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Amino Acids in Honeydews of the Rice Planthoppers and Leafhoppers (Homoptera : Delphacidae, Deltocephalidae)

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Amino acids in honeydews of three species of planthoppers (*Nilaparvata lugens*, *Laodelphax striatellus* and *Sogatella frucifera*) and two species of leafhoppers (*Inazuma dorsalis* and *Nephotettix cincticeps*) were examined by thin layer chromatography and on an automatic amino acid analyser. The honeydews excreted by *N. lugens*, *L. striatellus*, *S. frucifera* and *I. dorsalis* contained 18 kinds of amino acids, with a predominance in aspartic acid, asparagine, glutamic acid, glutamine and valine. On the contrary, the honeydew of *N. cincticeps* contained only a very small amount of most amino acids with the exception of aspartic and glutamic acid. Insects which suck rice seedlings in the germinating stage excreted comparatively fewer amino acids. Those sucking an artificial amino acid solution excreted the same kinds of amino acids as present in the ingested solution. When distilled water was sucked, only traces of amino acids were excreted. It seems likely that the free amino acids in honeydew excreted by the rice plant- and leafhoppers were mostly derived from the ingested plant sap. It is also considered that *N. cincticeps* preferentially sucks the xylem to the phloem when compared to the other species.

INTRODUCTION

It has been known that aphids and mealybugs excrete sweet liquid droplets, commonly referred to as honeydew, from their alimentary tract during sustained sucking, which attract ants and also cause the sooty mold diseases in their host plants. Since the high sugar content of honeydew has drawn the attention of many investigators, much data have been accumulated on its sugar compositions. GRAY (1952) and MALTAIS and AUCLAIR (1952) have also reported amino acids to be general constituents of honeydew. Detailed analytical data for honeydew of aphids and mealybugs were extensively reviewed by AUCLAIR (1963) and TAMAKI (1968). These reviews offer much of the available information concerned with sucking habits and nutritional requirements of these insects, causes of their sucking damage and varietal resistance of the host plants.

So far little has been studied on the honeydew of auchenorrhynchous species. The present paper deals with its amino acid constituents excreted by the rice plant- and leafhoppers.

MATERIALS AND METHODS

Insects. Female adults of the following species were used:

Delphacidae

Nilaparvata lugens (Stål), the brown planthopper

Laodelphax striatellus (Fallén), the smaller brown planthopper

Sogatella frucifera (Horváth), the white-back planthopper

Deltocephalidae

Inazuma dorsalis (Motschulsky), the zigzag-striped leafhopper

Nephotettix cincticeps (Uhler), the green rice leafhopper

The above mentioned insects were reared on rice seedlings in a constant temperature cabinet at 25°C, and subjected to a 16L : 8D photoperiod under fluorescent lamps.

Food sources for insects. The following plant materials and artificial media were offered to the insects. (a) Rice plants (var. Suzukaze) at the 4- to 5-leaf stage, which were grown in greenhouse at 25—30°C. (b) Rice seedlings (var. Suzukaze) at the germinating stage. (c) Amino acid solution, containing each 100 mg of alanine, arginine, asparagine, aspartic acid, glutamic acid and valine, and 2.5 g of sucrose in

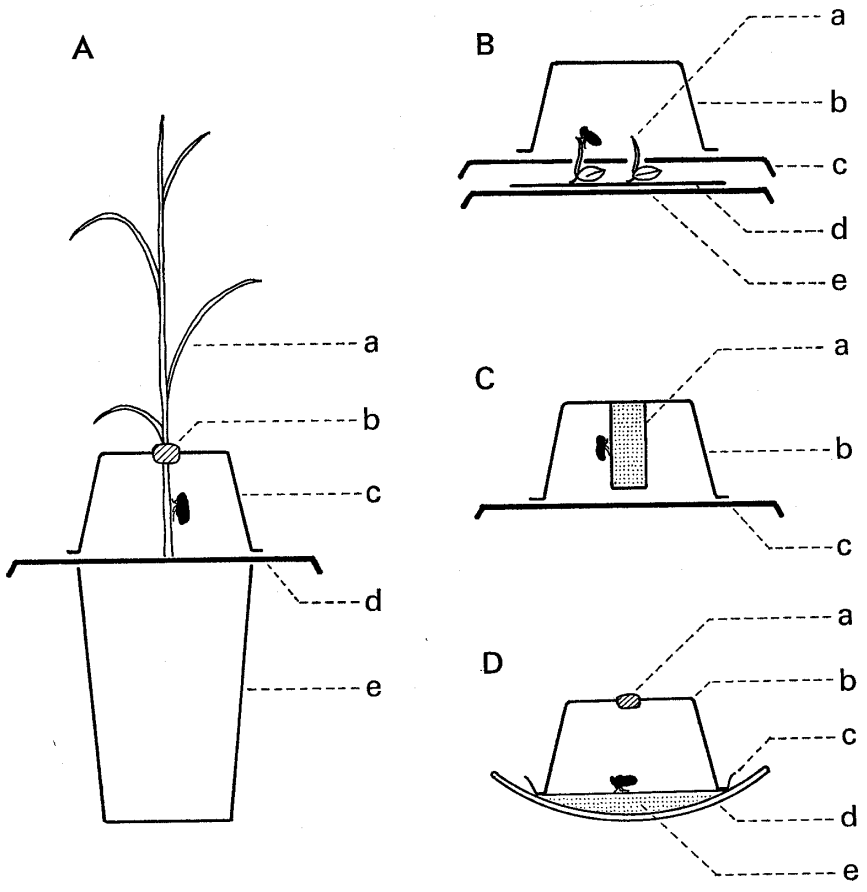

Fig. 1. Apparatus for collecting honeydews.

(A) a, rice plant at 4-to 5-leaf stage; b, cotton plug; c, plastic cup; d, plastic plate; e, soil container. (B) a, rice seedlings at germinating stage; b, plastic cup; c, e, plastic plate; d, moistened filter paper. (C) a, rolled filter paper absorbed amino acid solution or distilled water; b, plastic cup; c, plastic plate. (D) a, cotton plug; b, plastic cup; c, stretched parafilm; d, watch glass; e, amino acid solution or distilled water.

100 ml of distilled water, adjusted to pH 7 with an NaOH solution. (d) Distilled water, adjusted to pH 7 with a NaOH solution.

Apparatus for collecting honeydews. Four types of apparatus, shown in Fig. 1, were devised. The types A and B were employed for the plant materials. The amino acid solution and distilled water were given to the insects with apparatus C or D.

Procedures. Five to ten insects were introduced in each apparatus and allowed to suck the plants or artificial media for 24 hr at 25°C under fluorescent illumination. The insects were starved 2 to 3 hr prior to use, except for those used in the experiments with rice plants. Honeydew droplets discharged within the apparatus were collected with a capillary pipette directly or washed with a small volume of distilled water.

Amino acid analysis. The collected honeydew was mixed fourfold with ethanol and precipitant material was separated by centrifugation (1500 rpm). The supernatant was condensed with the rotary evaporator under reduced pressure at 50°C. When necessary, sugars in the samples were removed by passage through an ion-exchange column (Amberlite IR-120). Amino acids in honeydews were analysed by two-dimentional thin layer chromatography (TLC) with silica-gel G containing 5% corn starch. The plates were activated at 110°C for an hour prior to use. As solvent systems, phenol–0.1% ammonia water (4 : 1, v/v) and *n*-butanol–acetic acid–water (4 : 1 : 2, v/v) were used for the first and the second development, respectively. Amino acid spots were rendered visible with ninhydrin reagent. The honeydews excreted by the insects sucking the rice plants were also analysed by an automatic amino acid analyser (JEOL, Model JLC–5AH).

RESULTS

General appearance of honeydews

The green rice leafhopper apparently excreted more honeydew than any of the other species examined, and its honeydew always exhibited a translucent and dilute aqueous nature. Honeydew droplets of other species were generally observed as a colorless liquid, but occasionally displayed a yellowish coloration and a somewhat sticky consistency.

Amino acids in honeydews excreted by insects sucking plant sap

When rice plants of the 4- to 5-leaf stage were offered to insects as food, the brown planthopper, the smaller brown planthopper, the white-back planthopper and the zigzag-striped leafhopper discharged 18 kinds of amino acids, including asparagine, aspartic acid, glutamic acid, glutamine and valine as major compounds in the honeydew excretion (Table 1, Figs. 2 and 3). In honeydews of the five species, one unidentifiable compound was detected by both TLC and the automatic amino acid analyser. 14 kinds of amino acids were detectable in the honeydew of the green rice leafhopper (Table 1), but thin layer chromatograms indicated that their concentrations were usually strikingly lower with the exception of aspartic acid and glutamic acid than those in honeydew excreted by the above four species. In this regard, it is interesting to note that the total amino acid concentration of honeydew from the brown planthopper was estimated to be about 50 mM from the data obtained by the amino acid analyser, while that of the green rice leafhopper was only about 0.2 mM. Honeydews excreted by insects sucking on rice seedlings at the germinating stage were not as

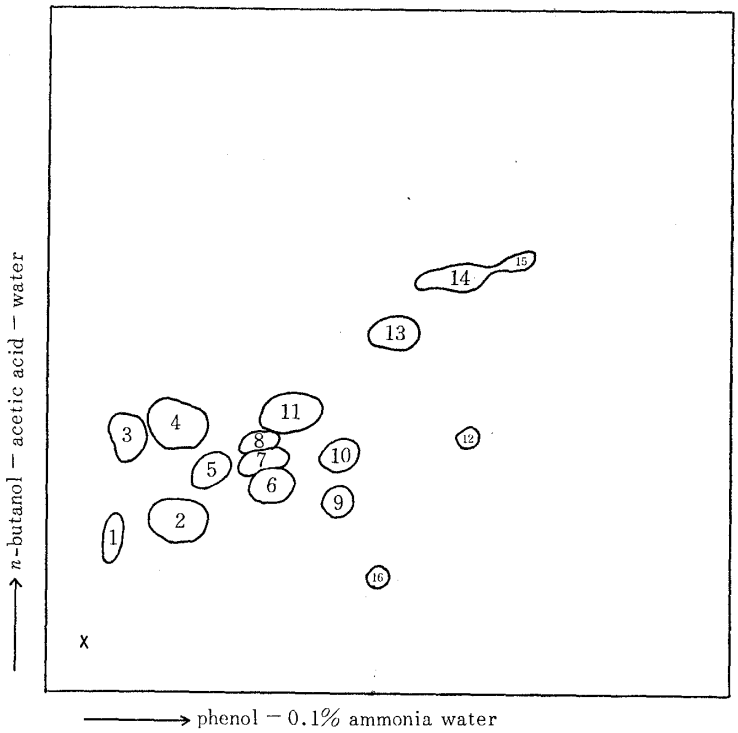

Fig. 2. TLC chromatogram of amino acids in honeydew of the smaller brown planthopper sucking the rice plant.
1, lysine; 2, arginine; 3, aspartic acid; 4, glutamic acid; 5, serine; 6, asparagine; 7, glycine; 8, threonine; 9, histidine; 10, glutamine; 11, alanine; 12, proline; 13, valine; 14, tyrosine+leucine+isoleucine; 15, phenylalanine+tryptophan; 16, unknown.

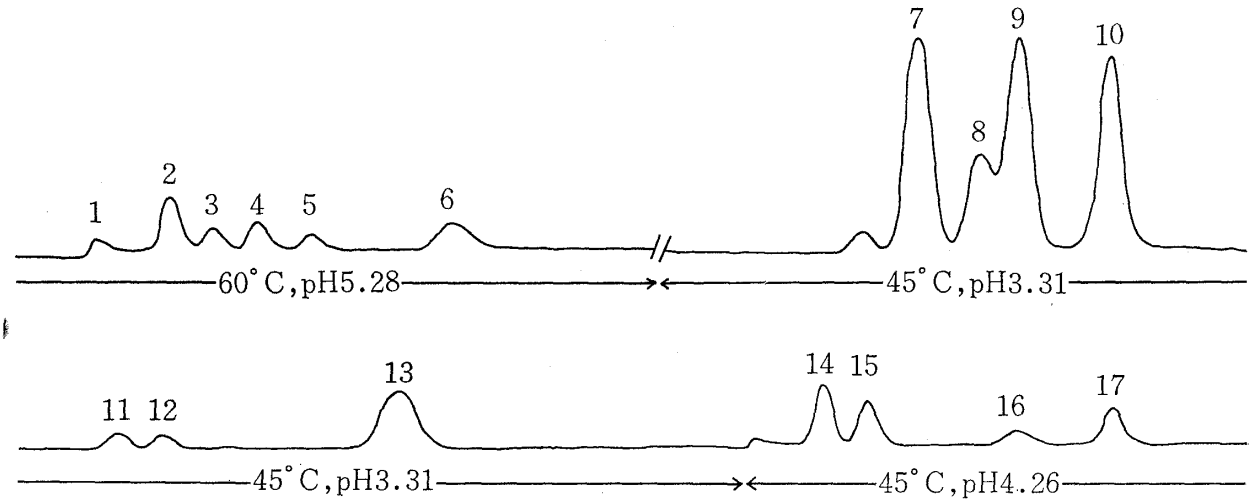

Fig. 3. Amino acids in honeydew of the brown planthopper detected by the automatic amino acid analyser.
1, tryptophane; 2, lysine; 3, histidine; 4, unknown; 5, ammonia; 6, arginine; 7, aspartic acid; 8, threonine; 9, serine+asparagine+glutamine; 10, glutamic acid; 11, glycine; 12, alanine; 13, valine; 14, isoleucine; 15, leucine; 16, tyrosine; 17, phenylalanine.

Table 1. Amino Acid Composition in Honeydews Excreted by the Planthoppers and Leafhoppers Sucking Rice Plants[a]

Amino acids	*N. lugens*	*L. striatellus*	*S. frucifera*	*I. dorsalis*	*N. cincticeps*
Alanine	+	+	+	+	±
Arginine	+	+	+	+	±
Asparagine	++	++	+	+	±
Aspartic acid	+++	+++	+++	+++	++
Cystine	−	−	−	−	−
Glutamic acid	+++	+++	+++	+++	++
Glutamine	+	++	+	+	+
Glycine	+	+	±	±	±
Histidine	+	+	+	+	±
Isoleucine	+	+	+	+	±
Leucine	+	+	+	+	−
Lysine	+	+	+	+	±
Methionine	−	−	−	−	−
Phenylalanine	+	+	+	+	±
Proline	+	+	±	±	−
Serine	+	+	+	+	±
Threonine	+	+	+	+	±
Tryptophan	±	±	±	±	−
Tyrosine	+	+	+	+	−
Valine	++	++	++	+	±
Unknown	+	+	+	+	+

[a] Number of + signs represents relative concentration of the amino acid; ±, trace; −, none detected.

complex in their amino acid composition as those excreted on the above-mentioned plant materials, where glutamine, valine, alanine and leucine (or isoleucine) were commonly detected, and asparagine, glutamic acid, aspartic acid and arginine were only occasionally detected.

Amino acids in honeydews excreted by the insects sucking artificial media

When the amino acid solution was given to the insects instead of rice plants, the five species excreted honeydews which showed amino acid compositions almost identical with that of the dietary solution. There was no significant difference in amino acid concentration among the examined species. On the other hand, the insects which sucked distilled water excreted very few amino acids, if any.

DISCUSSION

In the present experiments, a large variety of amino acids were identified in honeydews produced by five species of rice plant- and leafhoppers sucking on rice plants of the 4- to 5-leaf stage (Table 1). Those amino acids occurring most frequently included asparagine, aspartic acid, glutamic acid, glutamine and valine. These have been reported in honeydews of most species of aphids and mealybugs (Auclair, 1963; Tamaki, 1968). However, comparatively fewer amino acids were excreted by the insects

examined here when fed on the rice seedlings. When distilled water was offered as a sucking solution, they discharged only a negligible amount of amino acids in their honeydews. It is, therefore, considered that amino acids in honeydews originate primarily from the ingested plant sap. This is further supported by the fact that when fed on an artificial amino acid solution, the insects excreted the same kinds of amino acids as those present in the solution. In this respect, MITTLER (1953, 1958) also found that the same kinds of amino acids could be detected both in the honeydew of aphids and the phloem sap on which they fed.

SAXENA (1954) and SMITH (1940) pointed out an interesting relationship between the feeding behavior and nature of the fecal excrement of leafhoppers; the leafhoppers which suck the vascular elements excrete clear aqueous droplets, while those which suck the parenchymal cell sap deposit dark-colored fecal material. The honeydews of the plant- and leafhoppers examined here were all of a clear, aqueous type indicating that these insects ingest the vascular sap of rice plants. This seems to be consistent with results of histological observations of the stylet sheaths deposited by these insects within the rice plants (NAITO and MASAKI, 1967; Anonym., 1967; SŌGAWA, 1970a; SONKU and SAKURAI, 1973). However, it was noticed that the total amino acid concentration in honeydew of the green rice leafhopper was strikingly lower than those of the brown planthopper and the other species examined in spite of the same experimental conditions and use of the same host plant. This species was also observed to excrete much more honeydew than the others. These differences suggest that there is still some discrepancy in their sucking manner, although they belong to the same group of vascular feeders. The vascular bundles of the rice plants are functionally devided into two parts, namely the pholoem and the xylem. It is known that aphids usually insert their stylets into phloem elements (AUCLAIR, 1963), whereas on the other hand, several species of cicadellid and cercopid preferably suck the xylem sap (WITHYCOMBE, 1926; HOUSTON et al., 1947; WIEGERT, 1964). ESAU (1961) described that phloem feeders excrete a relatively small amount of honeydew rich in sugars, and that the xylem feeders discharged a large amount of diluted aqueous excrement in general. In view of these aspects it seems reasonable to conclude that the green rice leafhopper primarily suck the xylem sap, while the other species alternatively ingest both the phloem and xylem sap, as described by SŌGAWA (1970b) for the brown planthopper.

REFERENCES

ANONYMOUS (1967) IRRI Ann. Rep. Plant Pathology. p. 81–113.

AUCLAIR, J. L. (1963) Aphid feeding and nutrition. *Ann. Rev. Ent.* **8** : 439–490.

ESAU, K. (1961) *Plants, Viruses, and Insects.* Harvard University Press, Cambridge, 110 pp.

GRAY, R. A. (1952) Composition of honeydew excreted by pineapple mealybugs. *Science* **115** : 129–133.

HOUSTON, B. R., K. ESAU and W. B. HEWITT (1947) The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. *Pytopath.* **37** : 247–253.

MALTAIS, J. B. and J. L. AUCLAIR (1952) Occurrence of amino acids in the honeydew of the crescent-marked lily aphid, *Myzus circumflexus* (BUCK.) *Can. J. Zool.* **30** : 191–193.

MITTLER, T. E. (1953) Amino-acids in phloem sap and their excretion by aphids. *Nature* **172** : 207.

MITTLER, T. E. (1958) Studies on the feeding and nutrition of *Tuberolachnus salignus* (GMELIN) (Homoptera, Aphididae). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. *J. exp. Biol.* **35** : 74–84.

NAITO, A. and J. MASAKI (1967) Studies on the feeding behavior of green rice leafhopper, *Nephotettix cincticeps* UHLER. I. Insertion of the stylets into host plant. *Jap. J. appl. Ent. Zool.* **11** : 50–56. (In Japanese with English summary.)

SAXENA, K. N. (1954) Feeding habits and physiology of digestion of certain leafhoppers Homoptera : Jassidae. *Experientia* **10** : 383–384.

SMITH, F. F. (1940) Certain sucking insects causing injury to rose. *J. econ. Ent.* **33** : 658–662.

SŌGAWA, K. (1970a) Studies on feeding habits of the brown planthopper. I. Effects of nitrogen-deficiency of host plant on insect feeding. *Jap. J. appl. Ent. Zool.* **14** : 101–106. (In Japanese with English summary.)

SŌGAWA, K. (1970b) Studies on the feeding habits of the brown planthopper. II. Honeydew excretion. *Jap. J. appl. Ent. Zool.* **14** : 134–139. (In Japanese with English summary.)

SONKU, Y. and Y. SAKURAI (1973) Transmission of rice stripe virus by *Laodelphax striatellus* FALLÉN. I. Mode of stylet insertion and virus secretion. *Ann. Phytopath. Soc. Japan* **39** : 53–60. (In Japanese with English summary.)

TAMAKI, Y. (1968) Chemical composition of honeydew excreted or secreted by aphids and coccids. *Biological Science* (Tokyo) **20** : 17–25. (In Japanese.)

WIEGERT, R. G. (1964) The ingestion of xylem sap by meadow spittlebugs, *Philaenus spumarius* (L.). *Amer. midland Nat.* **71** : 422–428.

WITHYCOMBE, C. L. (1926) Studies on the aetiology of sugarcane froghopper blight in Trinidad. I. Introduction and general survey. *Ann. appl. Biol.* **13** : 64–108.