ATTRACTION OF LEAF ALCOHOL AND SOME ALIPHATIC ALCOHOLS TO THE ADULT AND LARVA OF THE VEGETABLE WEEVIL

STUDIES ON THE HOST PLANT DETERMINATION OF THE LEAF-FEEDING INSECTS V

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Leaf alcohol (β-γ-hexenol), as similar as leaf aldehyde (α-β-hexenal), possessing a fresh green leaf odor, is an important volatile compound which is present in many types of green leaves, herbs, and grasses (Takei, Sakato, Ōno & Kuroiwa 1938, Guenther 1949). Nevertheless, few attempts have been made to test the attraction of the compound to insects. As far as is known, the reports by Watanabe (1958, 1959) that the larvae of silkworm (*Bombyx mori* L.) and *Bombyx mandarina* Butler were attracted by leaf alcohol and leaf aldehyde are the first and only literatures hitherto on the attraction of these compounds.

In our previous papers (Sugiyama & Matsumoto 1957, 1959 a, b), it was shown that the newly-hatched larvae of the vegetable weevil (*Listroderes costirostris obliqus* Klug), which have a many of their host plant in Cruciferae and Umbelliferae, were attracted by mustard oils and umbelliferous essential oils and their principal constituents. But, in taking consideration of the fact that the present weevil is a potencially polyphagous insect, attacking many plants of more than about 30 families, 150 species (Table 1), it is quite conceivable that the weevil

Table 1. Food plants of the vegetable weevil

Compositae	45	Chenopodiaceae	4	Rosaceae	2	Crassulaceae	1
Cruciferae	22	Scrophulariaecae	4	Tropaeolaceae	1	Polemoniaceae	1
Umbelliferae	13	Oxalidaceae	3	Piperaceae	1	Papaveraceae	1
Labiatae	8	Oenotheraceae	3	Malvaceae	1	Violaceae	1
Leguminosae	8	Geraniaceae	2	Portulacaceae	1	Urticaceae	1
Polygonaceae	8	Plantaginaceae	2	Amaranthaceae	1	Loganiaceae	1
Caryophyllaceae	8	Convolvulaceae	2	Verbenaceae	1	Caricaceae	1
Solanaceae	7	Moraceae	2	Primulaceae	1		
Liliaceae	5	Cucurbitaceae	2	Borraginaceae	1		

Total: 34 families, 165 species

(Others unidentified: 8 species)

might be attracted by leaf alcohol and leaf aldehyde which are considered as a basic odor of green plants.

Fortunately the sample of leaf alcohol was generously supplied by Professor Ōno, and the author had the opportunity to experiment on attractiveness of the compound to the weevil. This paper reports on the attraction of leaf alcohol and some other aliphatic alcohols to the adult and larva of the weevil.

MATERIALS AND METHODS

Materials. ——— Two samples of leaf alcohol, one extracted from a natural source and the other synthesized (bp. 157°C, N_D^{20} 1.4419), were used for this experiment. In the preliminary experiments, leaf disc of 'Karasina' (*Brassica cernua* Boiss. : a Japanese form of black mustard) (1cm in diameter) and its juice (0.125cc) and water (0.125cc) were taken as test materials.

Larvae used were hatched within 24 hours prior to test under the conditions of 25°C, R.H. 100%, and darkness.

Adults which emerged at 20°C under normal-daylight on the day preceding testing, were held for about 24 hours with access to water, but not to food, in the dark at 20°C. Then from one to two hours just before test, they were transferred to dried Petri dishes in the light in order to increase their activity.

Testing method for adult. ——— The adults of the vegetable weevil have a strong thigmotactic behavior, i.e., when the adults are set free in any kind of container, they walk along the corner line of the container without fail. By making good use of this behavior, the author deviced the following simple method suitable for examination of attraction. Two watch glasses (6 or 9cm in diameter) were jointed together, making a shape of convex-lens. This convex-lens type container was placed horizontally, 40cm right under a fluorescent lamp. At the center of the container, a piece of filter paper (2 × 2cm) was placed and added by a small amount of test sample with a micro-pipette. As soon as one adult was released in this container, it began circular movement by its strong thigmotaxis along the contact line of upper and lower watch glasses. After a short time, the adult attacked the piece of the filter paper. This attacking behavior was observed for 6 or 9 minutes, and the time necessary for arrival of adult at the piece of filter paper was recorded as responding time with a stop watch. All of tests were run at 20°C.

The reason why the watch glass was used as the container in place of the common Petri dish is that the adults fall on their back frequently from the glass wall, which they climb up by their marked geotaxis and phototaxis, if they are set free in the Petri dish. Their climbing is checked by coating the glass wall with liquid paraffin, but in the present experiment it is not very satisfactory to use such chemical substance.

This simple method may be useful to olfaction test of other gressorial insects of medium or large size, such as cock-roach, cricket, cucurbit leaf beetle, peach curculio, and etc., when the size of watch glass is suited to every insect.

Testing method for larva. ——— A method similar to that used in the experiments on attraction of mustard oils and umbelliferous essential oils (Sugiyama & Matsumoto 1957, 1959 a, b) was adopted. Four small glass tubes (0.4 × 3cm) were placed crosswise and fixed with cellophane tape in a Petri dish (2 × 9cm), the mouths of which were faced to the center of dish.

At the bottom of each tube was placed a small piece of absorbent cotton wool, to which a small quantity of test samples was added to the experimental two tubes, facing each other, and not added to the other two, which were used as controls.

Thirty larvae were transferred with a moistened sable brush on a small disc of moistened filter paper (0.5cm in diameter) at the center of dish. A tightly fitting disc of filter paper (11cm in diameter) was placed in the lid of dish to aid in preventing the larvae from escaping. Five replicate Petri dishes were set up in each experiment and placed at 25°C in darkness. The number of larvae which entered into tubes was counted at intervals of one hour. This simple method was originated by Munakata, Saito, Ogawa & Ihii (1954, 1959), who worked on the isolation of attractive substance of the rice stem borer.

RESULTS

The typical responding behavior of adults is outlined as follows: the adults begin circular movements just after the setting of them in the container, as described before. When an odor substance exists there, after a short time the adults stop their walking for a moment, extending and holding up their antennae, in a tense manner. Then they slowly turn their head towards the odor substance, and walk to that, waving their antennae up and down. When they come near test material, they try to prick again and again the glass surface with the tops of antennae which are bended downwards at the pedicels. At the same time the head is lowered, and in some cases the mandible movements are fairly observed. Finally they reach to test sample and attack it.

The leaf disc and leaf juice of 'Karasina' (*Barassica cernua*) used in the preliminary experiment were attacked by adult, but water was scarcely, as shown in Table 2. In these preliminaty experiments, the tests were closed at the first attack of adults. From these results, it is quite apparent that the adult is able to discriminate between the odor and the water which emanate from

Table 2. Attractiveness of leaf disc, leaf juice of *Brassica cernua* Boiss. and water to adults

Insect No.	Responding time (sec)		
	Leaf disc	Leaf juice	Water
1	23	34	Not respond
2	5	25	〃
3	26	200	〃
4	57	85	〃
5	69	60	〃
6	79	75	325
7	92	150	Not respond
8	61	34	〃
9	64	10	〃
10	Not respond	25	〃
11	72	120	〃
12	92	50	〃
13	74	40	〃
14	21	65	325
15	78	62	Not respond
16	146	26	〃
17	Not respond	81	〃
18	61	109	〃
19	11	60	〃
20	56	130	〃

Watch glass size of the container: 6cm in diameter
Recording period: 6 minutes

test materials. But, if the adults were held for many days without water, they came to attack water (details of theses results will appear elsewhere).

The results of experiments on synthesized leaf alcohol attraction for adults and newly-hatched larvae were shown in Table 3 & 4. Similar results were obtained with leaf alcohol extracted from natural source. Leaf alcohol was found

Table 3. Attractiveness of leaf alcohol (synthesized) to adults

Insect No.	Responding time (sec)					
1	40					
2	90	210	325	395	440	500
3	33	150	240	300		
4	172	270	405	540		
5	31	83				
6	182					
7	38	140				
8	79	180	272	330	478	
9	298	360				
10	90	194	255			

Watch glass size of the container: 6cm in diameter
Recording period: 9 minutes
The above results were obtained while 31 adults were experimented.

Table 4. Attractiveness of leaf alcohol (synthesized) to newly-hatched larvae

Time (hr)		Dish No. 1	2	3	4	5	Sum
1	H	9	8	10	12	11	50
	C	0	2	1	1	0	4
2	H	10	7	14	13	14	58
	C	0	2	1	1	0	4
3	H	10	8	13	17	15	63
	C	0	2	1	1	0	4
4	H	10	8	13	17	15	63
	C	0	2	1	1	0	4

H: Leaf alcohol, about 0.0005cc
C: Control
Number of total larvae used: 30 × 5
25°C, darkness

to be attractive to both adult and larva, although the determinations of optimum dosage of the compound were not completed.

The adults, which reached to the filter paper piece in such a manner as above mentioned, advanced further to the center of the paper at which leaf alcohol was added in spot, and frequently bit the surface of the paper, especially at the

Table 5. Attractiveness of some aliphatic alcohols to newly-hatched larvae

n-Butyl alcohol	C_4H_9OH	75 : 11
n-Amyl 〃	$C_5H_{11}OH$	83 : 9
iso-Amyl 〃	$(CH_3)_2CHCH_2CH_2OH$	63 : 17
n-Hexyl 〃	$C_6H_{13}OH$	59 : 20
n-Heptyl 〃	$C_7H_{15}OH$	61 : 7
n-Octyl 〃	$C_8H_{17}OH$	27 : 7
β-Phenylethyl 〃	$C_6H_5CH_2CH_2OH$	82 : 7

Dosage of alcohol: 0.0002~0.0005cc
Number of larvae used: 30 × 5. 25°C, darkness

center, with their mandibles in excited manner. As a natural result of that the adults could not consume the paper because of the toughness of the fibers, they left the paper after a short time. It is of interest, however, that the attacking was repeated many times during the recording period as shown in Table 3. This repetition of attacking may be due to that the odor gradients which were appreciated by adults were kept for a considerable time in the experimental container. If the concentration of odor was too much high, the adults turned back on the way of attacking or cleaned the antennae with the fore legs.

Some other aliphatic alcohols, which are considered as common in the natural world, were tested for larvae. All of those tested were found to be attractive (Table 5), although the determinations of optimum dosage for attraction and the comparisons of effectiveness were not worked.

DISCUSSION

As has already been stated, leaf alcohol not only attracts the adult, but also causes it to display biting action in excited manner. This biting observed was an action which was held continuously for a considerable time, but was not an action on trial. It seems to be advisable that the present biting belongs to *continued feeding* rather than *biting response* which were offered by Dethier (1953). Therefore it can be concluded that the chemical stimulus of leaf alcohol plays an important part of host determination in the adult of the present weevil. This conclusion may be adopted too in the case of the larva, although the observation of larval biting action has not yet been completed.

In general the feeding of insect is induced by phagostimulant of the gustatory type, including either secondary substances or nutrients, while the food finding is taken by odoriferous attractive substances. With some species, however, the odors attracting insects also induce the feeding (Dethier 1953, Thorsteinson 1955). For instance, Dethier (1941) observed that umbelliferous essential oils and their main constituents not only attracted the larvae of *Papilio ajax*, but also stimulated their feeding. The Colorado potato beetle was induced to feeding by acetaldehyde (Hesse & Meier 1950), and the feeding response of the diamond back moth to diets containing sinigrin and nutrients was prompted by adding a little mustard oil (Thorsteinson 1953). The stimulation, which indicates both attraction and feeding induction, of leaf alcohol is a renewal of example of the dual effect of odoriferous substances playing roles of orientation to food and feeding. Watanabe (1958, 1959) reported the attraction of leaf alcohol, together with leaf aldehyde, to the larvae of silkworm and *Bombyx mandarina*, but whether the feeding stimulation followed or not was not studied.

Leaf alcohol is widely distributed in many types of green plants. Hence, we can draw a further conclusion that the polyphagy of the vegetable weevil may be partially due to the existence of leaf alcohol in green plants, although it has not yet been precise whether all the green plants always contain leaf alcohol and leaf aldehyde or not. According to Takei et al. (1938), leaf alcohol is much present especially in young leaves. This may be somewhat connected with the fact that the larvae are most found in the crown parts of vegetable plants. More attention should be paid to leaf alcohol and leaf aldehyde in the study of chemotactic response of green-leaf feeding insect.

The experiments on attraction and feeding stimulation of mustard oils and other volatile compounds for adults, which had already been found to be attractive for the larvae of vegetable weevil, are now underway.

The relation between the attraction of some other aliphatic alcohols and the host determination of the weevil was not discussed here, but, it is of interest that n-hexanol possesses an odor similar to leaf alcohol in human sense.

SUMMARY

Leaf alcohol is an important volatile compound, as similar as leaf aldehye, of wide botanical distribution. In view of the fact that the vegetable weevil is a polyphagous insect, attacking many plants of more than about 30 families, the experiments on attraction of leaf alcohol were carried out. A simple method deviced by the author was used for adult experiment. The compound was found to be attractive to both adults and newly-hatched larvae. In addition to the attraction, the feeding stimulation for adult was observed. From these results, it can be concluded that the chemotactic responses to leaf alcohol might play some roles of host finding and host acceptance in the vegetable weevil. More notice should be taken of leaf alcohol as well as leaf aldehyde in the study of host determination of phytophagous, especially polyphagous insect.

Some other aliphatic alcohols were also found to be attractive to larvae.

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