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Inhibition of Oviposition in the Bean Weevil (*Acanthoscelides obtectus* Say, Col., *Bruchidae*)

By

G. MUSCHINEK,[1] Á. SZENTESI and T. JERMY

Research Institute for Plant Protection, H-1525 Budapest, P.O. Box 102, Hungary

Bean weevil females laid, in choice tests, significantly more eggs on untreated dry beans as compared to those containing 0.234 mg and more $CuSO_4 \cdot 5\ H_2O$ per cm^2 of seed surface.

Keeping the weevils on dry beans treated with a 0.2 *M* solution of $CuSO_4$ caused: (a) reduction of fecundity (most probably by decreasing ovogenesis), (b) to a smaller extent, decrease of egg-viability, and (c) prolongation of the duration of oviposition period.

Thus the chemical qualities of the dry beans evoking oviposition in the weevil can be masked by $CuSO_4$.

The results indicate that the rate of multiplication can be reduced in this insect by oviposition inhibitors.

Many plants contain secondary substances inhibiting oviposition in phytophagous insects (GUPTA and THORSTEINSON, 1960; YAMAMOTO and FRAENKEL, 1960; BYRNE *et al.*, 1967; HSIAO and FRAENKEL, 1968; etc.). JERMY (1972) investigating the oviposition behaviour of the pea weevil (*Bruchus pisorum* L.) found that the females were not willing to lay eggs on the surface of pea-leaves touching the pea-pods, not even if the leaves were folded into the shape of a pod. However, they did oviposit close to the pea-pods on a glass surface providing indifferent tactile stimuli. Therefore, it could be supposed that the pea-leaves contained a substance inhibiting oviposition.

Selection of the oviposition site is based, similarly to food specialization, on a "two-way specialization" of the chemoreceptors (JERMY, 1965), thus, both stimulatory and inhibitory stimuli determine the behaviour of egg-laying females. This is obvious in the case of species the ovipositing females of which choose the food for the larvae by laying the eggs directly on or into the suitable substrate.

In the bean weevil, olfactory cues acting in a short range (POUZAT, 1974) and/or contact chemical stimuli play a decisive role in the selection of the food plant. First of all the receptors of the maxillary palpi but also those of the ovipositor participate in chemoreception. Tactile stimuli represented by the substrate,

[1] Present address: Ministry of Agr. and Food, Plant Protection Centre, H-1502 Budapest P.O.Box 127, Hungary.

e.g. shape (spherical surface and gap on a curved surface) perceived by the antennae and by the proprioceptors in the legs, are of secondary importance (SZENTESI, 1976).

Egg-laying can take place, though to a smaller extent, also on other leguminous plant seeds which do not provide optimal food for the larvae, as well as on substrates having the required shape and lacking of inhibitory substances, but absolutely unsuitable for larval feeding, for example on glass beads (SANDNER and PANKANIN, 1973; SZENTESI, 1976).

The chemical stimuli inducing oviposition in the bean weevil can be masked by applying various chemicals, e.g. bordeaux mixture, 2,4,6-trichlorophenoxy acetic acid, 2,4,6-trichlorophenoxy ethanol (JERMY, 1972) on the dry beans.

The present paper deals with experiments carried out applying copper sulphate for masking oviposition stimuli originating from the dry beans. The aim was to answer the question whether egg maturation, fecundity and fertility of bean weevil females were affected by the inhibitory stimuli.

Material and Methods

Virgin bean weevil adults were gained from a laboratory mass rearing (SZENTESI, 1972) by sieving just emerging adults in half an hour intervals.

Large white beans served as oviposition stimulus, and $CuSO_4 \cdot 5H_2O$ as inhibitory stimulus. 100 g dry beans (244 $\pm$ 4.5 seeds) were thoroughly mixed in a shallow glass dish with 2 ml of a solution containing 0.05, 0.1, 0.15 or 0.2 *M* $CuSO_4$, respectively, then dried in a hot (about 80°C) air stream under continuous shaking. The amount of $CuSO_4$ remaining in the glass dish was measured. By this means the seeds were uniformly covered by $CuSO_4$, the amount of which averaged 0.397 mg/seed or 0.234 mg/cm^2 of seed surface when treated with a 0.2 *M* solution. Dry beans treated in the same way with distilled water served as a control.

Experiment I

The dry beans were put in a one-seed layer into petri dishes of 10 cm in diameter the bottom of which was divided into four sections by a 3 mm high and 3 mm thick cross-shaped wall of paraffin wax.

The following three variants were applied:

A – In all four sections of the petri dish $CuSO_4$-treated dry beans ("total inhibition").

B – In two opposite sections of the petri dish $CuSO_4$-treated, in the other two sections water-treated dry beans.

C – In all sections water-treated dry beans.

Experiment II

One or two days old weevils were put into petri dishes containing one layer of $CuSO_4$-treated (0.2 *M*) beans and then transferred to untreated beans after 0, 3, 6, 9, 12, 15 and 18 days, respectively.

In both experiments 20 females and 20 males were put into each petri dish. Each variant was repeated 10 times. The dishes were kept at 23°C in total darkness. After the death of all females the number and viability of eggs laid, and the number of unlaid and retained eggs in the lateral oviducts were determined.

Results

Experiment I

In the variant B the overwhelming majority of the eggs (93.27 ± 13.2% in the case of 0.2 *M* $CuSO_4$) was laid on the water-treated beans, thus, copper sulphate strongly inhibited egg-laying. A hyperbolic curve was found to be characteristic for the percentage of the eggs laid on the $CuSO_4$-treated beans as a function of the concentration of the $CuSO_4$ solution used for seed treatment (Fig. 1).

The fecundity found in variant A was half of that in variant C, and differred significantly also from that in variant B ($P < 1\%$, t-test), owing to the fact that

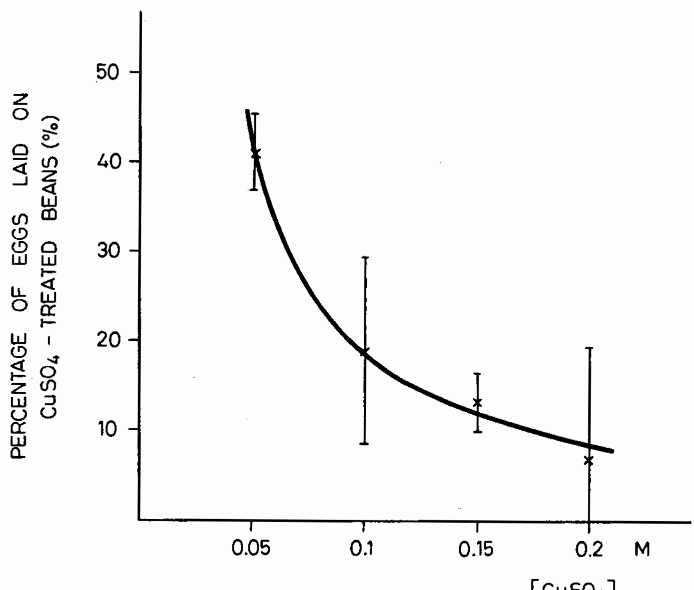

Fig. 1. The effect of the $CuSO_4$ concentrations on the percentage of eggs laid on treated beans

in the latter variant the females could choose between treated and untreated beans (Table 1).

Table 1

Effect of the presence of 0.234 mg/cm² $CuSO_4 \cdot 5H_2O$ on the surface of dry beans upon the fecundity and fertility of bean weevil females (10 replicates)

Variants	Fecundity, eggs/female	Percentage of non-viable eggs	No. of eggs retained in the lateral oviducts per female
A. treated beans only	10.3 ± 2.8	24.1 ± 9.3	2.44 ± 1.6
B. treated and untreated beans	16.6 ± 3.7	19.9 ± 7.1	1.36 ± 1.1
C. untreated beans only	20.7 ± 3.8	22.5 ± 8.3	0.84 ± 0.7

There was no difference in the percentage of non-viable eggs laid on treated and untreated beans, respectively, and there were only a few eggs retained in the lateral oviducts. The number of the latter showed a decreasing tendency according to the following order: treated beans > treated and untreated beans > untreated beans, although the differences were not significant (Table 1).

Experiment II

Total fecundity, i.e. the number of eggs laid both on treated and untreated beans, decreased with the time spent by the females on 0.2 *M* $CuSO_4$-treated beans, and there was a significant difference ($P < 0.1\%$) from the 12th day as compared to day 0 (Fig. 2, a). The number of eggs laid on treated beans (difference between *a* and *b* in Fig. 2) increased however, with a substantially lower rate than at optimal oviposition circumstances.

The percentage of non-viable eggs showed an increasing (but not significant) tendency till the 12th day and then remained almost at the same level (Fig. 3, columns-a). The percentage of non-viable eggs laid on treated dry beans did not change significantly (Fig. 3, columns-b). The same value increased continuously till the 15th day in the case of eggs laid after transferring the females to untreated dry beans (Fig. 3, columns-c), and there was a significant difference between the minimum and maximum values (16.7 ± 6.5 and 52.5 ± 24.3, $P < 1\%$).

The number of eggs retained in the lateral oviducts of females did not change with the duration of the inhibitory effect, it was at an equally low but not at a neglectable level (Fig. 2, c).

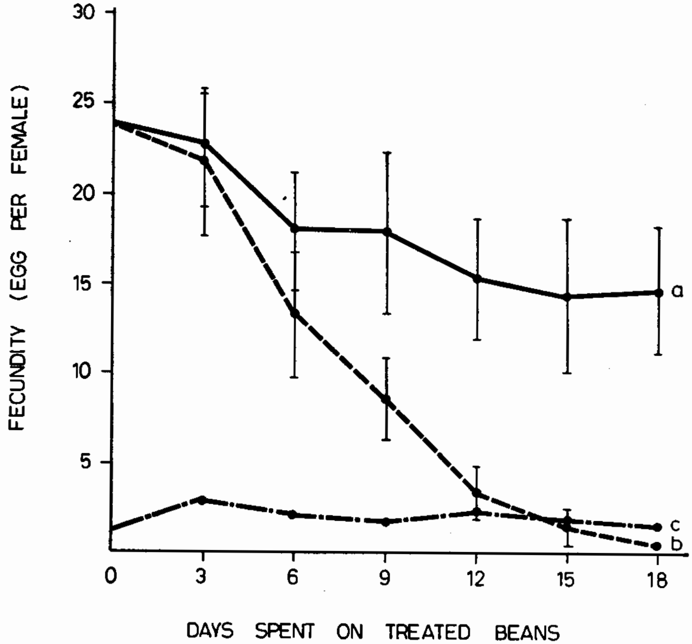

Fig. 2. The effect of duration (days) of inhibition on the fecundity and the number of eggs retained in the lateral oviducts per female: a: ——— total fecundity/♀; b: — — — — fecundity/♀ from the time of transmission to the untreated beans; c: —·—·— mature eggs retained in the lateral oviducts/♀

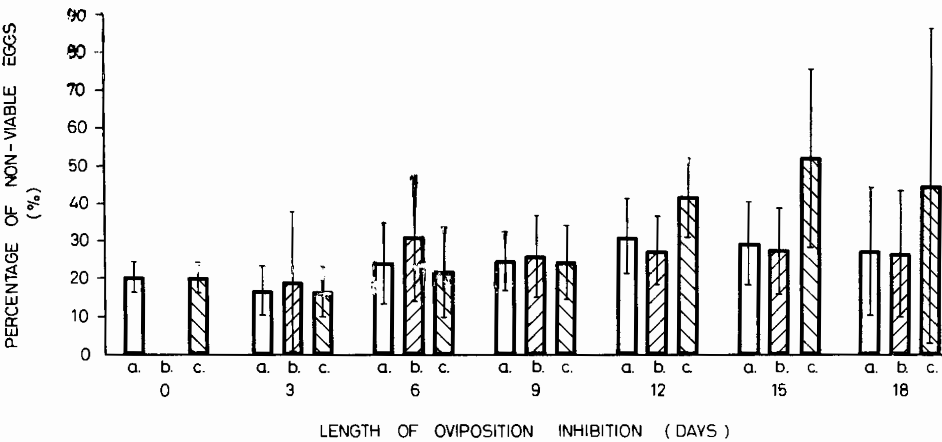

Fig. 3. The percentage of non-viable eggs: (a) laid during 18 days; (b) laid on treated dry beans; (c) laid on untreated dry beans

Discussion

Comparing *Exp. I* and *II*, there was no difference in the fecundity and fertility of females kept continuously on untreated or for one hour on treated dry beans. The same was found when females were egg-laying on treated dry beans during their lifetime or for 18 days.

The reduction in the fecundity with increasing duration of oviposition inhibition, (Fig. 2, a) and the generally low number of eggs retained indicate that the inhibition of oviposition (through the inhibitory state evoked by the $CuSO_4$) may affect ovogenesis either by slowing it down or by the resorption of immature eggs which can occur at the ovariole level (LABEYRIE, 1960). It can be supposed also that the vitellogenesis itself might be sensitive to the inhibitory stimuli (since the fecundity did not reach the normal level after the cessation of inhibition). However, these experiments are inadequate for revealing the physiological mechanisms of the influence of inhibition.

For the increase in the percentage of non-viable eggs laid after the transfer to untreated beans (Fig. 3, columns-c) several factors may be responsible: (a) the viability of eggs laid at the final period of oviposition decreases even under normal conditions (SZENTESI, 1975), (b) the inhibition of oviposition by $CuSO_4$.

It has been mentioned that inhibition may have affected the process of egg-laying also by slowing it down. Fig. 4 shows that 50% of eggs were laid on the treated beans by the 9th day. Under normal conditions and at the same temper-

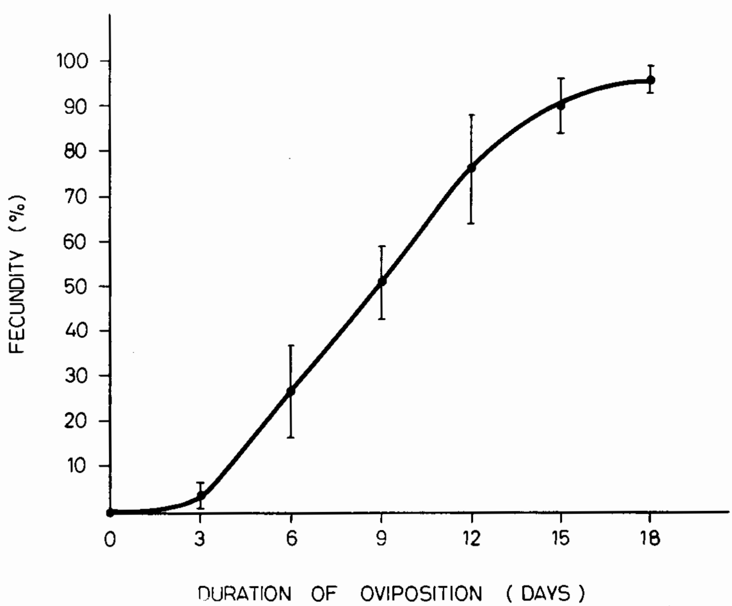

Fig. 4. Egg-laying rhythm in the presence of $CuSO_4 \cdot 5\,H_2O$

ature regime 50% egg-laying takes place by the 4th day (VUKASOVIĆ, 1949), and the same was found by SZENTESI (1972) although at higher temperature (28°C) and under crowded conditions (mass rearing).

It has been also shown by VUKASOVIĆ (1949) and SANDNER and PANKANIN (1973) that the lack of optimal egg-laying stimuli (absence of dry beans, replacing dry beans by glass beads, peas, etc.) extended considerably the beginning and duration of oviposition. Thus the presence of $CuSO_4 \cdot 5H_2O$ on the dry beans has an effect similar to the lack of optimal oviposition stimuli also regarding egg-laying rhythm.

In conclusion, $CuSO_4$ by masking optimal oviposition stimuli of dry beans caused:

(a) the decrease of fecundity,

(b) to a smaller extent, the reduction of egg viability, and

(c) the prolongation of the egg-laying period.

However, it has to be emphasized that there was no decrease in the fecundity when both treated and untreated dry beans were present, but, depending on the concentration of the $CuSO_4$ applied, the number of eggs laid on the treated dry beans proved to be significantly smaller as compared to those laid on the untreated ones.

Thus, the population dynamics of insects — at least of species having a reproduction biology similar to that of the bean weevil — can be influenced by applying an adequate quantity of oviposition inhibitors.

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