 mg a.i./l., to give the required dose range, 0.4, 2, 10 and 50 mg a.i./nymph. Eighty nymphs, approximately 40 females and 40 males, were used for each

dose. As a control the same number of nymphs were given leaves treated with 3 µl of distilled water. Nymphs that had not eaten the treated leaf piece after 24 h were discarded, the others were transferred to wooden cages (30x30x30 cm) with a 60 W bulb at a maximum of 40 nymphs per cage. The bioassay was conducted under the same conditions as the stock colony.

Mortality was recorded every 24 h until imaginal moult. This was necessary because at lower dose rates mortality may occur after the first moult of the treated nymphs (GROSSCURT and JONGSMA, 1987). The Abbott formula was used to correct mortality. Dose-response data were analysed by probit analysis (LeOra Software, Wageningen), the lethal doses (LD), for both females and males, were calculated as µg a.i./nymph and 95% confidence limits were determined for the $LD_{50}$. A test was made for parallelism (in LeOra Software) according to the relative potency estimation method (FINNEY, 1971).

The mean number of days to moult, whether successful or fatal (with 95% confidence intervals), was calculated for each treatment and were compared by a LSD test ($p<0.05$). The mean longevity of adults that developed from surviving nymphs, was calculated for each treatment and the values were compared by analysis of variance followed by a LSD test ($p<0.05$). Reproduction of adults coming from treated and control nymphs was studied by the number of days until the first copulation, the preoviposition period, the number of egg-pods per female and the number of eggs per egg-pod. Egg-pods were collected by inserting in the wooden cages (30x30x30 cm) 800-ml cups filled with wet sand.

## RESULTS

The mortality of IV instar, males and females, *D. maroccanus* nymphs, due to diflubenzuron doses are summarised in Table 1. The mortality increase directly with dosage on both males and females nymphs.

Table 2 shows the probit regression lines and associated statistics determined. From this table we found that although the females were more susceptible than males, the respective $LD_{50}$ values, were not significantly different, on the basis of overlap of 95 % fiducial limits. Further a test for parallelism showed that data could be represented by three parallel regression lines ($\chi^2$ = 1.6867, with 2 d.f.) with a common slope, b = 1.49 ± 0.126. The relative potency and the 95% fiducial limits of the diflubenzuron treatment on females, with respect to the treatment on males was 1.21470 (0.6303-2.3568); from these values we confirm that differences in susceptibility to diflubenzuron, between males and females *D. maroccanus* nymphs, are not statistically significant.

The most conspicuous effects of diflubenzuron observed in the treated nymphs, as early as 3 days after treatment, were incomplete moult (insect unable to cast the exuviae), hernia on the pronotum, haemolymph exudation and reduction of feeding. Most of the nymphs died in moult to V instar (IM5), some during the IV or V instar and a few (Fig. 1), at the lower dose, in moult to adult (IMA). The mortality occurring in moult to V instar is directly related with the dose and it is significantly higher than the mortality on the other two cases (Table 3).

The duration of the IV instar was significantly prolonged on treated nymphs however the duration of the V instar did not show any clear difference between treated and control nymphs (Table 4).

Adult females coming from surviving diflubenzuron treated nymphs were morphologically normal, however the preoviposition period was prolonged, produce as many egg-pods as the control but abnormal in shape and with significantly fewer eggs number (Table 5). On the other hand, the mean longevity of adults, which had been either untreated or treated as nymphs, varying between 25 and 30 days, was not significantly different.

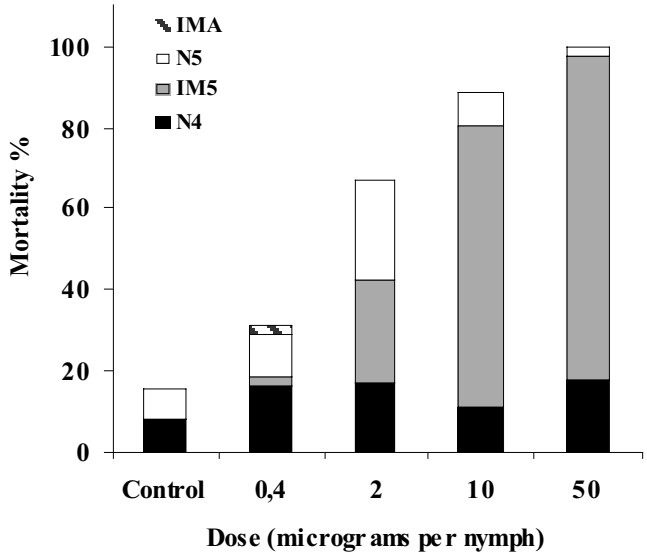


Fig. 1 - Relative mortality between classes by dose rate. N4 and N5 mortality occurring during IV and V instar respectively; IM5 and IMA mortality occurring in moult to V instar and to adult respectively.

Table 1 - Mortality of 0-24 h old IV instar nymphs of *D. maroccanus* treated with diflubenzuron.

| Dose (µg a.i./nymph) | Males | | | | Females | | | | Both sexes | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | n | % Mort. | % Abbott's Mort. | N | n | % Mort. | % Abbott's Mort. | N | n | % Mort. | % Abbott's Mort. |
| Control | 36 | 2 | 5.5 | 0.0 | 43 | 10 | 23.2 | 0.0 | 79 | 12 | 15.2 | 0.0 |
| 0.4 | 43 | 12 | 27.9 | 23.7 | 38 | 13 | 34.2 | 14.3 | 81 | 25 | 30.9 | 18.5 |
| 2.0 | 40 | 25 | 62.5 | 60.3 | 36 | 26 | 72.2 | 63.8 | 76 | 51 | 67.1 | 61.2 |
| 10.0 | 40 | 33 | 82.5 | 81.5 | 37 | 35 | 94.6 | 92.9 | 77 | 68 | 88.2 | 86.2 |
| 50.0 | 43 | 43 | 100.0 | 100.0 | 37 | 37 | 100.0 | 100.0 | 80 | 80 | 100.0 | 100.0 |

Table 2 - Probit Statistics for the Acute Dose-Responses of IV instar *D. maroccanus* nymphs to diflubenzuron

| | Regression line | s.e. of slope | $LD_{50}$ (± 95% f.l.) (µg per nymph) | $\chi^2$ (2 d.f.) significance[1] |
|---|---|---|---|---|
| Males | Y=1.32X + 4.79 | 0.2083 | 1.43 (0.77-2.30) | $1.9097^{ns}$ |
| Females | Y=1.83X + 4.74 | 0.3896 | 1.38 (0.60-2.34) | $0.2277^{ns}$ |
| Both sexes | Y=1.52X + 4.74 | 0.1956 | 1.47 (0.91-2.16) | $1.7899^{ns}$ |

[1] ns=not significant

Table 3 - Mortality occurring during IV and V instar respectively; IM5 and IMA mortality occurring in moult to V instar and to adult respectively.

| Dose (µg a.i./nymph) | N4 | | IM5 | | N5 | | IMA | |
|---|---|---|---|---|---|---|---|---|
| Control | 7.94 ±7.87 | A | | | 7.78 ±4.27 | A | | |
| 0.4 | 15.75 ±11.39 | A | 2.56 ±3.62 | A | 9.98 ±4.01 | A | 2.56 ±3.62 | A |
| 2.0 | 16.25 ±5.30 | A | 22.50 ±7.07 | A | 25.00 ±3.53 | A | | |
| 10.0 | 10.54 ±7.53 | B | 67.52 ±2.43 | A | 10.32 ±7.15 | B | | |
| 50.0 | 16.47 ±12.95 | B | 81.08 ±9.50 | A | 2.50 ±0.08 | BC | | |

Data followed by the same letter in each row are not significantly different in a test LSD (P>0.05).

## DISCUSSION

The insecticidal activity of diflubenzuron against *D. maroccanus* IV instar nymphs must be considered as high if we compare the $LD_{50}$ obtained, 1.47 µg/nymph, with that determined for the II instar nymphs of *Schistocerca gregaria* (Forskål), 68 µg/nymph (COOPEN and JEPSON, 1996a). Nevertheless, it is

Table 4 - Mean duration (days± s.d.) of IV and V instar after a diflubenzuron dose applied to the IV instar.

| Dose (µg a.i./nymph) | Mean duration of instar (days) | | | |
|---|---|---|---|---|
| | n | IV instar | n | V instar |
| Control | 74 | 5.36 ± 0.65[a] | 72 | 8.25 ± 1.52[a] |
| 0.4 | 60 | 6.33 ± 0.93[b] | 44 | 7.72 ± 1.24[b] |
| 2.0 | 50 | 6.78 ± 1.41[c] | 19 | 8.57 ± 1.38[a] |
| 10.0 | 64 | 6.78 ± 1.42[c] | - | - |
| 50.0 | 44 | 6.09 ± 1.05[b] | - | - |

Data followed by the same letter in each column do not show any statistical differences in a test LSD ($p<0.05$).

Table 5 - Reproduction of *D. maroccanus* adults coming from treated IV instar nymphs[1].

| Dose (µg a.i./nymph) | n | Days to 1st copulation | Preoviposition period | Egg-pods/ female | Eggs/ egg-pod[2] |
|---|---|---|---|---|---|
| Control | 13 | 5.0 | 11 | 1.15 | 16.7 ± 8.2[a] |
| 0.4 | 20 | 5.5 | 15 | 1.05 | 16.6 ± 8.3[a] |
| 2.0 | 10 | 6.0 | 16 | 1.00 | 10.1 ± 11.3[b] |

[1]n= Number of pairs per cage, due to the small number of nymphs reaching the adult state there was only 1 cage per dose. [2]Data are mean ± s.d. Data followed by the same letter in each column do not show

necessary to consider the weight differences between both species although the used instars have similar length. Our results indicate that diflubenzuron is toxic to IV instar of *D. maroccanus* nymphs exposing to treated foliage for only 1 day. Changes in the duration of exposition are liable to affect toxicity as has been shown in *Melanoplus sanguinipes* (ELLIOT and IYER, 1982) and *Schistocerca gregaria* (Forskål) (COOPEN and JEPSON, 1996b). It is therefore necessary further research to evaluate the effect of duration of exposure on the toxicity of diflubenzuron to Mediterranean locust nymphs.

Moreover, in the IV instar nymphs of *D. maroccanus* the timing of the exposure during the inter-moult period does not play the same role on the insecticidal action of diflubenzuron than in the II instar nymphs of *S. gregaria* (COOPEN and JEPSON, 1996b). Thus, a significant proportion of the mortality occurred during the first moult after the treatment on the nymphs of *D. maroccanus* received the dose on Day 1 of the inter-moult period, while on treated nymphs of *S. gregaria* the significant proportion of mortality occurred after the first moult following the treatment when nymphs were dosed on the Day 1. The pharmacodynamics of diflubenzuron into the body of each other species and instar nymphs could be the responsible of this difference.

The duration of IV instar nymphs diflubenzuron treated is prolonged as normally occurs in other locusts (WEILAND *et al.,* 2002) but does not accumulate in the insect (COOPEN and JEPSON, 1996b), so the duration of the V instar nymphs is not disturbed. This effect will cause longer periods of exposure to lethal doses of the chemical and increasing the susceptibility of weakened nymphs to natural enemies, predators, parasites, etc., of *D. maroccanus* (HERNÀNDEZ-CRESPO, 1993; HERNÀNDEZ-CRESPO and SANTIAGO-ALVAREZ, 1997).

It is known the effect of diflubenzuron on various aspects of the locust reproduction when applied directly to adult stage (MARIY *et al.*, 1981; WEILAND *et al.* 2002). The reduction in fecundity showed by *D. maroccanus* females coming from treated nymphs is related with the production of abnormal egg-pods. In this case diflubenzuron can affect the thickening of the ovipositor (KER, 1977; SARASUA and SANTIAGO-ALVAREZ, 1983).

Our results indicate that diflubenzuron is a promising insecticide for *D. maroccanus* preventive control by each other application techniques: "barrier spraying" or full-cover spraying because the acute lethal effects and the debilitation of survivors could ultimately reduce their reproductive capacity and be as significant for effective control as direct death itself.

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## RIASSUNTO

VALUTAZIONI DI LABORATORIO SULLA TOSSICITÀ ACUTA E CRONICA DEL DIFLUBENZURON CONTRO NINFE DELLA CAVALLETTA MEDITERRANEA *DOCIOSTAURUS MAROCCANUS* (THUNBERG)

Sono stati valutati mediante saggi di laboratorio gli effetti letali di benzoylphenyl urea diflubenzuron contro ninfe di *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae: Gomphocerinae). Dosi singole sono state applicate su pezzetti di cariossidi di grano e, dopo l'ingestione, la vita delle ninfe è stata monitorata fino al raggiungimento dello stadio adulto. I dati ottenuti hanno evidenziato una $DL_{50}$ di 1.47 µg a.i./ninfa. La risposta delle ninfe maschio e femmina al diflubenzuron non ha evidenziato alcun significato statistico anche se la popolazione delle femmine trattate ha mostrato una certa tolleranza. L'analisi della mortalità relativa ad ogni singola dose utilizzata ha evidenziato che la mortalità ottenuta nel passaggio al secondo stadio di ninfa aumentava in maniera direttamente proporzionale alla dose utilizzata. La durata del I stadio ninfale è stata significativamente maggiore dopo il trattamento con diflubenzuron a tutte le dosi, mentre la durate del II stadio ninfale non ha mostrato alcuna differenza tra ninfe trattate e non trattate. Il maggiore effetto subletale sulle femmine adulte è stato la riduzione del numero di uova che ha influenzato il successivo sviluppo della popolazione di *D. maroccanus*.


Parole chiave: Dociostauro, controllo, effetti letali, effetti subletali, riproduzione.

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