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RH-5849, a nonsteroidal ecdysone agonist, does not mimic makisterone-A in *Dysdercus koenigii*

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Abstract. The response of final instar nymphs of *Dysdercus koenigii* to topical application of the non-steroidal ecdysone agonist, RH-5849, was dose dependent. The candidate compound produced mortality even at moderate doses, but precocious adult development was not observed. Similar results were obtained after oral administration or injection. Conversely, injections of makisterone-A (the principal moulting hormone of *Dysdercus*) into 5th instar nymphs resulted in precocious adult development within 4 days. We conclude that RH-5849 does not mimic makisterone-A, as is the case with ecdysone, and that toxicity is mediated instead through non-endocrine targets in this insect species.
Key words. Dysdercus keonigii; ecdysone mimic RH-5849; makisterone-A; juvenilizing effect; toxicity.

RH-5849 (1,2-dibenzoyl-1-tert-butylhydrazine) is a non-steroidal ecdysone agonist[1-3]. This compound deters feeding by larval lepidopterans and induces premature lethal moulting which has been attributed to a direct action on target tissues[3].

Various studies with this compound have shown activity in larvae of many lepidopteran species[1,3-8], some dipteran[1,2,9], a few coleopteran[1,10], and dictyopteran[9] species. Effects on reproduction in some lepidopteran, coleopteran and dipteran species[1,11] have also been reported and such effects are consistent with the putative ecdysonergic mode-of-action of RH-5849[3].

RH-5849 has also been shown to cause neurotoxic symptoms[8,12] in insects by blocking potassium channels, which points to the dual action of this compound. This can help delay or prevent development, especially if several types of K^+ channels, encoded by different genes, are affected[12].

In the study described here RH-5849 was administered to nymphs of the red cotton bug, *Dysdercus koenigii* F. (Heteroptera: Pyrrhocoridae). The aim of the study was to evaluate the toxicity of RH-5849 against this hemipteran and also to determine if this compound mimics makisterone-A, the principal moulting hormone in these insects[13-15].

Materials and methods

The insects were taken from a laboratory culture maintained at 27 ± 1 °C and 16:8 LD photophase[16]. Most experiments were conducted with 5th instar nymphs. However, in a few cases 4th instar nymphs were also treated.

RH-5849 (Rohm & Haas Co., USA) was a gift from Dr. M. B. Isman, University of British Columbia, Canada and makisterone-A was kindly provided by Dr. A. K. Raina, USDA, Beltsville, USA.

Bioassays were initially performed with 0–20 h-old 5th instar nymphs, which were topically treated with 1.25–5.00 µg/nymph of RH-5849 dissolved in 1 µl of acetone. In each treatment 20 nymphs/dose were used. Controls received 1 µl of carrier alone. In another set of experiments, 4th and 5th instar nymphs were treated topically from day 0 to day 4 of their development. The doses applied were similar to the ones mentioned above, with $N = 20$ in each treatment. Incidences of moulting and mortality were recorded for all groups.

Potential behavioural effects of RH-5849 were investigated by giving the chemical orally to 5th instar nymphs in their drinking water at various concentrations of 1–50 ppm. RH-5849 is soluble in water up to 5×10^{-2} g/l ($= 65$ ppm). The fate of 5th instar nymphs on various days (day 0 through day 6) after chronic exposure for 24, 48, 72, 96 and 120 h respectively, using 5 different cohorts of insects ($N = 20$ in each case) was also recorded. After each interval, treatment was removed and insects were allowed to feed and grow normally.

RH-5849 was also dissolved in 10% ethanol and injected into 0-day-to 4-day-old nymphs at doses of 1, 2, and 5 µg/insect in 1 µl of the solvent after making further dilutions in double distilled water. In this experiment 5 cohorts of 10 insects each were used. Controls were injected with 1 µl of carrier alone. Makisterone-A (the principal moulting hormone of *Dysdercus*) was injected into 5th instar nymphs ($N = 10$) in a similar manner on various days at a dose of 5 µg/insect in 1 µl of 10% ethanol. Controls received 1 µl of carrier alone.

Results and discussion

RH-5849 is the first non-steroidal ecdysone agonist which induces premature cuticle synthesis[2,4,11]. It also competes with ponasterone A for high affinity ecdysone

Table 1. Effect of RH-5849 on 4th and 5th instar *D. koenigii* nymphs after topical application

Treatment µg/nymph	Mortality (%) after: 24 h		48 h		Moulting (%)	
	4th	5th	4th	5th	4th	5th
0.0	0.0	0.0	0.0	0.0	100	100
1.25	10.0	0.0	15.0	0.0	85	100
2.5	30.0	25.0	40.0	35.0	60	65
5.0	50.0	40.0	80.0	65.0	20	25

receptor sites[3], particularly in lepidopterans. In the absence of any detailed report regarding the effects of RH-5849 against hemipterans we studied the effects on *D. koenigii*, since moulting is controlled by makisterone-A and not the ecdysones, for which RH-5849 is a very effective agonist.

As *D. koenigii* is a very sensitive test insect for juvenoids, RH-5849 was topically applied to 5th instar nymphs to see if any juvenilizing effect is induced as is seen in the lepidopteran *Galleria mellonella*[17]. The response of final instar larvae to RH-5849 was dependent on the age of the nymphs, the dose applied, and the mode of application. Nymphs 0–20 h old exhibited 25–40% mortality at 1.25 to 5.0 µg/nymph dose within 24 h and 35–65% after 48 h (table 1). 25–100% of adult formation was observed in this treatment (table 1) but no supernumerary moulting occurred during the course of development. In our earlier studies no direct interaction of RH-5849 with JH-I or JH-II was observed in lepidopterans[8]. Therefore, the production of supernumerary instars in *G. mellonella* appears to be exceptional. In addition, the suggestion that young final instar larvae of this species produce supernumerary instars due to stress at moderately high doses of RH-5849 does not conform to the present data, as the mortality increases considerably with the increase in dose to *D. koenigii*. Similar results were observed when treatment was given to 4th instar larvae by topical application (table 1). After injection of RH-5849 into 0–20 h-old 5th instar larvae at 1 to 5 µg/nymph, a similar effect was seen. At 2 and 5 µg, 50 to 100% mortality was recorded respectively, with no indication of any precocious moulting or a juvenilizing effect (fig. 1). Nymphs that survived moulted normally into adults. Continuous feeding of the candidate compound to nymphs at 1 to 50 ppm in their drinking water also produced high mortality. Above 20 ppm, all insects died within 48 hours (fig. 2). At 10 and 20 ppm, insects were dead by the 5th and 2nd day respectively. At 5 ppm, 80% mortality was recorded after 7 days. Surviving larvae moulted normally into adults after 9 days, representing a delay of 3 days. In this experiment insects were never observed to feed continuously and remained weak, culminating in death of these nymphs. RH-5849 is well known to reduce feeding regardless of larval age

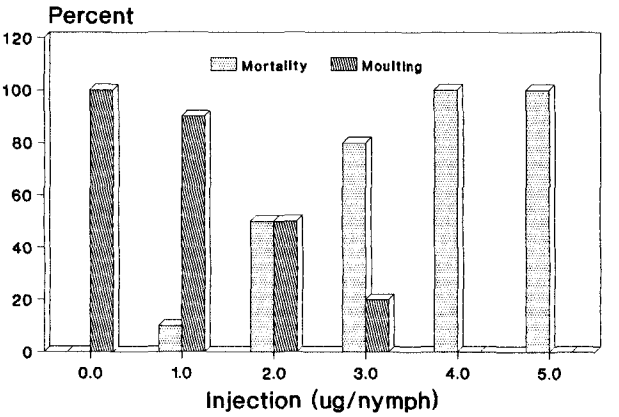

Figure 1. Effect of RH-5849 administered via injection on 5th instar nymphs.

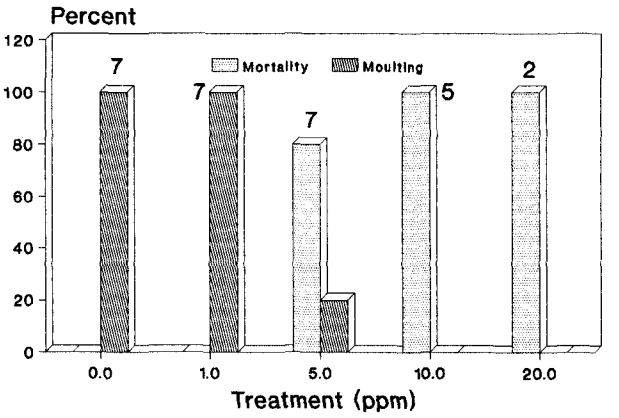

Figure 2. Chronic effect of RH-5849 after oral feeding to 5th instar nymphs 0–20 h old. Numbers on the columns denote the days on which the mortality or moulting occurred.

and is highly efficacious when ingested[1]; *D. koenigii* appears to be no exception in terms of the behavioural effect of this compound. In order to determine whether precocious moulting (a characteristic of this ecdysone agonist) occurs following ingestion, nymphs were fed RH-5849 for 24–120 h, followed by a normal diet after various treatment intervals (table 2). Some nymphs died

Table 2. Fate of 5th instar nymphs of *D. koenigii* after oral administration of RH-5849 for different time intervals

Treatment time (h)	RH-5849 (ppm) 0		5		10	
	Mort	Moult[a]	Mort	Moult	Mort	Moult
24	0.0	100.0	0.0	100.0	0.0	100.0
48	0.0	100.0	10.0	90.0[b]	30.0	70.0[b]
72	0.0	100.0	20.0	80.0[b]	50.0	50.0[b]
96	0.0	100.0	100.0	0.0	100.0	0.0
120	0.0	100.0	100.0	0.0	100.0	0.0

Mort, mortality; Moult, moulting.
[a]Moulting occurred mostly late on 6th day in all treatments.
[b]Moulting occurred on day 9 after treatment.

Table 3. Fate of 5th instar nymphs after administration of makisterone-A at 5 µg/nymph dose on various days

Treatment (days) after last nymphal ecdysis	Moulting (%)	Mortality (%)
0.0	70.0[a]	30.0
1.0	100.0[b]	0.0
2.0	100.0[c]	0.0
3.0	100.0[d]	0.0
4.0	100.0[d]	0.0

[a]Moulted to adults on day 4 but moulting was partial.
[b]Though moulting occurred on day 4, more than 50% moulting was partial.
[c]Complete moulting to adults in 4 days.
[d]Normal moulting to adults on late day 5 and day 6.

in their half cast moults after 7 days of treatment. This type of effect is produced by some phytochemicals as well[16,18]. However, at no stage of treatment was premature moulting observed. Thus the above results clearly show that RH-5849 was not able to stimulate the moulting process in *D. keonigii* and the effects observed were instead a consequence of either behaviour or toxicity.

In injection experiments there was a clear indication that moderate doses also induced a slow death with hyperactivity or paralytic symptoms in the legs, suggestive of neurotoxic action. A neurotoxic insecticidal action of this compound due to potassium channel block in neurons and muscles has been shown in cockroaches and houseflies[12] and recently observed in some lepidopteran species as well[8].

It is well known that makisterone-A is the principal moulting hormone in *Dysdercus*. Injections of makisterone-A to 5th instar nymphs of different ages at 5 µg/nymph exhibited very interesting results. Makisterone-A administered to 0-h- and 1-day-old nymphs led to precocious moulting after 4 days (table 3). In the former treatment, precocious moulting occurred in 70% of the nymphs and 30% died as 5th instars only. Half of those moulting failed to complete ecdysis. This indicated the presence of a partial stimulus for moulting in these insects; however this is only speculation as failure of ecdysis is a common event with IGRs and could be due to a number of causes. 48-h-old larvae when injected with makisterone-A in a similar fashion all moulted to adults on the 4th day, which is interesting since there was already an endogenous stimulus for initiation of moulting after day 2, when the level of makisterone-A begins to rise in *Dysdercus*[19] before reaching a peak at day 4. A subsequent drop in titre almost to zero after five and a half days leads to the final ecdysis. When we inject makisterone-A on day 2, the initial stimulus for this hormone in the haemolymph is already there and exogenously administered makisterone-A provides the elevated level normally expected at day 4, forcing the nymphs to undergo ecdysis prematurely. In the latter treatments, larvae injected on day 3 and day 4, moulting did not appear to be precocious and occurred normally on day 5 or day 6. This shows that a normal level of makisterone-A was already available to nymphs by the time exogenous makisterone-A reached its receptor sites: therefore, normal moulting occurred. However, under these circumstances hyperecdysonism should have occurred, which was not observed in either case (i.e. day 3 and day 4 injections of makisterone-A). A simple explanation for this could be that the dose injected (5 µg/nymph) was not sufficient to

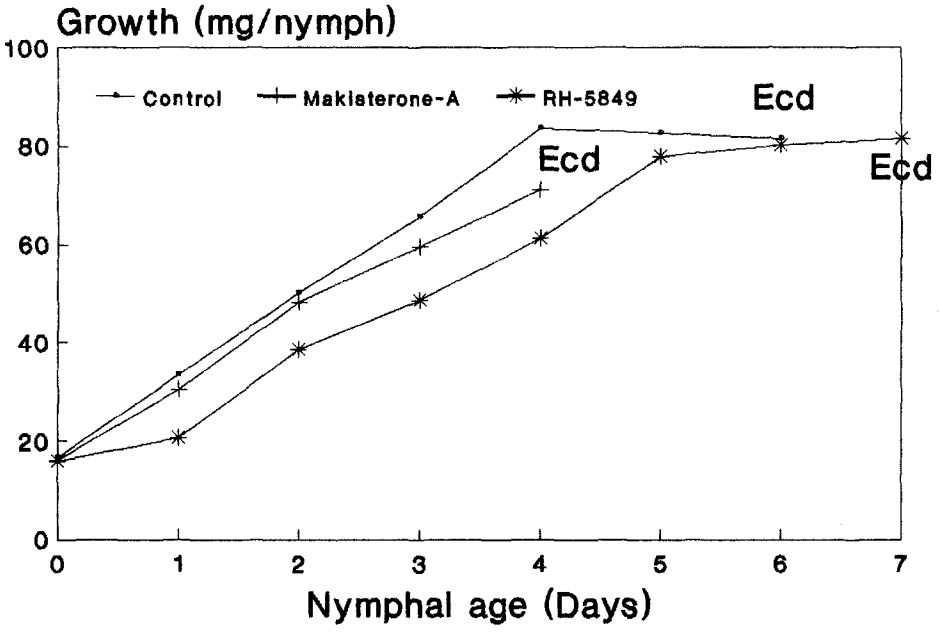

Figure 3. Growth of 5th instar nymphs (48-h-old) on various days after the administration of makisterone-A and RH-5849 at 5 µg/nymph and 2 µg/nymph respectively. Ecd = ecdysis.

elevate the makisterone-A level beyond the physiological tolerance of the insect.

Conversely, as shown above, RH-5849 injected in a similar fashion did not induce any premature moulting. In fact there was not much deviation in nymphal weights from controls when 2 µg of RH-5849 was injected (at 5 µg level 100% mortality occurred), and 40–50% larvae died on various days (fig. 3). In contrast, nymphs treated with makisterone-A remained between 68–75 mg, even though ecdysis occurred prematurely (fig. 3). The only other example of the effect of RH-5849 on a heteropteran species is that of *Oncopeltus fasciatus*, where LC_{50} of 12.71 µg/cm^2 against second instar nymphs has been recorded. These studies show the formation of half sized nymphal adult intermediates with small forewings. However, these studies do not record any precocious moulting due to RH-5849 treatment[20].

We conclude that RH-5849, a known ecdysone agonist, does not mimic the action of makisterone-A in *D. koenigii* and the toxicity of the candidate compound seems to be strongly species- and stage-dependent[20]. Apparently RH-5849 is able to mimic ecdysones only and not the ecdysteroids in general, although cross reactivity of makisterone-A with ecdysone is reported to be of the order of 8.65[13].

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