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Report on Bioassay of Compounds Sent by Dr. G. Matkocsy, September 20, 1991

Materials and Methods

Manduca sexta larvae were reared and treated with the compound on the first day of the 3rd instar as described previously (Ujvary *et al.* 1989). Assay larvae were then monitored daily until death or pupation, whichever came first. Both spiracle and crochet anomalies were scored as previously described (Ujvary *et al.* 1989).

Compounds were also tested in the black larval assay (Fain and Riddiford, 1975) for JH activity.

Results

Spiracular inhibition

Table 1 shows that at 100 µg/larva, only two compounds, Pierce 21555 and Pierce 21524, had effects on spiracular development. At this dose the compounds were toxic and only about 40% molted to the 4th instar.

When lower doses were given, the effects on the spiracles were found to be dose-responsive. Low doses of Pierce 21555 (1-50 µg/larva) (Table 2) caused damage in 25-40% of the larvae tested, whereas the higher dose of 100 µg/larva affected 71% of the treated larvae. The average spiracular score indicating degree of damage was low and showed little dose-responsiveness.

By contrast, Pierce 21524 was active on the spiracles of most larvae even at 1 µg/larva and showed a dose-dependency in the spiracular score. (Table 3 and Fig. 1). The ED₅₀ for the 4th instar score (*i.e.* a score of 2.5) is 40 µg.

Crochet development

Only the 2 Pierce compounds 21555 and 21524 had adverse effects on crochet development. (Tables 2 and 3). In both cases the % with damaged crochets was low until 50-100 µg were given at which point about half of the treated larvae showed some crochet damage.

Table 2 shows that even at 100 µg/larva Pierce compound 20590 had little effect on larval development and pupation. NKI 43766b, NKI43833b and Pierce 20664 at 100 µg/larva were toxic to 25-40% of the treated larvae who died before the molt to the 4th instar. Since those that molted showed no spiracular or crochet anomalies, they were not tested at lower doses.

Both Pierce 21555 and Pierce 21524 showed dose-dependent effects on larval and pupal development (Tables 2 and 3). Doses above 10 µg/larva slowed growth resulted in 50% or less molting to the 5th larval stage. Many of those treated with 5 µg or more were unable to complete the 5th instar and died before pupation. Growth was noticeably slowed in these.

JH Effects

All compounds were tested for JH activity at the high dose of 100 µg/black larvae. Only the NKI compounds showed any JH activity. When NKI 43833b was tested at lower doses, the ED₅₀ was found to be 30 µg/larvae (Fig. 2). This is about 166 times less active than JH III in this assay.

Pierce 21524 & spines

to 5:00

Fig 1

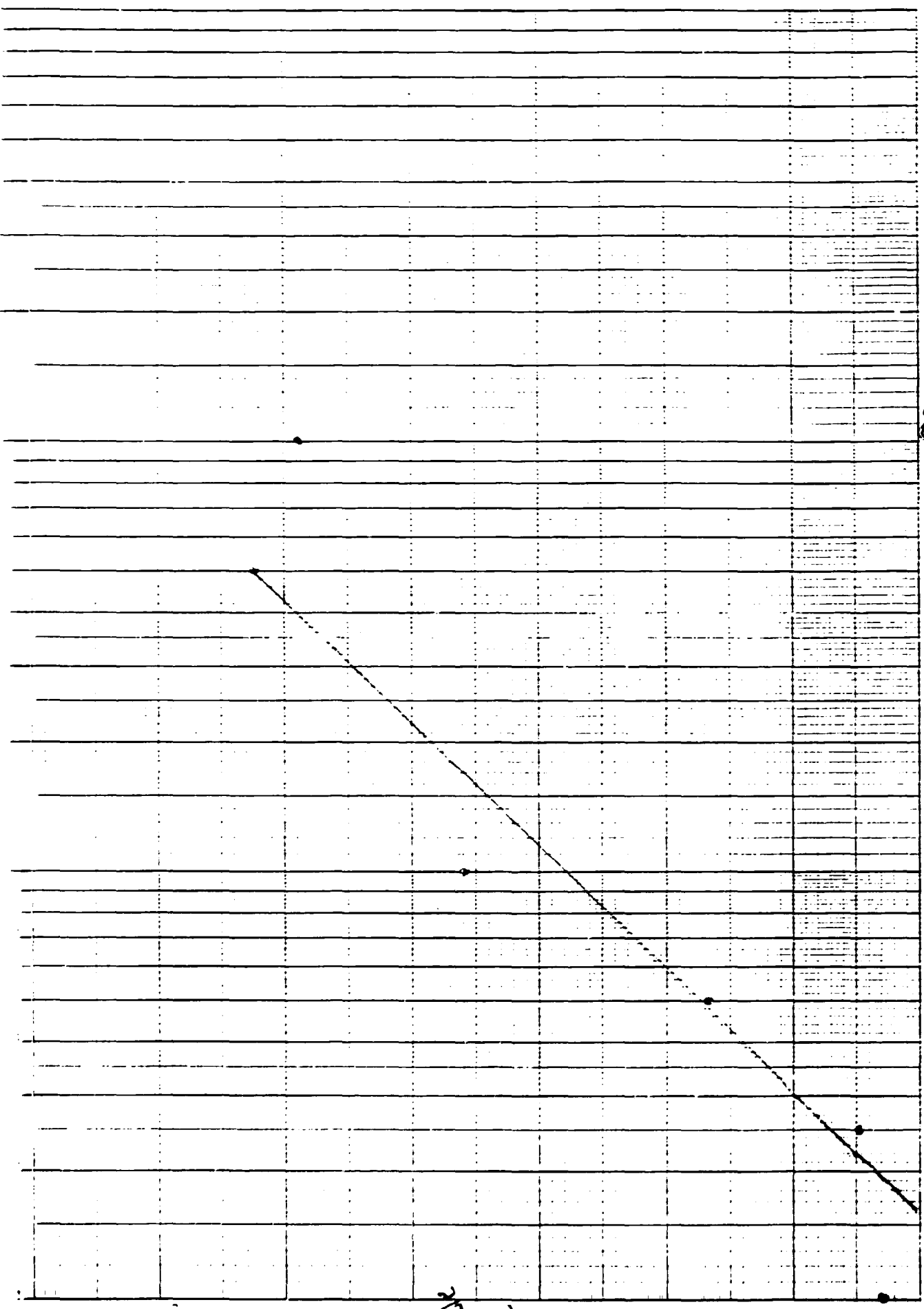
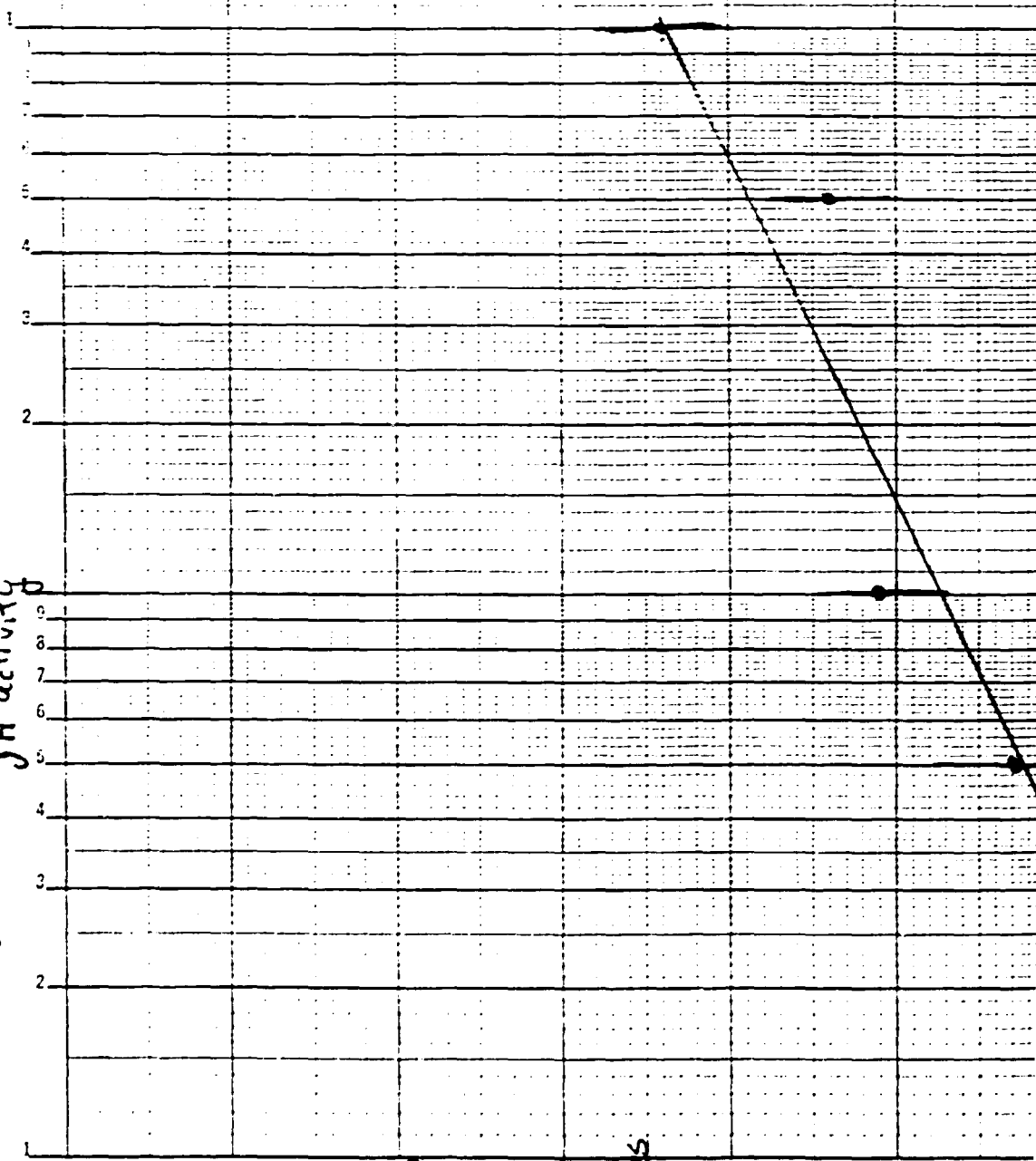


Fig 2
NKI 43833b
JH activity



33
2

Table 1: Effects on Development, Spiracles, and Crochets

Compound	Dose ($\mu\text{g}/\text{larva}$)	No.	% to 4th	% to 5th	% to 6th	% pupated	% spiracle damaged larvae (4th)	% spiracle damaged larvae (5th)	% crochet damaged larvae (4th)	% crochet damaged larvae (5th)
A) NKI-43833b	100*	12	75	67	0	50	0	0	0	13
B) NKI-43765b	100*	12	58	58	0	58	0	0	0	14
C) Pierce 20664	100**	12	75	75	8	58	0	11	0	0
D) Pierce 21555	100***	20	35	20	10	5	71	50	43	25
E) Pierce 20590	100*	12	92	92	0	92	0	0	0	0
F) Pierce 21524	100*	20	85	40	0	25	94	100	33	38

* Dissolved in 1 μl acetone and topically applied

** Dissolved in 1 μl H_2O and injected

*** Dissolved in 1 μl DMSO and injected

Table 2: Dose-responsiveness of Pierce 21555*

Dose ($\mu\text{g}/\text{larva}$)	No.	% to 4th	% to 5th	% to 6th	% pupated	% spiracle damaged larvae (4th)	% spiracle damaged larvae (5th)	% crochet damaged larvae (4th)	% crochet damaged larvae (5th)
1.0	20	100	95	0	95	25	16	5	0
2.5	20	100	100	5	80	40	30	10	5
5.0	20	95	30	0	65	26	28	21	17
10.0	20	90	90	5	13	39	39	11	11
50.0	20	60	25	0	10	42	40	42	60
100.0	20	35	20	10	5	71	50	43	25

*Pierce 21555 (dissolved in DMSO and 1 μl was injected)

Table 3: Dose-responses of Pierce 21524

Dose ($\mu\text{g}/\text{larva}$)	No.	% to 4th	% to 5th	% to 6th	% pupated	% spiracle damaged larvae (4th)	% spiracle damaged larvae (5th)	% crochet damaged larvae (4th)	% crochet damaged larvae (5th)
1.0	20	100	95	0	70	60	11	0	5
2.5	20	100	95	0	80	55	47	5	5
5.0	20	90	75	0	55	72	79	22	14
10.0	20	100	80	0	50	100	100	20	6
50.0	20	75	50	0	10	100	100	33	30
100.0	20	85	40	0	25	94	100	33	38

*Pierce 21524 (dissolved in acetone and 1 μl was topically applied)

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January 30, 1992

Dr. George Matolcsy
Plant Protection Institute
Hungarian Academy of Sciences
H-1525 Budapest
P. O. Box 102
HUNGARY

Dear George:

Enclosed is the final report of the compounds that you sent for testing. Sorry that it took so long to get all the tabulations done, then for me to analyze it. This winter quarter has been somewhat easier than the fall, but I still have two graduate courses to deal with and have had visiting scientist house guests for the last two weeks.

I apologize for the state of the Tables. They will be put in typed format and sent next week. I hope that these Results are informative.

If you have any more compounds to test, we will do so and not ask for more funds unless there are many. Enclosed is the letter I wrote to UNIDO asking that they send a letter saying that they would not pay the overhead and the reply. Since they would not state that they did not want to pay overhead, my University required that we put it in. I am sorry that it happened that way as I told you in good faith the \$2000 based on the absence of overhead for the previous subcontract.

Sincerely,

Lynn M. Riddiford
Professor of Zoology