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Effects of Nutrient Compounds on Sucking Response of the Brown Planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae)

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The feeding preference of the brown planthopper, *Nilaparvata lugens*, for various nutrient constituents of artificial diets were studied using a radioactive tracer. The fluid intake in *N. lugens* was markedly enhanced on 20% sucrose solution near neutrality. The acceptability of 20% sucrose solution was further improved when incorporated with 0.5—1.0% amino acids, 0.007—0.014% vitamins, 0.07% mineral salts (0.005% KH_2PO_4 and 0.02% $MgCl_2$ or $MgSO_4$), or 0.0035% metal sequestrenes.

INTRODUCTION

Since MITTLER and DADD (1962) have first succeeded in the artificial rearing of the green peach aphid, *Myzus persicae*, on a chemically defined diet by introducing the parafilm membrane technique for holding the diet, many attempts have beem made to rear the plant-sucking insects on synthetic diets with this method, and consequently some synthetic diets for permanent culture of aphids have been developed. MITSUHASHI and KOYAMA (1971) have developed a synthetic diet for the smaller brown planthoppers, *Laodelphax striatellus*, and the white-back planthopper, *Sogatella furcifera*, by referring to the composition of the aphid diets. Recently several workers have also tried to rear the leafhoppers on similar synthetic diets (KOYAMA, 1973; HOU and BROOKS, 1975).

This experiments were conducted to determine the influence of various nutrient constituets included in synthetic diets, especially the MED-4 diet for *L. striatellus* (MITSUHASHI and KOYAMA, 1972), on the sucking preference of the brown planthopper, *Nilaparvata lugens*, in order to elucidate available aspects to formulate the synthetic diet for this species.

MATERIALS AND METHODS

Insects: The planthoppers used in the present experiments were the macropterous female adults which were collected from the stock colony reared successively on the rice seedlings for several years in the laboratory.

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Test solutions: Nutrient constituets were classified into five groups, namely sucrose, amino acids, vitamins, mineral salts, and trace metals. All the constituents of each group excepting sucrose were incorporated in 20% sucrose solution containing radioactive ^{32}P-H_3PO_4 at about 10^5 cpm/ml.

(1) Sucrose; Sucrose solutions were tested at five different concentrations ranging 5 to 25%(w/v). Influence of pH values was also examined appling 20% sucrose solution adjusted at pH 1 to 9 with 0.1 M HCl or 0.1 M NaOH.

(2) Amino acids; Two types of amino acid mixture were tested varying their overall concentrations. The one was the same as that in MED-4 diet for *L. striatellus* (MITSUHASHI and KOYAMA, 1972). The other one was formulated on the basis of the amino acid composition in the honeydew excreted by *N. lugens* (NODA et al., 1973) (Table 3). The solutions were neutralized with 0.1 M NaOH.

(3) Vitamins; The mixture of B-vitamins and ascorbic acid in MED-4 diet was tested at different total concentration without modifying the composition.

(4) Mineral salts; Several combinations of potassium phosphate and magnesium salts were tested (Table 6).

(5) Trace metals; The mixture of ferric, cupric, zinc, manganese and calcium chloride which were used in MED-4 diet, and the mixture of metals chelated with ethylenediamine tetraacetic acid (EDTA) which was used in the synthetic diets for aphids (e.g. DADD and MITTLER, 1966) were comparatively tested.

As a control, 20% sucrose solution was used in most of the experiments.

Bioassay: A parafilm sachet (MITTLER and DADD, 1964) enclosing 0.2 to 0.3 ml of the radioactive nutrient solution was set so as to close the bottom opening of a glass ring cage (27 mm in inner diameter, 20 mm in height). The top opening was covered by a sheet of tetoron gauze. Five female planthoppers were introduced in each cage through a small opening in the tetoron gauze. The cages were maintained in an incubator of 27°C and 100% RH for 24 hr under continuous illumination with fluorescent lamps. At the end of this period the insects were frozen to death, and the radioactivity of the insects' body was directly measured with an Aloka model-2 G. M. counter. The evaluation of relative acceptability of each nutrient solution was based on the amount of ^{32}P absorbed during the feeding of insects on each nutrient solution.

RESULTS

Sucrose

Ingestion was low on 5 and 10% sucrose, raised to a maximum at 20% and declined again at higher concentration (Table 1). The pH of the sucrose solution influenced

Table 1. RELATIVE INTAKE OF SUCROSE SOLUTION AT DIFFERENT CONCENTRATIONS

Replication	Radioactivity cpm/insect				
	5	10	15	20	25%[a]
1	32	43	156	214	35
2	53	45	60	149	8
Average	43	44	108	182	22
Ratio	1.0	1.0	2.5	4.2	0.5

[a] Concentration of sucrose.

Table 2. RELATIVE INTAKE OF 20% SUCROSE SOLUTION AT DIFFERENT pH VALUES

Replication	Radioactivity cpm/insect				
	pH 1	3	5	7	9
1	13	87	178	228	241
2	11	69	305	149	97
3	23	123	126	238	117
Average	16	93	203	205	151
Ratio	0.1	0.5	1.0	1.0	0.7

Table 3. RELATIVE INTAKE OF 20% SUCROSE SOLUTION CONTAINING THE TWO TYPES OF AMINO ACID MIXTURES (MED-4 AND HONEYDEW TYPES) AT TWO DIFFERENT CONCENTRATIONS

Replication	Experiment I[a]			Experiment II[b]		
	Radioactivity cpm/insect			Radioactivity cpm/insect		
	Control	MED-4	Honeydew[c]	Control	MED-4	Honeydew[c]
1	61	9	13	201	263	110
2	68	12	24	127	134	207
3	52	25	21	154	477	486
Average	60	15	19	161	291	268
Ratio	1.0	0.3	0.3	1.0	1.8	1.7

[a] Total amino acid concentration is about 3.8%.

[b] Total amino acid concentration is reduced to one-fourth, i.e. about 1.0%.

[c] Amino acid composition of the honeydew type mixture: 1% asparatic acid and glutamic acid, 0.6% alanine, 0.4% asparagine, 0.2% valine, 0.05% arginine, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine and threonine, 0.01% glutamine, tryptophan and tyrosine.

the planthopper sucking, and the solutions of pH 5 to 7 were more accepted (Table 2).

Amino acids

The amino acid mixture of MED-4 type was sucked as little as only one-third of the amino acid-free control at the original concentration, i.e. about 3.8%. The honeydew type mixture was also rejected at the same overall concentration. However, the both mixtures exhibited a phagostimulatory effect when their concentration was reduced to one-fourth, i.e. about 1.0% (Table 3). The latter mixture was most accepted at the concentrations ranging from 0.5 to 1.0% (Table 4).

Vitamins

The vitamin mixture inhibited markedly the sucking of *N. lugens* at about 0.23% in its overall concentration, which was usually adopted in the synthetic diets for aphids. The inhibitory effect was reduced as the concentration decreased, and the stimulatory effect was recognized when diluted to 0.014 to 0.007% (Table 5).

Mineral salts

The mixture of 0.5% KH_2PO_4 and 0.2% $MgCl_2$, which was used in MED-4 diet,

Table 4 Relative Intake of 20% Sucrose Solution Containing the Amino Acid Mixture of Honeydew Type at Different Concentrations

Replication	Radioactivity cpm/insect				
	Control	1/1	1/4	1/8	1/16[a]
1	246	90	352	532	238
2	164	190	620	512	132
3	150	46	508	690	716
Average	186	109	493	578	362
Ratio	1.0	0.6	2.7	3.1	1.9

[a] Rate of dilution of the total amino acid concentration; The original concentration is about 3.8%.

Table 5. Relative Intake of 20% Sucrose Solution Containing Vitamin Mixture at Different Concentrations

Replication	Radioactivity cpm/insect						
	Control	1/1	1/4	1/8	1/16	1/32	1/64[a]
1	144	24	138	160	406	312	222
2	300	60	116	170	538	360	226
3	360	84	154	236	470	—	308
Average	268	56	136	189	471	336	252
Ratio	1.0	0.2	0.5	0.7	1.8	1.3	0.9

[a] Rate of dilution of the total vitamin concentration; The original concentration is about 0.226%.

Table 6. Relative Intake of 20% Sucrose Solution Containing Mineral Salts at Different Concentrations

Replication	Experiment I	Experiment II			
	Radioactivity cpm/insect	Radioactivity cpm/insect			
	Control	KH_2PO_4 (500)[a] $MgCl_2$ (200)	KH_2PO_4(500) $MgCl_2$ (200)	KH_2PO_4(250) $MgCl_2$ (200)	KH_2PO_4(50) $MgCl_2$ (20)	KH_2PO_4(50)[a] $MgSO_4$ (20)
1	108	71	38	82	136	291
2	86	36	72	76	170	193
3	88	44	72	58	206	164
Average	94	50	61	72	171	216
Ratio	1.0	0.5	1.0	1.2	2.8	3.5

[a] The values in the brackets indicate the concentrations of each salt in mg/100 ml.

deterred considerably the sucking of *N. lugens*. Among the four types of the mixtures of potassium phosphate and magnesium salts, the mixture of 0.05% KH_2PO_4 and 0.02% $MgCl_2$ or $MgSO_4$ gave better acceptability (Table 6). If KH_2PO_4 was replaced with K_3PO_4, the insect sucking tended to decrease.

Trace metals

The mixture of metal chlorides inhibited strongly, but the mixture of metal sequestrenes rather stimulated the sucking of *N. lugens* (Table 7).

Table 7. RELATIVE INTAKE OF 20% SUCROSE SOLUTION CONTAINING THE MIXTURES OF METAL CHLORIDES AND METAL SEQUESTRENES

Replication	Experiment I		Experiment II	
	Radioactivity cpm/insect		Radioactivity cpm/insect	
	Control	Metal chlorides	Control	Metal sequestrenes
1	108	20	295	444
2	86	34	148	463
3	88	24	180	448
4	59	20	—	—
Average	85	25	208	432
Ratio	1.0	0.3	1.0	2.1

DISCUSSION

It has been known that sucrose is essential for the plant-sucking insects as a phagostimulant as well as a nutriment. MITTLER and DADD (1963), MITTLER (1967), and ARN and CLEERE (1971) have reported that the aphid, *Myzus persicae* ingests none or little diets whose sucrose concentration is below 5%, maximal intake is obtained in the diets with 10 to 20% sucrose, and the diet ingestion declines progressively with sucrose concentration above 20%. Similar conclusion concerning the importance of sucrose as a phagostimulant was drawn from the experiments with *N. lugens*. The rate of fluid intake of *N. lugens* was the highest on 20% sucrose solution, and decreased definitely below 10% sucrose. It seems likely that such high sucrose concentration is equivalent to that encountered in the phloem saps of host plants, on which the insects feed. ZIMMERMANN (1960) has mentioned that the phloem saps contain 10 to 25% sucrose. However, MITSUHASHI and KOYAMA (1971) have succeeded to rear the phloem-feeding *L. striatellus* on the diet containing 5% sucrose.

A number of amino acids have been demonstrated to function as important phagostimulants for the aphids (MITTLER and DADD, 1964; MITTLER, 1967; KLINGAUF and NOCKERWENZEL, 1972) and for *N. lugens* (SŌGAWA, 1972). The rate of fluid intake by the aphids is also markedly affected by the overall level of dietary amino acids. According to MITTLER (1967), the fluid intake by *M. persicae* was poor or nonexistent on 15% sucrose solution containing amino acids of less than 1% in the total concentration, increased with the concentration up to 3% and then decreased slightly. SRIVASTAVA and AUCLAIR (1974) have reported that the diet containing 2.5 to 3.5% amino acids were most accepted by the aphid, *Acyrthosiphon pisum*. In the case of *N. lugens*, 20% sucrose solution containing such higher concentration of amino acids was not accepted, but a pronounced sucking was obtained when the amino acid concentation was reduced to 0.5 to 1.0%. HOU and BROOKS (1975) have pointed out that the amino acid requirement of the leafhopper, *Macrosteles fascifrons*, is only one-fifth that of aphids, i.e. 0.84%. These evidence indicates that the plant- and leafhoppers prefer the fluid containing relatively lower concentration of amino acids. With regard to the level of free amino acids in the phloem sap of plants, ZIMMERMANN (1960) has stated that it usually lies between 0.03 to 0.40%.

It was evident that *N. lugens* ingested the liquid only poorly if 20% sucrose solution was added with the vitamin mixture of the same composition as that of the MED-4

diet. However, the vitamin mixture exhibited the sucking stimulatory effect at lower concentrations such as 0.007 to 0.014%. B-vitamin concentration in the diets for the aphid, *Rhopalosiphum maidis*, and the leafhopper, *M. fascifrons*, was one-half, i.e. 0.06%, of that in the aphid diets developed previously (KLECKHEFER and DERR, 1967; HOU and BROOKS, 1975).

A definite reduction in fluid intake of *N. lugens* on 20% sucrose solution was recorded when monobasic potassium phosphate and magnesium chloride were added to it in the same level as that in the MED-4 diet. However, these mineral elements exerted a phagostimulatory effect when the concentration was reduced to one-tenth. The aphid diet formulated by KIECKHEFER and DERR (1967) contains these salts in similarly reduced level.

In a view point of acceptability to *N. lugens*, the mixture of metal sequestrenes was found to be apparently superior to that of metal chlorides.

The present demonstration that the fluid intake of *N. lugens* was markedly affected by the overall level of major nutrients indicated their important roles in the insect feeding at a prenutritional level, and seemed to offer useful information in order to develop the synthetic diet for *N. lugens*.

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