Ent. exp. & appl. **30** (1981) 219—226. *Ned. Entomol. Ver. Amsterdam*

PHEROMONE-LIKE SUBSTANCES AFFECTING HOST-RELATED BEHAVIOUR OF LARVAE AND ADULTS IN THE DRY BEAN WEEVIL, *ACANTHOSCELIDES OBTECTUS*

Á. SZENTESI
Research Institute for Plant Protection, H-1525 Budapest, Pf. 102, Hungary

Males and females of the dry bean weevil, *Acanthoscelides obtectus* deposit chemically unknown substance(s) on beans during various activities (most probably during defecation), under laboratory conditions. The marking results in avoidance of these beans by other females for oviposition. Also, first-instar larvae are influenced to disperse more by the deposited substance(s), so that they are more likely to find unmarked beans to enter. No specific marking behaviour by adults was noted. That the deterring substance(s) might originate from microorganismic activity after deposition has occurred, is improbable. Possible explanations for sources of marking substance(s) and the significance of the corresponding behaviour in this species are discussed.

KEY WORDS: *Acanthoscelides obtectus* - dry bean bruchid - oviposition behaviour - epideictic pheromone - egg and larval distribution.

Distribution of trophic resources and space by parents for their progeny by chemical marking after oviposition has been frequently recorded in insects since Salt (1937) first reported such behaviour in parasitoids. As a result, conspecific ♀♀ detecting the substances deposited, do not continue laying eggs into the same substrate provided alternative egg-laying sites are available. Basically such a behaviour could be considered to be a specific type of territoriality, in which benefit is gained by the marking individual, increasing its fitness. In general, the mediating substances are termed "epideictic pheromones", and they provide information about population size for individuals present on a limited resource (Prokopy, 1981).

In Bruchidae, on the basis of behavioural observations with *Callosobruchus chinensis* L., Utida (1943) considered the possible reasons for even distribution of eggs by ♀♀. Later, the egg-laying behaviour and the production of oviposition-deterring substances associated with it were investigated thoroughly by Yoshida (1961), Umeya (1966) (both cited in Umeya & Kato, 1970), Oshima *et al.* (1973) and Yamamoto (1974). With *C. maculatus* (F.) a similar oviposition behaviour was described by Yoshida (1961), Umeya (1966) and by Mitchell (1975), stating that ♀♀ avoided ovipositing on beans already "conditioned" by other ♀♀. Another bruchid, *Zabrotes subfasciatus* Boh. has a similar behaviour (Umeya, 1966).

The dry bean weevil, *Acanthoscelides obtectus* is an important granary pest. Its life cycle, in Hungary, is divided into outdoor and indoor phases (Szücs, 1959; Jávor, 1970). In the field, females gnaw holes along the suture of ripening bean pods and insert several eggs. In stores, eggs are scattered at random in small clusters around beans and will only be distributed evenly when adult density is high (Umeya & Kato, 1970). Competition for food among larvae in overcrowded circumstances can be severe (Sandner, 1962), though modified by the size of seed (Szentesi, 1972); from a single, 19 mm sized bean as many as 40 adults (A. Szentesi, unpubl.) may emerge. Only first-instar larvae have legs and they walk to find a place to enter the beans. In *A. obtectus*, Umeya & Kato (1970) found experimentally that ♀♀ were unable to differentiate between beans "unconditioned" and "conditioned" (already used for egg-laying by other ♀♀).

Bean weevil cultures usually have a very characteristic odour, mainly due to a male substance thought to be a pheromone (Hope *et al.*, 1967). The presence of some other chemicals is also highly probable, and therefore deposited substance(s) may play a definite role in such phenomena, as (1) the even distribution of eggs at higher adult densities (Umeya & Kato, 1970), and (2) a significant divergence from the otherwise random distribution of bore holes made by the first-instar larvae (Labeyrie, 1960). Aggregation of larvae before entering the beans has also been observed (Labeyrie, 1965).

This paper considers the above problems and attempts to demonstrate under laboratory circumstances that substance(s) which we may call deterring pheromone(s), do indeed exist in *A. obtectus*. It also suggests to find benefits that might be gained by a population having such a behaviour.

MATERIALS AND METHODS

Adults of *A. obtectus* were obtained from a laboratory culture maintained for about 10 years (Szentesi, 1972). Seeds of beans which were considered to be pheromone-marked by adults were obtained from regularly used egg-laying cylinders, unless otherwise stated, in which 150 to 200 g beans and ca. 10,000 adults were confined for 3 weeks. Virgin adults were collected using a method described elsewhere (Szentesi *et al.*, 1973).

Surface rinses of pheromone-marked (PM) and unmarked (UM) beans, as well as extracts of adults were prepared and tested. In order to cover the surface of beans by extracts, a method described by Muschinek *et al.* (1976) was used. In short: known quantities of solutions were poured onto cleaned, dry beans in a glass container and, while shaking continuously, the solutions were rapidly dried onto the surface of seeds under a cool or warm airstream.

The ability of first-instar larvae to enter PM-beans was investigated and direct observations on behaviour possibly associated with marking were also made.

In all test *Phaseolus vulgaris* cv. "Harvester" and "Közép fehér" beans of the year 1977 were used. For Exps. 1 to 4, 10 ♂♂ and 10 ♀♀ of freshly emerged *A. obtectus* were used per Petri dish, to examine ovipositional responses to PM- and UM-beans. All experiments had 3 to 10 replicates (= Petri dishes or vials). Oviposition tests were conducted in total darkness with a temperature of 26° ± 2° and a relative humidity of 60-70%, unless otherwise stated. The tests were usually terminated after 10 days, except for Exp. 5, and others where stated. Oviposition preference tests were evaluated by counting the number of eggs laid in respective sectors or vials, then by using the so-called discrimination quotient (DQ) (David & Van Herrewege, 1970) which is given by the formula:

$$\mathrm{DQ} = \frac{\text{No. of eggs laid on UM-beans - No. of eggs laid on PM-beans}}{\text{No. of eggs laid on UM-beans + No. of eggs laid on PM-beans}}$$

Multiplication by 100 gives the quotient as a percentage. Preference for an unmarked substrate is indicated by positive values of DQ and aversion by negative values. The DQ values can range between +100 and −100. In choice tests, treatments inside the Petri dishes were not independent, therefore it was not possible to apply calculations of significance. Thus, in such cases only the DQ values are given indicating the level of responses. In experiments with independent treatments, student's *t*-test was used.

The following experiments were carried out:

Exp. 1. Preliminary test with beans marked by a group of adults. The purpose of the test was to obtain an indication of marking on beans. Petri dishes (9 × 2 cm) were divided into 4 sectors by paper walls (87 × 7 mm) attached by melted paraffin wax. PM- and UM-beans (24 in each) were put into 2 opposite sectors.

Exp. 2. Oviposition on beans marked by virgin and mated, ♂♂ and ♀♀, resp. Egg-laying cylinders of 13 × 11 cm were prepared. Test insects were collected daily and stored at 6°. Altogether ca. 2100, 1-7-day-old ♂♂ and ♀♀ were allowed to mate for 1 day (a period adequate for the majority of ♀♀ to be inseminated) at 26°, then cooled down to 6°, sexed again, and confined in egg-laying cylinders with 160 g beans at 26°. Honey water was provided as food. Approx. the same number of virgin ♀♀ and ♂♂ received the same treatments (cooling, etc.) except that they were always kept separate. The 4 groups of adults were allowed to remain on the beans for 5 days. Beans from the cylinders were tested in choice tests as described above. Prior to the tests any eggs on the surface of the beans were removed.

Exp. 3. Oviposition on surface rinses of PM-beans. Samples of beans collected over 3 months from egg-laying cylinders and stored in a refrigerator at −30°, as well as UM-beans (1570 g each) were washed either with 200 ml n-hexane (purified by vacuum dist.) or with the same quantity of dist. water, for 30 sec. The solutions were sifted, centrifuged at 6000 rpm for 5 min, then stored at −30° and +5°, resp.

As the solutions had to be stored (for technical reasons) for ca. 1 month, just prior to the experiment fresh rinses of beans, pheromone marked for 5 days, were also prepared. The rinses were applied at a dosage equivalent to 8 g PM-beans per Petri-dish sector on fresh seeds, as this quantity had already proved to be quite effective in Exp. 1. Here also choice tests were used.

Exp. 4. Responses of ♀♀ to extracts of virgin and mated, ♂♂ and ♀♀. The four kinds of adults, 5—10 days old (no. = 1017 ± 58), were washed with n-hexane (purified) at room temperature. The extracts were stored at −30° until use. In choice tests, beans treated with 1, 10 and 100 adult equivalents (AE) of the extracts were tested.

Exp. 5. Response of newly hatched larvae to PM-beans. In 4 separate series of Petri dishes (9 × 2 cm) 90 beans of the same variety and age were placed as follows: (1) PM-beans only, (2) UM-beans only, (3) PM-beans and UM-beans in 2 sectors, but not isolated by paper walls, and (4) PM- and UM-beans mixed randomly. Consequently, variations (1) and (2) are no-choice types, while (3) and (4) are choice experiments. Over the beans, 836 ± 157 newly laid eggs per Petri dish were distributed randomly. After hatching of the larvae the percentage of egg mortality was determined. Besides this, other factors (see Table IV), considered to be indicators of the effects of PM-beans, were also evaluated. To determine the number of bore holes per bean by first-instar larvae (L_1), all beans were thoroughly examined. The % of L_1, dying before entering the beans was established for each Petri dish or dish-sector. After terminating the tests all the beans were dissected and dead insects were counted.

Exp. 6. Observations on behaviour of both sexes on beans. ♂♂ and ♀♀ (23 altogether), mated, 3 to 4 days old, and deprived of egg-laying (kept without beans) at 23° and 75% r.h., were put individually into vials (5 × 2.5 cm) containing a single bean. The exploratory and host-acceptance activities of adults were closely followed through a binocular microscope from the first contact with the seed till depositon of the first 3 to 4 eggs or for ca. 45 min. Each seed and adult was used only once.

Exp. 7. Experiment to exclude the role of microorganismic activities. Ca. 2800 mated ♂♂ and ♀♀ (see the method at Exp. 2), 4 days old and deprived of egg-laying sites during this period, were allowed to visit and mark 207 g beans for 2 hr only. Immediately afterwards, all the eggs deposited on the beans were removed and then 6.4 ± 0.3 (S.D.) g seeds/Petri dish sector or vial were tested in choice and no-choice tests, resp. Each test lasted 6 hr. For the choice test, 10 ♂♂ and 30 ♀♀ per Petri dish and for the no-choice tests, 5 ♂♂ and 15 ♀♀ were used. In the latter, PM- and UM-beans were placed in separate vials. Tests conditions were: total darkness, 23° and 85% r.h. After terminating the test, the number of eggs deposited was counted. No. of replicates: 9.

RESULTS

In Exp. 1. ovipositing ♀♀ showed a strong preference for UM-beans, the DQ value was + 71.6 ± 7.3 (S.D.)%, i.e., oviposition was inhibited on PM-beans. There was an average of 26.8 ± 5.3 (S.D.) eggs laid per ♀ on UM- and 4.3 ± 0.6 on PM-beans.

In Exp. 2 ovipositing ♀♀ again definitely preferred UM-beans, and preferred those marked by either virgin or mated ♀♀ to those marked by either virgin or mated ♂♂ (Table I). DQ values differed substantially from each other, and though significance tests could not be applied, differences between S.D.-s may indicate reliability.

Distilled water rinses of PM-beans (Exp. 3), either stored or freshly prepared, conferred a considerable level of deterrence on treated beans. The rinse made with n-hexane produced ca. half as much response (Table II).

There is some indication that at high doses extracts from adults are slightly deterrent. However, seemingly even 100 AE was not high enough to give a definite inhibition response. At 1 AE there was a weak stimulative effect by the extract of mated ♂♂ (Table III).

Newly hatched larvae preferred UM-beans and made significantly (P = 0.1%) more bore holes in the no-choice situations (Table IV, Exp. 5). In choice tests similar preference and difference between the numbers of holes on PM- and UM-beans were noted. The % of L_1 dead per Petri dish was significantly (P = 0.1%) higher in the presence of PM-beans, while in choice tests it was lower. Significantly (P = 0.5%) more adults emerged from UM-beans. By contrast, there was no significant difference between PM- and UM-beans in % of dead instars inside the beans (other than L_1). There was a minimum 3.3 ± 0.6 % and a maximum 3.6 ± 0.6 % of egg-mortality in the

TABLE I

Oviposition response of naive A. obtectus ♀♀ *(in pairs with ♂♂) to beans pheromone-marked by adults of different sexes and reproductive state. 10 replicates*

Sex and no. of adults marked		No. eggs laid by ♀♀ UM-beans	PM-beans	Discrimination quotient (%)
V[1]♂	1059	55.0 ± 7.6[3]	3.4 ± 1.6	+ 88.3 ± 5.0
V ♀	957	53.1 ± 7.5	14.7 ± 8.0	+ 58.0 + 17.5
M[2]♂	1074	57.8 ± 8.4	4.4 ± 3.6	+ 85.9 ± 11.4
M ♀	978	48.4 ± 8.9	15.5 ± 5.7	+ 51.4 ± 14.9

[1] V = virgin; [2] M = mated; [3] S.D.

TABLE II

Oviposition response of naive A. obtectus ♀♀ *(in pairs with ♂♂) to surface rinses of pheromone-marked beans in choice test. 7 replicates*

Surface rinse		No. eggs laid per ♀	Discrimination quotient (%)
Solvent: dist. water			
Beans:	UM[1]	55.8 ± 6.2[3]	+ 61.5 ± 5.8
	PM[1]	13.5 ± 3.6	
	UM[2]	63.6 ± 11.6	+ 78.2 ± 6.1
	PM[2]	7.7 ± 2.1	
Solvent: n-hexane			
Beans:	UM[1]	50.0 ± 5.1	+ 32.8 ± 7.7
	PM[1]	25.3 ± 3.7	

[1] Stored at + 5° or − 30° for ca. one month; [2] Prepared freshly; [3] S.D.

four variants, and they were not significantly different.

No identified "marking behaviour" was observed in Exp. 6 with either ♂♂ or ♀♀, while on beans, nor was excretion or deposition of any specific substances noted. However, touching of the surface of beans with the slightly extruded ovipositor or with the tip of it, did occur with ♀♀ before and after egg-laying.

In Exp. 7, a test designed to exclude the role of micro-organismic activities on PM-beans, both in choice and no-choice tests, there was an oviposition depressing effect noted on freshly marked PM-beans. In the choice test, ♀♀ preferred laying eggs on UM-beans (129.7 ± 36.2 eggs vs. 50.1 ± 18.4). The DQ value was + 43.5 ± 17.3%. In independent treatments, ♀♀ laid significantly (P = 0.5%, t_{cal} = 3.352, DF = 16) more eggs also on UM-beans (148.3 ± 46.2 eggs vs. 86.2 ± 30.8).

DISCUSSION

It is clearly demonstrated that *A. obtectus* adults deposit chemicals on beans, both whilst ovipositing and just moving on them. Beans attended by the adults highly deterred new ♀♀

TABLE III

Oviposition response of naive A. obtectus ♀♀ *(in pairs with ♂♂) to n-hexane extracts from adults of different sexes and reproductive states. 7 replicates. AE = Adult equivalent*

Extract[1] Dose:	Discrimination quotient (%) 100 AE	10 AE	1 AE
Virgin ♂♂	+ 6.6 ± 12.8[2]	+ 12.1 ± 17.8	+ 10.4 ± 20.8
Virgin ♀♀	+ 6.5 ± 15.1	+ 2.0 ± 26.2	− 1.9 ± 14.3
Mated ♂♂	+ 2.7 ± 22.7	+ 0.4 ± 22.2	− 22.4 ± 7.7
Mated ♀♀	+ 15.5 ± 10.9	− 7.2 ± 15.7	+ 17.7 ± 16.0

[1] Made of 1017 ± 58 adults; [2] S.D.

TABLE IV

Responses of first-instar larvae of A. obtectus *to pheromome-marked beans. 5 replicates*

Quality of beans Petri dish	No. (± S.D.) L_1 bore holes per seed	% (± S.D.) mortality of L_1 before entering the beans	% (± S.D.) mortality of other instars inside beans	% (± S.D.) adult emergence
1. UM only	0.9 ± 0.1a	15.0 ± 4.8c	2.6 ± 0.6e	61.6 ± 11.5f
2. PM only	0.5 ± 0.1b	26.8 ± 6.2d	1.6 ± 1.0e	34.5 ± 9.3g
3. UM and PM in 2 sectors each	0.9 ± 0.2 0.4 ± 0.1	8.7 ± 1.5 2.6 ± 0.1	2.3 ± 1.2 0.6 ± 0.7	48.5 ± 3.4 17.3 ± 4.4
4. UM and PM randomized	1.0 ± 0.1 0.5 ± 0.1	17.6 ± 3.2	1.3 ± 1.0 0.3 ± 0.3	38.9 ± 6.4 13.3 ± 3.3

1 and 2 are no-choice, 3 and 4 are choice experiments. a–b: P = 0,1%; f–g: 0.5%; c–d: 1.0%; e–e: not significant.

from repeated egg-laying. Our finding is in contradiction with that of Umeya & Kato (1970), who found that 200 beans conditioned for 24 hr by 200 ♂♂ and ♀♀ separately did not hinder further egg-laying of 3 or 10 pairs of adults.

Further, substance(s) deposited on the surface of beans can be washed off by either aqua dist. or n-hexane. It is also reasonable to suppose that among the active constituents the majority are polar. Similarly, with tephritids such substances were also found to be water-soluble (Katsoyannos, 1975).

Regardless of sex and reproductive state (i.e., virgin or mated) adults may produce substance(s) having similar effects but different levels of inhibiting influence on oviposition (Tables I, III). It should be noted that: (1) the substances are not necessarily the same with ♂♂ and ♀♀; (2) it is not unambiguous that the chemicals involved are used for oviposition-deterring "purposes" only; (3) prior to and after mating the nature of the chemicals does not seem to change. The level of oviposition inhibition by substances deposited either by mated or virgin ♀♀, was not as high as in the case of beans marked by ♂♂, but still significant deterrence occurred. By contrast, Oshima *et al.* (1973) found greater activity with an extract obtained from ♀♀ of *Callosobruchus chinensis* L. than from ♂♂.

First-instar larvae also differentiated between PM- and UM-beans. Labeyrie (1960) found that the distribution of bore holes by L_1s on uncontaminated dry beans was totally random. Therefore, the preference for entering UM-beans in choice tests must have been elicited by a factor present on the surface of PM-beans (Table IV). Egg-mortality values showed that substance(s) on PM-seeds did not affect egg-hatch. The differences in the number of bore holes per bean-seed were not due to differential egg-mortality. The % mortality in L_1s, however, was different in PM- or UM-beans, i.e., in the no-choice situation the wandering period must have been longer, while in choice tests few larvae remained in PM-sectors and aggregated more under UM-beans. We consider that marking substance(s) increased larval mortality, no matter how indirect the process was. Thus, the young larvae must have kept moving around as a result of the deterring effect, which in turn enhanced the rate of mortality, probably by increasing the risk of desiccation. Marking substance(s) did not interfere with development inside the beans. The number of adults emerged merely reflected the preference by L_1s.

The egg-laying habits of *A. obtectus* ♀♀ and larval behaviour together make it possible for newly hatched larvae to avoid marked beans at low population levels. The majority of eggs are usually laid in clusters under single seeds or nearby (Umeya & Kato, 1970). Larvae are not obliged to enter the beans on or under which egg-laying occurred; they possess legs and actively move among the beans. The oviposition and larval characters are thought to be more ancestral ones (Pfaffenberger & Johnson, 1976) than the egg-laying habits of some other species, where eggs are mostly placed singly on beans at low adult density, and hatching larvae enter the beans directly (e.g., *C. maculatus* F.; Mitchell, 1975). Thus, by detecting substances on beans, both larvae and adults of *A. obtectus* may confer benefit by dispersal, as they produce a more even exploitation of beans, supposing that avoidance of larval competition is advantageous for the population. With *A. obtectus* at least, such marking substances may have more than one function, because they influence larval dispersal, too.

The lack of a conspicuous "marking behaviour" by adults (Exp. 6) suggests other ways of marking, e.g., through defecating on beans. It seems likely that the primary source of marking material is, or can be found among the defecated substances.

It could be supposed also that the oviposition-deterring effect was the results of growth of microorganisms on the deposited substance(s). However, a test (Exp. 7) carried out within a reasonably short-time period showed that a 2-hr marking and a 6-hr testing resulted in a significantly decreased oviposition response by naive ♀♀. This indicated that the effect is not the consequence of microbial activity.

It was shown with tephritid fruit fly species that the effect of deterring pheromones depended on several population characters, like density, on availability of food for larvae and on the oviposition drive of ♀♀ (Prokopy, 1972, 1977, 1981). Such substances were usually effective only at low density.

There are some features in the population and behavioural ecology of *A. obtectus* adults, which, we feel, one should also take into account when considering the origin, use and significance of substance(s) deposited on beans. Firstly, one can consider the pre- and post-copulatory behaviour of *A. obtectus*. Both ♂♂ and ♀♀ are continuously present at mating and egg-laying sites, at least in stored beans. Males permanently produce an allenic ester (Horler, 1970), which appears to be an aphrodisiac-type pheromone and supposedly influences receptivity of ♀♀ to mate (Halstead, 1973). He also found the ester to be extremely stable in hexane or benzene solutions. Therefore, together with a real, water-soluble oviposition deterring pheromone produced by the ♀♀, there are at least two different substances present, which, depending on ratios and concentratons might have multiple functions. Secondly, polyphagous insects also deposit oviposition-deterring pheromones (Prokopy, 1981); consequently, the use of such substances must be correlated with the size of a given unit of food and only to a lesser extent with the degree of food specialization. Still, the evolutionary significance of oviposition-marking substances seems to be more evident in the case of mono- or almost monophagous species (Prokopy *et al.*, 1977). *A. obtectus* is an oligophagous species. This character theoretically decreases the importance of marking, especially if alternative food plants were simultaneously present. However, both in field and store conditions in Central Europe, infestation is largely restricted to beans; therefore, the significance of deterring substances might be considerable. Thirdly, at high infestation levels, numerous bean weevil larvae can develop in a single seed, though they also show an aggregative behaviour in the colonization of seeds (Labeyrie, 1965). This does not suggest that the effects of larval competition are severe. Such circumstances may also indicate an increased threshold of sensitivity to substances present in this species.

It is also worth mentioning that, unlike many other phytophagous insect species in the temperate zone, the bean weevil usually lives in stored beans, i.e. in limited quantities of seeds removed from the pods. The available food will then eventually be completely used up and only empty seed-shells will remain. The adults move almost continuously among the beans and during walking and egg-laying they probably defecate (mark) all beans present even if only a portion would actually still support developing larvae. In this way, the adults seem to produce a "super territory" (Verner, 1977) during early stage of colonization. As the population increases, the self-deterring effect should be more and more influential. What kind of advantage could the population, as a whole, enjoy by marking in such a situation? One can suppose that the deterring pheromone, at low population levels, provides a more or less even distriburion of eggs and L_1 only, while at high abundace, it would also initiate migration of adults and help them to discover new food sources. We are not going to give the impression that the oviposition marking substrate of the bean weevil is the adaptive consequence of life in stores. The possible importance of the same behaviour in the field is also emphasized; however, there are only speculations available here. Oviposition-site marking may regulate infestation patterns and decrease the probability of overloading bean pods with eggs, especially in marginal zones of bean fields when the beans intercrop with maize, where the highest infestations are usually found (Labeyrie & Maison, 1954; Szentesi & Pálfi, 1975).

The author thanks R. J. Prokopy and V. Labeyrie for criticism on an earlier version; the members of the Zoology Department of the Research Institute for Plant Protection, Budapest, Hungary, and especially Dr. T. Jermy for comments and Mrs. A. Mydló for technical assistance.

RÉSUMÉ

Comportement de ponte d' Acanthoscelides obtectus: *on évidence de l'existence d'une pheromone répulsive et discussion de ses fonctions*

Les mâles et les femelles d'*A. obtectus* déposent des substance(s) encore non-identifiées sur les grains du haricot au cours de leurs diverses activités. Ce comportement de marquage fait que les autres femelles hésitent à pondre des œufs sur ces grains. De plus, les substance(s) déposée(s) poussent les larves du 1er stade à se disperser plus vigoureusement, par conséquent, elles sont enclines positivement à entrer dans les grains sans marque. On n'a observé aucun comportement marqueur spécifique chez les adultes. La possibilité d'une origine microbiologique des substance(s) répulsive(s) a été eliminée. Les origines possibles des substance(s) de marquage et la signification de ce comportement sont discutés.

REFERENCES

David, J. & Van Herrewege, J. (1970). Choix d'un site de ponte chez *Drosophila melanogaster*: Technique d'étude et variabilité. *Rev. Comp. Anim.* **4** : 82—84.

Halstead, D. G. H. (1973). Preliminary biological studies on the pheromone produced by male *Acanthoscelides obtectus* Say (Col., Bruchidae). *J. stored Prod. Res.* **9** : 109—117.

Hope, J. A., Horler D. F. & Rowlands, D. G. (1967). A possible pheromone of the bruchid *Acanthoscelides obtectus* Say. *J. stored Prod. Res.* **3** : 387—388.

Horler, D. F. (1970). (-)-Methyl n-tetradeca-trans-2, 4, 5-trienoate an allenic ester produced by the male-dried bean beetle, *Acanthoscelides obtectus* Say. *J. Chem. Soc. C,* 859—862.

Jávor I. (1970). Babzsizsik. The bean weevil. In: Növényvédelmi Karantén Kézikönyv, [*Handbook of Quanratine Plant Protection*], D. M. Sebestyén & M. Várady (Eds.). Mezögazd. Kk., Budapest, 155—163 (in Hungarian).

Katsoyannos, B. I. (1975). Oviposition-deterring male arresting fruit marking pheromone in *Rhagoletis cerasi. Environ. Entomol.* **4** : 801—807.

Labeyrie, V. (1960). Nature de la répartition des larves d'*Acanthoscelides obtectus* Say. *C. R. Acad. Sc.* **251** : 149—151.

Labeyrie, V. (1965). Etude de la répartition des larves néonates d'*Acanthoscelides obtectus* Say. *Proc. XII Int. Congr. Ent.*, London, 1964, 310—311.

Labeyrie, V. & Maison, P. (1954). De l'influence du microclimat sur la ponte de la bruche du haricot (*Acanthoscelides obsoletus* Say.) dans la nature. *A.A.F. Extr. Séance, 733.*

Mitchell, B. (1975). The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). *Ecology,* **56** : 696—702.

Muschinek, G., Szentesi, Á. & Jermy, T. (1976). Inhibition of oviposition in the bean weevil (*Acanthoscelides obtectus* Say, Col., Bruchidae). *Acta Phytophathol. Acad. Sci. Hung.* **11** : 91—98.

Oshima, K., Honda, H. & Yamamoto, I. (1973). Isolation of an oviposition marker from azuki bean weevil, *Callosobruchus chinensis* (L.). *Agr. Biol. Chem.* **37** : 2679—2680.

Pfaffenberger, G. S. & Johnson, C. D. (1976). Biosystematics of the first-stage larvae of some North American Bruchidae (Coleoptera). *USDA Techn. Bull.* **1525** : 75.

Prokopy, R. J. (1972). Evidence for a marking pheromone deterring repeated oviposition in apple maggot flies (*Rhagoletis pomonella*, Dipt., Tephritidae). *Environ, Entomol.* **1** : 326—332.

Prokopy, R. J. (1975). Oviposition-deterring fruit marking pheromone in *Rhagoletis fausta. Environ. Entomol.* **4** : 298—300.

Prokopy, R. J. (1977). Stimuli influencing trophic relations in Tephritidae. *Coll. Int. C.N.R.S.* **265** : 305—334.

Prokopy, R. J. (1981). Epideictic pheromones regulating spacing patterns of phytophagous insects. In: *Semiochemicals: Their Role in Pest Control.* D. A., Nordlund, R. L. Jones, W. J. Lewis (Eds.) Wiley, New York (in press).

Prokopy, R. J., Greany, P. D. & Chambers, D. L. (1977). Oviposition-deterring pheromone in *Anastrepha suspensa. Environ. Entomol.* **6** : 463—465.

Salt, G. (1937). Eperimental studies in insect parasitism. V. The sense used by *Trichogramma* to distinguish between parasitized and unparasitized hosts. *Proc. Roy. Soc. London*, B, **122** : 57—75.

Sandner, H. (1962). Badania nar rola i mechanizmem dzialania czynnika zageszczenia w populacjach strakowca fasolowego *Acanthoscelides obtectus* Say). (Studies on the role of the density factor and the manner of its action on populations of *A. obtectus.*) *Ekol. Pols.*, B, **8** : 179—186 (in Polish).

Szentesi, Á. (1972). Studies on the mass rearing of *Acanthoscelides obtectus* Say (Col., Bruchidae). *Acta Phytopathol. Acad. Sci. Hung.* **7** : 453—463.

Szentesi, Á., Jermy, T. & Dobrovolszky A. (1973). Mathematical method for the determination of sterile insect population competitiveness. *Acta Phytopathol. Acad. Sci. Hung.* **8** : 185-191.

Szentesi, Á. & Pálfi, D. (1975). Szabadföldi védekezési kisérletek a babzsizsik ellen (*Acanthoscelides obtectus* Say, Col., Bruchidae), steril imágók felhasználásával. Field experiments on the control of the bean weevil by the release of sterile adults. *Növénytermelés,* **24** : 249—257 (in Hungarian).

Szűcs, J. (1959). A. babzsizsik (*Acanthoscelides obtectus* Say) Magyarországon. The bean weevil in Hungary. *Bács-Kiskun megyei Nyomda, Kecskemét*, 32 (in Hungarian).

Umeya, K. (1966). Studies on the comparative ecology of bean weevils. I. On the egg distribution and the oviposition behaviours of three species of bean weevils infesting azuki beans. *Res. Bull. Pl. Prot. Japan,* **3** : 1—11.

Umeya, K. & Kato, T. (1970). Studies on the comparative ecology of bean weevils. V. Distribution of eggs and larvae of *Acanthoscelides obtectus* in relation to its oviposition and boring behaviour. *Res. Pop. Ecol.* **12** : 35—50.

Utida, S. (1943). Studies on experimental populations of the azuki bean weevil, *Callosobruchus chinensis* (L.). VIII. Statistical analysis of the frequency distribution of the emerging weevils on beans. *Mem. Coll. Agric. Kyoto Imp. Univ.* **54** : 1—22.

Verner, J. (1977). On the adaptive significance of territoriality. *Amer. Nat.* **111** : 769—775.

Yamanoto, I. (1974). Approaches to insect control based on chemical ecology-case studies. In: *Environmental Quality and Safety*. Vol. 5. *Global Aspects of Chemistry, Toxiocology and Technology as Applied to the Environment.* F. Coulston, & F. Korte (Eds.). Georg Thieme Publ., Stuttgart, 73—77.

Yoshida, T. (1961). Oviposition behaviors of two species of bean weevils and interspecific competition between them. *Mem. Fac. Lib. Arts. Educ., Miyazaki Univ.* **11** : 41—65.

Accepted: April 4, 1981.