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Effects of Neem Oil on Mating and Oviposition Behaviour of Azuki Bean Weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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Abstract: Effects of neem oil on mating and oviposition behaviour of *C. chinensis* grown on azuki bean under laboratory conditions were studied. Mating frequency, copulation duration, fecundity and survivorship of the next generation progenies were significantly decreased by neem oil. Mating frequency was always found to be lower in the oil-treated pair than in the control pair, but it was increased with time after the oil application. A lower mating frequency and shorter copulation duration results in lower fecundity and lower emergence rate. Fecundity and emergence rate were always lower in oil-treated conditions. The effects of neem oil were always greater on male-treated pairs than on female-treated pairs.

Key words: Stored product pest, neem oil, mating success, copulation duration and oviposition behaviour

Introduction

Azuki bean is a favourite and commonly used bean in Japan. The increased public concern for possible contamination of foodstuffs with pesticide residues has led to greater emphasis on developing non-pesticidal methods for protecting stored products against insect damage (Bhaduri *et al.*, 1990). The use of plant materials in the control of stored product pests is an ancient measure in many parts of the world (Saxena, 1986). The mixing of plant oils is traditionally practiced in Asia and Africa. This type of pest control was abandoned with the advent of modern synthetic insecticides. However, due to the problems encountered with the use of the insecticides, interest in the use of biocides from plants has been revived (Stone, 1992). This is because insecticides derived from plants like pyrethrum from *Chrysanthemum* spp. or Morgason-O from neem, *Azadirachta indica,* found to be non-persistent in the environment, have low mammalian toxicity and is relatively safe to other non-target organisms (Rejesus *et al.*, 1990). A large number of studies have been carried out on the effectiveness of different kinds of plant oils against bean weevils, *Callosobruchus* spp. (Khaire *et al.*, 1992; Ahmed *et al.*, 1993; Pacheco *et al.*, 1995). Previously we reported that neem oil completely inhibited multiplication of azuki bean weevil, *C. chinensis* population and suggested that the effectiveness of neem oil might be physical or chemical (Ahmed *et al.*, 1999).

There are many specific reciprocal activities between sexes in insect mating behaviour, which are usually initiated by one sex and assisted by chemical, visual, tactile or other cues. Sex pheromone became a pertinent detail in the mating behaviour of *Callosobruchus* spp. (Rup and Sharma, 1978; Qi and Burkholder, 1982). In *C. chinensis*, female sex pheromone has been clarified as a cue for the encounter with a conspecific mate (Tanaka *et al.*, 1981). If neem oil can disrupt communication in mating behaviour of *C. chinensis*, unsuccessful mating could lead to a lower fecundity and ultimately lower the population. Communication disruption by neem oil can be a safer and more economical method of pest control than killing because the dose of oil necessary for communication disruption seems to be lower than that for killing. However, possible effects of plant oils on communication disruption in mating behaviour have not so far been assessed.

Therefore, the present study was undertaken to evaluate the effects of neem oil on mating behaviour, fecundity and emergence rate of *C. chinensis* grown on azuki bean under laboratory conditions.

Materials and Methods

The experiment was conducted in the laboratory of Entomology, Faculty of Agriculture, Kagawa University, Japan from May to October 2000. Azuki bean weevils, *C. chinensis* were reared on azuki beans in an incubator at 25°C under 70±5% R.H. and 14L: 10D photoperiod. Single-egg containing beans were kept individually in petri dishes (6 cm in diameter) to obtain unmated adults, which were sexed according to the criteria given by Southgate (1958) and then kept separately in petri dishes.

Cold pressed 100% pure neem oil (The Original Neem Company, USA) was initially dissolved in a small amount of acetone, then a 5% (v/v) neem oil suspension was made by adding distilled water. Unmated males and females were kept separate in a petri dish whose base was covered with 5% neem oil-soaked filter paper for 2 minutes. Then the unmated-treated males and females (T♂ and T♀) were kept separately on a fresh filter paper in a petri dish for 2 hours. To get unmated-normal males and females (N♂ and N♀), beetles were kept separately on solvent (acetone + water) soaked filter paper for 2 minutes.

The effect of neem oil on mating was studied at 4 different combinations (T♂x T♀, T♂x N♀, N♂x T♀ and N♂x N♀), and mating was observed in 100 pairs of beetles for each combination. Mating was also observed in 20 pairs of N♂x N mated♀. For each pair, 5-minute observation was carried out on 0, 1, 2, 3, 4, 7 and 10 days after oil-treatment (DAT) and the number of mating pairs were counted. On 2 DAT, copulation duration was also recorded for the mating pairs.

To compare the fecundity, normal virgin females, the mated females on 2 DAT from above 4 combinations, and normal mated females (which were treated with neem oil after mating, N♂x N♀→T♀) were kept separately in 6-cm petri dishes containing 50 fresh azuki beans (5 females per petri dish). The number of eggs deposited by the females and adult emergence rate were recorded. Five replications were made for each experimental group.

The data of oil effects on mating frequency in different pairs on different DAT was analyzed with three-way ANOVA using Sistat-9 for windows and H statistic was adjusted by the method of Scheirer *et al.* (1976) and Sokal and Rohlf (1995). The data of mating duration, fecundity and survivorship were analyzed with two factors completely randomized design and the means were compared using Turkey's test. The data for egg deposition (Y) was transformed to log (Y+1) and emergence rate (p) was transformed to arcsine (√p) and subjected to the tests.

Results and Discussion

In the whole mating process (from the sex attraction to termination of the genital coupling), male active behaviour has an important role. The male attempted several times to mate whereas the female stands still or walks sometimes a few steps. The female usually controls mating chemically by the

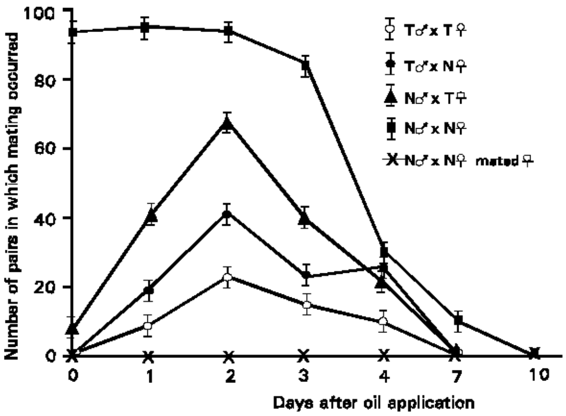

Fig. 1: Effects of neem oil on mating frequency in T♂x T♀ (n = 100), T♂x N♀(n = 100), N♂x T♀(n = 100), N♂x N♀(n = 100) and N♂x N mated♀(n = 20), pairs on 0, 1, 2, 3, 4, 7 and 10 days after oil-treatment (DAT). T indicates oil treated and N not treated (control). Vertical bars show standard deviations.

secretion of sex pheromone (Tanaka *et al.*, 1981) and is able to inhibit mating physically by the extension of her hind legs backwards when she has been already mated. So the mating success of an unmated pair depends on the chemical activity (production of sex pheromone) of females and the physical behavioural activity (response to sex pheromone) of males.

Oil treatments had significant effects on the mating frequency of males and females and the degree of oil effects varied with the time after treatment (Table 1, Fig. 1). In the control pair (N♂x N♀), more than 95% of beetles were mated within 5 minutes up to 2 DAT, and thereafter mating frequencies were greatly reduced. Mated females never mated again. Under normal conditions, the beetle completely failed to mate after 9 days of their emergence, due to lethargic nature of the

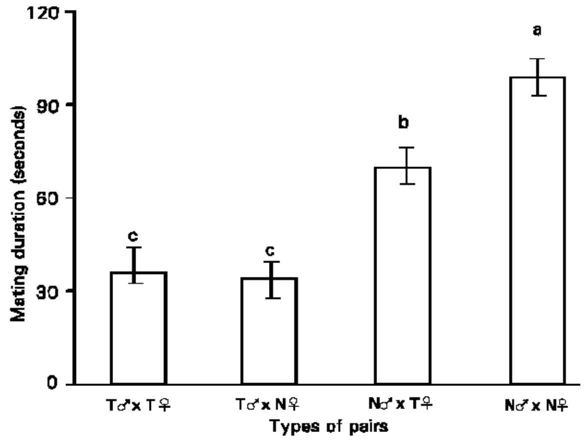

Fig. 2: Effect of neem oil on mating duration (in second) of *C. chinensis* in T♂x T♀, T♂x N♀, N♂x T♀ and N♂x N♀ pairs (n = 20 in each group). T indicates oil treated and N not treated (control). Vertical bars show standard errors. Values with the same letter are not significantly different (P<0.05, multiple comparison Tukey`s test).

males (Rup, 1986). In the treated pair (T♂x T♀), the beetles abstained from mating on 0 DAT. Mating frequency in 3 treated pair combinations (T♂x T♀, T♂x N♀ and N♂x T♀) increased with time after oil application up to 2 DAT, but from 3 DAT it started to decline and from 7 DAT beetles completely failed to mate. This is due to the reduction in behavioural activity of males possibly by the toxicity of the oil and/or its pungent odour; the treated beetles cannot perform normal mating behaviour because the oil may chemically hamper the pheromone production by females and the males may fail to recognize the pheromone in the presence of neem oil.

Comparing the oil effect between only female-treated (N♂x T♀) and only male-treated (T♂x N♀) pairs, mating frequency was always lower in only male-treated pairs. This result suggests that the disturbance of pheromone recognition by males is more plausible than that of the inhibition of pheromone production by the treated females and the effect of neem oil on mating is greater in males than in females.

Mating activity was found to peak on 2 DAT. So, data for copulation duration, egg deposition and adult emergence were recorded on 2 DAT only.

Copulation duration was significantly affected by the oil treatment (Fig. 2). In both-normal pairs (N♂x N♀), the beetles stayed in copula for 98.2±3.8s whereas, 35.4±2.3s in both-treated pairs (T♂x T♀) and 35.7±1.5s in only-male-treated pairs (T♂x N♀). But in only-female-treated pairs (N♂x T♀) the

Table 1: Results of 3-way rank-order ANOVA about oil effects on mating frequency†

Source of variation	Sum of squares	df	Adjusted H (χ^2)	Probability
Days after treatment (DAT) (0, 1, 2, 3, 4, 7 or 10)	203445000	6	903.9155	<0.001
Males (treated or untreated)	121722000	1	540.8164	<0.001
Females (treated or untreated)	56062300	1	249.0874	<0.001
DAT × Males	67746000	6	300.9986	<0.001
DAT × Females	27683600	6	122.9995	<0.001
Males × Females	22932700	1	101.8910	<0.001
DAT × Males × Females	30864400	6	137.1319	<0.001
Error	543057000	2772		
Total	1073513000	2799		

†By the method of Scheirer *et al.* (1976) and Sokal and Rohlf (1995)

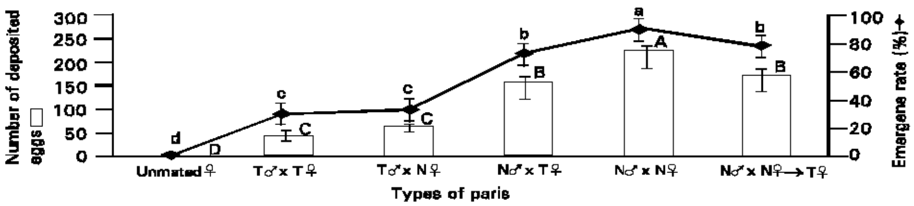

Fig. 3: Number of deposited eggs by 5 *C. chinensis* females from T♂x T♀, T♂x N♀, N♂x T♀, N♂x N♀ and N♂x N♀T♀ pairs, and the eggs survivorship to adults (emergence rate). T indicates oil treated and N not treated (control). Vertical bars show standard errors. Number of deposited eggs (Y) was transformed to log (Y+1) and emergence rate (p) was transformed to arcsine (vp) and subjected to the tests. Values with the same letter are not significantly different ($P<0.05$, multiple comparison Tukey`s test).

copulation period (65.3±3.2s) was longer than only-male-treated pairs. Therefore, it is likely that due to oil toxicity the male stayed shorter in copulation, resulting in incomplete or unsuccessful mating.

The normal virgin female cannot lay eggs at all (Fig. 3). So fecundity must depend on successful mating. We found that fecundity was always significantly lower in male-treated pairs than in female-treated pairs. This tendency was also found in the adult emergence rate. These results suggest that an insufficient number of sperm were transferred due to a short copulation period, the oil has some spermicidal effect on males (Riar *et al.*, 1990 and Sharma *et al.*, 1996), or treated males produce lower number of sperm.

In the case of oviposition behaviour, there was no significant difference in fecundity between females from (N♂x T♀) and (N♂x N♀→T♀) pairs (Fig. 3). But the females of these groups deposited a significantly lower number of eggs than normal females (N♂x N♀). This difference in fecundity between treated and normal females is due to the oil effect on oviposition behaviour of the females. Ahmed *et al.* (1999) and Khaire *et al.* (1992) reported similar results that neem oil has an effect on fecundity and survivability of the beetle, but they treated the oils only after mating and did not observe the mating behaviour of beetles. The present results suggest that the oil has significant effects on mating and egg laying behaviour, even when the oils are applied before mating.

The present study shows that the adverse effect of neem oil on mating is greater in males than in females. The oil significantly reduced mating frequency and copulation duration, leading to a lower fecundity and lower emergence rate. The results suggest that neem oil has significant effects on mating behaviour of *C. chinensis*. To elucidate the mechanism(s) responsible for these oil effects, further studies should be carried out.

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