Sugars as Phagostimulants for Larvae of the Onion Fly, *Hylemya antiqua* MEIGEN (Diptera: Anthomyiidae)

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(Received May 16, 1985)

Phagostimulants for the larvae of the onion fly, *Hylemya antiqua* MEIGEN were isolated from onion bulbs. Among the basic, acid and neutral fractions of the methanolic extract, only the neutral fraction showed phagostimulatory activity. This fraction contained glucose, fructose and sucrose at 0.09 M, 0.07 M and 0.06 M, respectively. Fructose and sucrose were effective at 0.1 M, but glucose was not effective at any concentration. The mixture of these sugars at 0.05 M each was also effective. Fructose and sucrose in onion bulbs were determined to act as phagostimulants additively to the larvae of the onion fly, suggesting the importance of the fructose moiety.

INTRODUCTION

Newly-hatched larvae of the onion fly, *Hylemya antiqua* MEIGEN are attracted to sulfur compounds specific to onion and the 3rd-instar larvae are attracted to its microflora (MATSUMOTO and THORSTEINSON, 1968; IKESHOJI et al., 1980). YAMADA et al. (1981) reported that the larvae may need both olfactory and contact chemostimuli for the correct orientation to their host plant. But the feeding behavior of larvae on onion bulb has not been studied. Much work has been done on the feeding response of phytophagous insects to plant chemicals, however, little has been reported about the larvae of cyclorrhaphous diptera. MIYAKAWA and his group (MIYAKAWA, 1981, 1982; MIYAKAWA et al., 1980) studied gustatory response of *Drosophila* larvae to sugars, amino acids and monovalent salts, although the species *Drosophila* are not phytophagous insects.

In the present work, we attempted to isolate phagostimulants for the larvae of the onion fly from onion bulb with a new bioassay devised by the first author, and we obtained phagostimulation by fructose and sucrose but not glucose, suggesting the importance of the fructose moiety.

MATERIALS AND METHODS

Insects. Insects used for experiments were from a strain established at Hokkaido National Agricultural Experiment Station and were reared on an artificial diet devised by ISHIKAWA et al. (1983) in our laboratory.

Sample preparation. Onion bulbs (600 g) were cut into small pieces and immersed in 95% methanol for 5 days. This methanolic extract was filtrated and concentrated under reduced pressure at 45°C. The extract was fractionated to basic, acid and neutral

Fig. 1. A special trench-type device. *Left*: before start of the test, indicating the trench and two pieces of diet, one is control. *Right*: under testing, indicating two pieces of diet set right above the trench contiguously.

fractions with ion-exchange columns. One milliliter of the extract was passed through a column (diam. 1 cm $\times$ length 10 cm) of Amberlite IR 120B (Organo) cation-exchange resin (H^+ form) and continuously eluted with distilled water. The unadsorbed basic-neutral fraction was neutralized with $NaHCO_3$ and then concentrated to 1 ml *in vacuo*. It was further passed through an anion-exchange column of DE 52 (Whatman) cellulose resin (OH^- form). The acid and basic fractions adsorbed on each column were eluted with 4% NH_4OH . Each fraction was neutralized with 1 N HCl or $NaHCO_3$ and concentrated to 1 ml as above.

Qualitative and quantitative analyses of sugars. Sugars in the methanolic extract were analyzed by high performance liquid chromatography (HPLC) (Shimadzu, LC 4A). Column: zorbax NH_2 (Du Pont) 4.6 mm $\times$ 25 cm, mobile phase: 72% acetonitrile/28% water, flow rate: 0.8 ml/min, temperature: ambient, detector: RI.

Bioassay. A special trench-type device was used for bioassay based on the larval characteristic behavior of boring into the food (usually an onion bulb) from its bottom surface. A 3 mm thick paraffin-layer having a trench (length 30 mm $\times$ width 2 mm $\times$ depth 3 mm) at the center lined the bottom of a 9 cm petri dish (Fig. 1, left). Ten larvae at early or mid 3rd-instar starved for 1 hr before the tests but with access to water were placed in a line in the trench, and then two pieces of agar diet, test and control (30 $\times$ 10 $\times$ 5 mm) were set right above the trench contiguously (Fig. 1, right). Finally, the petri dish was covered with a lid and kept in the dark at 22°C. After 1 hr the number of larvae which burrowed into and took each of the diets were counted. The two diets were respectively dyed red and black, and larval intake of each diet was determined by the color of dye in the larval guts which was easily visible through the cuticle (Fig. 2).

Basal agar diet was made of 1.2 g cellulose powder, 0.3 g agar, 13 ml distilled water and 1 ml food-red or India ink. Each test sugar or extract fraction was added to the diet at a temperature below 65°C in the agar diet preparation process.

RESULTS

Of the basic, acid and neutral fractions of the methanolic extract, only the neutral fraction was effective (Table 1). Sugars in the neutral fraction were further analyzed

Fig. 2. The upper larva has dye in the gut (arrow). The lower larva does not.

Fig. 3. HPLC chromatogram of the methanolic onion extract.

Table 1. Feeding response of the onion maggot to extracts of onion

Fraction added to diet <i>A/B</i>	Larval preference index ^a		Difference
	<i>A</i>	<i>B</i>	
MeOH extract/D.W. (control)	8.9	0.5	8.4**
Basic fr./D.W.	4.6	4.5	0.1
Acid and neutral fr./D.W.	6.3	3.1	3.2**
Acid fr./D.W.	2.4	5.8	-3.4
Neutral fr./D.W.	7.1	2.4	4.7**
Sugar mixture ^b /D.W.	7.4	2.0	5.4**
Sugar mixture ^b /neutral fr.	5.8	3.9	1.9
MeOH extract/sugar mixture ^b	8.6	1.3	7.3**

^a Mean number of larvae burrowing into and taking each diet (8 replicates).

^b Glucose 234 mg, fructose 185 mg, sucrose 270 mg in 15 ml water.

** $P < 0.01$ (*t*-test).

by HPLC; the chromatogram is shown in Fig. 3. The principal ingredients were glucose, fructose and sucrose, yielding 234 mg, 185 mg and 270 mg, respectively, in 16.5 g fresh onion, corresponding to 0.09 M, 0.07 M and 0.06 M in onion juice. When the mixture of these three sugars at the equivalent concentration was tested, it was found to be as effective as the neutral fraction, though, less effective than the methanolic extract (Table 1).

Table 2. Feeding response of the onion maggot to sugars

Sugar		Larval preference index ^a		Difference
		Test	Control	
Glucose	0.5 M	4.6	5.4	-0.8
	0.1	3.9	5.9	-2.0
	0.05	4.1	5.3	-1.2
Fructose	0.5	5.0	4.8	0.2
	0.1	7.0	2.6	4.4*
	0.05	5.0	4.8	0.2
Sucrose	0.5	4.1	5.9	-1.8
	0.1	6.8	3.0	3.8**
	0.05	4.0	5.5	-1.5
Mannose	0.5	5.1	4.7	0.4
	0.1	3.7	5.2	-1.5
	0.05	4.3	5.5	-1.2
Galactose	0.5	3.5	4.9	-1.4
	0.1	5.7	4.6	1.1
	0.05	5.2	4.6	0.6
Maltose	0.5	4.6	4.8	-0.2
	0.1	3.7	3.4	0.3
	0.05	4.1	4.9	-0.8
Lactose	0.5	5.3	4.2	1.1
	0.1	4.9	4.5	0.4
	0.05	4.0	5.4	-1.4
Raffinose	0.5 ^b	—	—	—
	0.1	2.9	6.3	-3.4
	0.05	3.5	5.0	-1.5

^a See footnote to Table 1.^b Raffinose at 0.5 M does not completely dissolve in water.* $P < 0.05$, ** $P < 0.01$ (t -test).

Table 3. Feeding response of the onion maggot to sugar mixtures

Sugar mixture	Larval preference index ^a		Difference
	Test	Control	
Glucose 0.05 M + fructose 0.05 M	5.4	4.1	1.3
Glucose 0.05 M + sucrose 0.05 M	5.1	4.8	0.3
Fructose 0.05 M + sucrose 0.05 M	6.5	3.4	3.1*

^a See footnote to Table 1.* $P < 0.05$ (t -test).

Each of the three sugars was tested at 0.05 M, 0.1 M and 0.5 M concentrations. Fructose and sucrose were apparently effective at 0.1 M, but not at 0.05 M or 0.5 M. Glucose was not effective at any of the three concentrations. Other sugars, mannose,

galactose, maltose, lactose and raffinose which are not detected in onion (BACON, 1959) showed no activity (Table 2).

When the two mixtures out of the three sugars were tested at 0.05 M each, only the mixture of fructose and sucrose showed a significant activity (Table 3).

DISCUSSION

Sucrose, fructose and glucose have been known as good phagostimulants for many insects (BERNAYS and SIMPSON, 1982). In this experiment, fructose and sucrose were found to stimulate the larval feeding of the onion fly, but glucose did not; nevertheless, it is present in the highest concentration (0.09 M) in onion bulb. Fructose and sucrose were singly effective only at 0.1 M. Since the mixture of 0.05 M fructose and 0.05 M sucrose was as stimulative as 0.1 M fructose or sucrose, and the onion extract contained 0.07 M fructose and 0.06 M sucrose, these sugars act as a phagostimulant additively to the larvae of the onion fly. Onion bulbs contain water-soluble oligosaccharides, fructans, which have fructose moiety (BACON, 1959; DARBYSHIRE and HENRY, 1978). Sucrose also has a fructose moiety. Therefore, all of these fructose moieties of fructans may additively stimulate the feeding of the onion fly larvae.

As shown in Table 1, phagostimulative activity by sugars was lower than that of the methanolic extract so that other co-factors might exist in the methanolic extract. Further experiments are needed to elucidate these co-factors.

ACKNOWLEDGEMENTS

We thank Dr. T. IKESHOJI for his critical reading of the manuscript.

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