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MATERIALS ATTRACTIVE OR REPELLENT TO *LAR VAE OF TROGODERMA GRANARIUM*

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ABS T R A C T

Attraction and repulsion of the khapra beetle larvae to various foods, vitamins and organic compounds was tested in an apparatus that allowed the larvae to choose between a material and control. The most attractive foods were sesame seeds, fish meal, almonds and peanuts; most other foods tested were also attractive. Ether extract of sesame seeds, allyl alcohol and paraldehyde were highly attractive. The most attractive vitamin was choline chloride. Most of the fatty acids and 2 esters: ethyl adipate and ra-butyl acetate as well as 7 out of 191 synthetic compounds tested were attractive. A number of compounds were repulsive.

INTRODUCTION

The khapra beetle larvae is destructive to a wide variety of foods in many parts of the world. It is not easily killed by cold or starvation, and is very difficult to detect in incipient infestations. This study was undertaken to examine the behavior of the larvae towards various compounds and foods for the purpose of finding good attrac- tants to the larvae that may improve the effectiveness of routine in- spections and control or the eventual development of a repellent. Since *Trogoderma* adults live only a short time and are not known to feed, all references to food habits are concerned with larvae. Very little work has been done on the attraction of the khapra beetle larvae, as well as other stored product insects, to various compounds and foods. Spangler (1965) found that fish meal was attractive to the khapra

beetle larvae while alcohols containing from 3 to 10 carbons were repellent. Stanic et al. (1973) found that the larvae of the khapra beetle were attracted by the acetates of *cis*-5,-7, and -9-tetradecen-*1*-ols and were repelled by the acetates of *cis* and -7-hexadecen-1-ols, 8 pentyloxy-1-actanol and *cis*-4-dodecen-1-ol. Finger et al. (1965) have shown that traces left by crawling larvae of the khapra beetle lure other larvae of the same species more than various forms of wheat tested.

MATERIALS AND METHODS

The rearing medium of the khapra beetle consisted of 200 gr of semolina and 2% dry Brewer's yeast. It was placed in a glass container (21 cm in diameter) and about 50 newly emerged adults were added and left for 2-3 days,. About 600–800 larvae were thus obtained in each container. When about 1/3 of the larvae had pupated, the medium was sifted and the retained larvae and pupae were put on a circular metal sheet (18 cm in diameter) which was placed in the culture container a few cm above its bottom. An electric light above the container caused the larvae to move and fall into the container, whereas the pupae which remained on the metal sheet were placed in containers for emergence. This procedure was repeated 2 or 3 times at intervals of a few days.

Apparatus. The apparatus used for studying attraction or repulsion is a modification of the apparatus used by Laudani and Swank (1954) which is a modification of an apparatus used by Loschavio (1952). It consists of a circular brass plate (50 cm in diameter) having a 7 cm brass rim. The plate, which has been ground by a strong spray of sand to roughen it a little for facilitating the larvae to walk on it, has 12 holes (3.5 cm in diameter) equidistantly spaced along the outer edge, like the numbers of a watch. A petri dish (5 cm in diameter and 1.4 cm high) can be attached and removed below each hole with the aid of a spring. This setup is placed in a room of constant temperature (29°C $\pm$ 1°C) inside a tin box (65 x 65 x 30 cm) painted black in the inside and the top of which can be removed. The top, which has an edge of 7 cm, does not close tightly but leaves a space about 1 cm around the edge of the top of the box. This permits air to circulate freely in and out of the box. To insure complete darkness, the box is covered with a black cloth.

Test method. About 200 2nd or 3rd instar larvae which had been starved for 1 to 2 weeks were placed in the center of the arena inside a glass ring (3–5 cm in diameter) for half an hour, after which the ring was removed to enable the larvae to move freely in the arena. Larvae that entered a petri dish could not get out. After 2½ hours (including the half hour of preconditioning) the number of larvae in each petri dish was counted. In each test the 12 petri dishes below the holes of the brass plate were used. In 6 of them the test material was placed and the other 6, in alternate positions, were empty (control). Thus, in any one test 6 replicates were carried out. This procedure was repeated at least twice. After each test the brass plate was washed with acetone to remove any possible traces of the larvae.

An Index of Reaction (I.R.) is calculated from the formula 100(M–C)/N where M and C are the average number of larvae in the material and control respectively and N the total number of larvae in the material and control. A positive number and its magnitude (the maximum is 100) indicates attraction and its relative attractiveness. Similarly, a negative value (the maximum is - 100) indicates repulsion. Over 95% of the larvae entered the petri dishes at the end of an experiment (2½ hours).

Materials tested

1. *Food materials.* All the food materials that were tested are given in Table 1. Those that were not in the form of meal or powder were coarsely ground.

2. *Vitamins.* All the vitamins tested are given in Table 1.

3. *Organic compounds.* Many of the organic compounds tested were chosen because of their attractiveness to various insects (Green et al., 1960; Lehman, 1932). The following is a list of the compounds tested. In Table 2 only those compounds that were found attractive are given. Those which were highly repulsive are given in a separate list in the Results.
   - *Acids.* acetic acid, *n*-butyric acid, capric acid, caproic acid, isobutyric acid, lactic acid, megatomic acid, propionic acid. *Alcohols.* allyl aclcohol, *n*-butanol, isoamyl alcohol, 2-methyl-3-butyn 2-ol, 2-methylcycolhexanol.
   - *Aldehydes.* acetaldehyde, anisaldehyde,, benzaldehyde, butyraldehyde, furfural, paraformaldehyde, paraldehyde, 2-phenyl propanal, propionaldehyde, *n*-valeraldehyde.

- *Amines.* Diethylamine.
- *Amino acids.* DL-alanine, L-arginine, L-asparagine, L-cysteine, L-citruline, cystine, glutamic acid, glutamine, glycine, histidine, Ls-hydroxyproline, L-isoleucine, L-leucine, L-lysine · HCl, L-methionine, ornithine, L-phenylalanine, L-proline, L-serine, threonine, L-tryptophan, L-tyrosine, DL-valine.
- *Esters.* benzyl acetate, *n*-butyl acetate, ethyl benzoate, ethyl acetate, ethyl adipate, ethyl cyanoacetate, ethyl formate, ethyl levulinate, iso-amyl acetate, methyl anthranilate, methyl caproate, methyl laurate, methyl myristate, methyl palmitate, phenylalanine methyl ester, vinyl acetate.
- *Ethers.* Anisol.
- *Fats.* tripalmitin, tristearin.
- *Fatty acids.* caproic acid, caprylic acid, lauric acid, linolenic acid, myristic acid, oleic acid, palmitic acid, stearic acid, undecyleric acid, valeric acid.
- *Hydrocarbons.* cumene, *o*-cymene, isoheptene, kerosine, *a*-methylstyrene monomer, turpentine.
- *Ketones.* cyclopentanone, diacetyl, ethyl methyl ketone, ethyl pentanone.
- *Miscellaneous.* acetic anhydride, allyl isothiocyanate, caseine hydrolisate, dimethyl acetamid, eugenol, lactolbumin hydrolisate, malonaldehyde bis dimethyl acetal, nitromethane, phenothiazine, urea, yeast extract.

4. *Synthetic compounds* (Obtained from the USDA). One hundred and ninety-one synthetic compounds obtained from the USDA were tested. Only those that were attractive are given in Table 2. Some were repulsive (see Results). A complete list of all the synthetic compounds tested can be obtained on request from the author or looked up in a Final Research Report (Bar-Zeev, 1975) to the USDA.

RESULTS AND DISCUSSION

Results on the effect of various foods and vitamins are given in Table 1 in descending order of attractiveness. Most of the foods tested were attractive; carob meal, however, was repulsive. The most

A. Foods

Amount of food per dish 1 gr. Twenty-four replicates.

Food	Av. no. of larvae per dish ± stand. dev.		Index of Reaction (I.R.)
	in food	In Control	
Sesame seeds	37.1 ± 29.3	5.2 ± 2.6	75.4**
Fish meal	31.0 ± 15.2	4.4 ± 3.3	75.1**
Almonds	26.3 ± 9.8	7.0 ± 3.1	57.9**
Peanuts	27.1 ± 14.2	10.5 ± 6.7	44.1**
Pecan nuts	22.2 ± 5.9	10.1 ± 4.2	37.4**
Wheat bran	35.7 ± 17.1	16.1 ± 9.0	37.8**
Bean	26.1 ± 14.9	13.9 ± 7.7	30.5**
Alfalfa meal	28.5 ± 15.3	16.1 ± 12.9	27.8**
Soya bean oil meal	29.4 ± 12.7	16.7 ± 7.5	27.5*
Soya bean protein	29.0 ± 11.1	16.5 ± 8.3	27.5**
Chick pea	24.3 ± 10.8	14.8 ± 7.1	24.2**
Rice	21.5 ± 8.4	14.1 ± 6.3	20.8**
Powder milk	22.4 ± 10.2	17.2 ± 10.1	13.1
Corn	17.0 ± 8.2	14.3 ± 6.8	8.6
Lentils	18.5 ± 7.1	20.4 ± 8.5	-4.8
Feather meal	18.3 ± 9.7	20.7 ± 13.5	-6.1
Carob meal	10.3 ± 7.0	21.0 ± 9.9	-34.1**

Table 1. Effect of various foods and vitamins on the larvae.

A positive I.R. (maximum 100) indicates attraction

A negative I.R. (maximum - 100) indicates repulsion

* Significant at the 5% level

** Significant at the 1% level

B. Vitamins

Amount of vitamin per dish ½ gr. Twelve replicates

Vitamin			
Choline chloride	29.1 ± 9.0	12.0 ± 5.8	41.6**
Folic acid crystalline	28.3 ± 15.6	14.3 ± 8.8	32.8*
Nicotinic acid (Niacin)	20.7 ± 10.7	11.3 ± 4.6	29.3*
L-Ascorbic acid	21.8 ± 9.3	14.4 ± 6.4	20.4*
D-Calcium pantothenate	24.6 ± 10.5	17.8 ± 8.4	16.0
Riboflavin (vitamin B_2)	19.6 ± 12.1	15.5 ± 9.1	11.6
i-Inositol	25.0 ± 17.4	21.8 ± 12.2	6.8
D-Biotin crystalline	20.0 ± 12.6	18.9 ± 12.1	2.8
Pyridoxine hydrochloride	14.0 ± 13.2	17.8 ± 9.7	-11.9

Table 1. Continued

Compound	Amt used	Av.no. of larvae per dish±stand. dev.		Index of Reaction (I.R.)
		In compound	In control	
FATTY ACIDS				
Oleic acid	0.12 ml	23.7 ± 22.4	7.0 ± 4.0	54.3*
Oleic acid	0.03 ml	19.4 ± 10.2	9.5 ± 6.9	34.2*
Undecylenic acid	0.12 ml	32.8 ± 8.1	8.6 ± 1.8	58.4**
Undecylenic acid	0.03 ml	26.3 ± 18.8	7.6 ± 4.0	55.1*
Linolenic acid	0.12 ml	24.0 ± 6.8	9.1 ± 4.2	45.0**
Linolenic acid	0.03 ml	22.1 ± 5.1	13.1 ± 4.0	25.9**
Linoleic acid	0.12 ml	21.4 ± 12.2	11.3 ± 5.8	30.8**
Linoleic acid	0.03 ml	23.8 9.3	6.6 ± 3.5	56.8**
Lauric acid	0.5 gr	23.8 ± 13.3	10.0 ± 5.6	40.8**
Stearic acid	0.5 gr	18.5 ± 6.3	11.0 ± 4.5	24.1**
Palmitic acid	0.5 gr	18.8 ± 8.1	12.0 ± 4.8	22.0*
ESTERS				
Ethyl adipate	0.12 ml	28.0 ± 8.1	8.4 ± 3.9	53.8**
Ethyl adipate	0.03 ml	18.7 ± 10.4	12.4 ± 6.2	20.2
n-Butyl acetate	0.12 ml	21.7 ± 8.3	12.0 ± 5.4	28.7**
n-Butyl acetate	0.03 ml	27.0 ± 8.0	7.5 ± 4.7	56.5**
ALCOHOLS				
Allyl alcohol	0.12 ml	28.6 ± 17.6	4.5 ± 3.0	72.8**
Allyl alcohol	0.03 ml	30.5 ± 12.6	6.0 ± 3.9	67.1**
ALDEHYDES				
Paraldehyde	0.12 ml	32.6 ± 23.7	7.1 ± 3.3	64.2**
Paraldehyde	0.03 ml	29.0 ± 10.8	7.5 ± 3.3	58.9**

TABLE 2. Verious organic compounds found attractive to the larvae (Twelve replicates)

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MISCELLANEOUS			
Malonaldehyde bis dimethyl acetal	0.12 ml 16.8 ± 7.5	10.0 ± 5.8	25.3*
"	0.03 ml 18.7 ± 6.9	12.6 ± 6.6	19.4*
Nitromethane	0.12 ml 25.6 ± 12.6	15.0 ± 7.1	26.1*
Nitromethane	0.03 ml 21.0 ± 6.7	13.2 ± 5.3	22.8**
EXTRACTS			
Ether extrac of sesame seeds Concentration: 1 ml = 8.4 gr	0.8 ml[1] 30.2 ± 8.9	4.0 ± 5.2	76.6**
	0.4 ml[1] 33.5 ± 10.3	3.3 ± 2.2	82.1**
	0.2 ml 27.1 ± 6.8	8.3 ± 4.2	53.6**
SYNTHETIC COMPOUNDS (obtained from the USDA. In parenthesis Ent. No. of USDA).			
cis-3-Hexen-1-ol (25091)	0.03 ml 28.6 ± 10.0	7.5 ± 3.7	58.4**
1-(2-Methylcyclohexane carbonyl) piperidine (35768)	0.03 ml 24.3 ± 11.0	8.5 ± 1.2	48.1**
Pentyl 2-nonynoate (35803)	0.03 ml 24.5 ± 7.3	8.7 ± 3.2	47.5**
3-Chloropropionic acid, pentamethylene ester (18793)	0.03 ml 22.8 ± 9.7	8.4 ± 5.0	46.1**
n-Hexylamine (16554)	0.03 ml 18.8 ± 8.4	7.3 ± 4.3	44.0**
Bromoacetic acid, 1-methyl-trimethylene ester (18932)	0.03 ml 25.0 ± 11.3	10.2 ± 4.7	42.0**
Phenol (1814)	10 mg 21.9 ± 13.8	9.1 ± 6.6	41.3*

[1] All the larvae which were in the petri dish in contact with the material, were dead by the end of the experiment.

Table 2. continued

attractive foods were sesame seeds and fish meal. Ether extract of sesame seeds was also very attractive. It appears that foods rich in fats (sesame seeds, fish meal, almonds, peanuts) are very attractive to the larvae. A chemical analysis of the most attractive foods and identification of the attractive compounds should be a further step in this study. Of the vitamins tested 4 were attractive; the most attractive one was choline chloride. In Table 2 are given only the organic compounds which were found attractive. Allyl alcohol and paraldehyde were highly attractive. Most of the fatty acids tested were attractive. Of the 191 synthetic compounds tested, 7 were attractive.

A number of compounds were repulsive, i.e. the average number of larvae entering the petri dishes containing the chemical was significantly lower than that of the control. It should be recalled, however, that the chemicals tested were not combined with an attractant, but were compared as such to controls since the original purpose was to search for their attractiveness. Under these conditions, a relatively weak repellent may show significant repellency. In order to determine their practical value as repellents, they should be further tested with an attractant food. The following is a list of those chemicals which were highly significantly repulsive and which gave an I.R. of -80 to -99.

Compounds repellent to the larvae giving an I.R. of -80 to -99.

The compounds are listed in descending order of repellency.

- *Organic compounds.* (Amount of material used: 0.12 ml) Furfural, anisaldehyde, 2-phenyl propanal, benzaldehyde, methyl caproate, priopionic acid, benzyl acetate, *α*-methylstyrene monomer, ethyl benzoate, cumene, methyl myristate, *n*-valeraldehyde, *o*-cymene, methyl laurate.

- *Synthetic compounds.* (Amount of material used: 0.03 ml. In parenthesis, Ent. number of USDA).

2,4-Hexadienyl acetate (32962-b), heptyl butyrate (21505), 2,4-hexadienyl butyrate (32960-e), phenethyl propionate (18544), dipentylamine (15326), hexyl pivalate (20486), ethyl 2-heptynoate (33391-b), benzyl tiglate (5784), isosafrole (2068), safrole (514), angelica seed oil (21302-XS), (E)-2-hexenyl butyrate (35967), citronellal (203), ethylheptenone (35815), 3,7-dimethyl-6-octen-1-ol (25080), citral (1011), 2,4-hexadien-1-ol (30249), (E)-2-hexenyl propionate

(35969), octyl methacrylate (8767), (Z)-3-hexenyl isobutyrate (33348-a), (Z)-3-hexenyl α-methylbutyrate (33349-a), octyl 2-methylbutyrate (33625-b), chrislure (21466-b), 2,4-hexadienyl valerate (33181-c), melanal (33278), octyl butyrate (24204), 2,4-hexadienyl hexanoate (33180-c), (Z)-3-hexenyl butyrate (33202-b), ethyl 1,4-benzodioxan-2-carboxylate (26022-b).

The khapra beetle larvae are very suitable to study attractants and repellents by the method used, due to their tendency to move and enter the petri dishes. As pointed out, 95% or more of the larvae entered the petri dishes in 2 hours. Similar experiments with *T. glabrum* were not possible because of this beetle's tendency to remain motionless.

The observations in this study indicate that the khapra beetle larvae perceive attractants and repellents from at least short distances by olfaction. It has also been observed by Stanic and Shulov (1972) that the larvae of *T. granarium* respond to olfactory stimuli. Since repellents generally act on the olfactory senses, the introduction of a repellent that has no ill effect on humans and animals into infested foods merits further studies. An effective repellent may cause the larvae to move away from infested food. This assumption is based on behavioural observations made by various investigators. *T. granarium* larvae were described to leave foods under unfavorable conditions such as high temperature, quality of food, dense population, etc. (Burges, 1959; Nutting & Gerhard, 1964; Stanic & Shulov, 1972).

Our results showed that most of the fatty acids tested were attractive to the larvae. This agrees with the findings of Stanic & Shulov (1972) that the attractive power of faeces of the larvae is mainly due to their fatty acid content. It is interesting to note that oleic acid, which was among the most attractive fatty acids (Table 2), was also found attractive to the adults (Cohen et al., 1974). The organic compounds that were attractive (Table 2) were more effective at the larger amounts (0.12 ml) than at the lower (0.03 ml) except for 2 compounds, linoleic acid and *n*-butyl acetate, which were more attractive at the lower amounts. Some attractants are known to become less attractive or even repulsive at high concentrations (Dethier, 1947).

Seven of the synthetic compounds were shown to be attractive to the larvae (Table 2). No common chemical structure can be observed in these compounds. They may, however, serve as leads for the development of future better synthetic attractants. It is noteworthy

that a relatively large number (about 15%) of the synthetic chemicals were highly repulsive to the larvae.

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