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Effect of Organic Rice Farming on Leafhoppers and Planthoppers 2. Amino Acid Content in the Rice Phloem Sap and Survival Rate of Planthoppers

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Nitrogen content of rice plants was compared among chemically fertilized, poultry-manured, non-fertilized and organically farmed rice fields to clarify factors of a comparatively lower population density of planthoppers in the latter. The nitrogen content of rice plants was found to be the lowest in the organically farmed field. The content of amino acids in the phloem sap of potted rice plants was compared between the chemically and the organically fertilized cultivations. The amount of total amino acids tended to be smaller in the plants from the organic cultivation. The content of the asparagine was significantly lower in the organically cultivated plants. In pot experiments, the survival rate of the nymphs of *Nilaparvata lugens* and *Sogatella furcifera* was lower in the organic plant than in the chemically cultivated ones.

Key words: *Nilaparvata lugens, Sogatella furcifera,* organic farming, amino acid, phloem sap

INTRODUCTION

A rapid growth in the global population in the last few decades, together with the depletion of cultivated land area, has required a substantial increase in crop yield and produce quality to supply the growing demand for food. Agro-chemical products have played a key role in the improvement of the yield of major crops. However, in recent years, their extensive use has raised numerous environmental and health-related concerns. Recently, proposals have been made to develop sustainable agricultural systems to avoid deleterious effects of modern agricultural practices (REGANNOLD et al., 1990). Natural or organic farming, a traditional agricultural system in Japan, has been conducted by a few farmers and could be regarded as a sustainable agriculture (ANDOW and HIDAKA, 1989). In such traditional farming, frequency of insect pest outbreaks has been believed to be low by the farmers. However, systematic research has scarcely been conducted on the population dynamics of insect pests in organically fertilized farm lands (SUGIMOTO et al., 1984; HIDAKA, 1990).

Recently, we found that the population densities of the brown planthopper, *Nilaparvata lugens* (BPH) and the white-backed planthopper, *Sogatella furcifera* (WBPH) in an organically farmed field were much lower than those in the other fields (KAJIMURA et al., 1993). It seemed that the low densities of planthoppers were probably due to specific growth patterns and unique nutritional requirements rather than the actions of natural enemies.

This prompted us to further investigate the relationships between the reproductive rate of these planthoppers and their nutritional requirements. Amino acids are considered to be of particular importance in the population increase of insects that feed on phloem sap

(BRODBECK and STRONG, 1987). Since BPH and WBPH feed on phloem sap of rice plants, we used amino acid as a marker for population change.

We assumed that the nitrogen content of rice plants, particularly that of amino acids, was one of the major factors affecting the population density of planthoppers. We therefore compared the nitrogen content among rice plants cultivated by different methods. Furthermore, amino acid content in the phloem sap of the potted rice plants cultivated with soil and seedlings of the chemically fertilized or the organically grown operations was analyzed, and the survival rate of planthoppers on the potted rice plants was estimated.

MATERIALS AND METHODS

1. Field experiments

Experiment fields: The field experiments were conducted in chemically fertilized, poultry-manured and non-fertilized rice fields, and in an organically farmed rice field located at the Okayama Prefectural Agricultural Experimental Station in Sanyo-cho, Okayama and in Okayama city, respectively, in 1991 (for detail see KAJIMURA et al., 1993). The rice plants, *Oryza sativa* L. var. Akebono, were cultivated in all fields as described previously (KAJIMURA et al., 1993). The fertilizers were applied as follows.

In the organic field, dried poultry manure (1.75 t/ha), rapeseed meal (0.2 t/ha) and straw wastes (ca. 7 t/ha) (80 kg/ha in N base) were applied in December 1990, about 6 months before transplantation. The field was plowed 3 or 4 times during the winter and following spring.

Chemical fertilizers were applied for basal dressing (N, P, K = 65, 30, 55 kg/ha) and for top dressing at ear formation (N, K = 32, 40 kg/ha) in the chemically fertilized field. In the poultry-manured field, 5 t/ha of dried poultry manure (ca. 100 kg/ha in N base) was applied only for basal dressing. No fertilizers have been applied in the non-fertilized field since 1989.

Detection of total nitrogen content in the leaves and stems of rice plants in the fields: Twenty stems with leaves were collected randomly 5 times between July and September from all fields tested in 1991. The samples collected were washed with distilled water, dried at 90°C for 48 h and powdered. Total nitrogen content in the samples was measured with a CN analyzer (Yanaco, MT-300).

2. Experiment on potted rice plants

Cultivation: Rice plants (var. Akebono) were cultivated in plastic pots (10 cm dia. × 16 cm ht.) in 1993 as follows.

1) Chemical cultivation: Thirty-one-day-old chemically fertilized seedlings (N, P, K = 0.02, 0.02, 0.033% of soil weight) cultivated in a seedling box were transplanted to pots from fields with N, P, K applications of 66 kg/ha on June 19.

2) Organic cultivation: Forty-day-old seedlings obtained from the organic field were potted on the same soil on June 24.

3) Chemical-organic cultivation: Thirty-one-day-old chemically fertilized seedlings were transplanted to the pots with the organic farm soil on June 19.

The soil from the organic field was taken in mid-May. In all experiments, 3 seedlings were transplanted to each pot. The pots were kept in a container (51 × 36 × 29 cm) with 10 cm of water under field conditions.

Collecting the rice plant phloem sap and amino acid analysis: Three to 5 BPH adults were confined in a small plastic cage (1 × 1 cm, 7 cm ht.) with a mature leaf sheath near the base of the potted rice plants. The sap was collected on July 13 to 15 in 1993 as follows. While the planthoppers were feeding on a rice plant, their stylets were severed with a YAG laser beam (NEC, SL443Nd) (KAWABE et al., 1980). Sap exuded from the cut and formed a drop. This was collected with a 5-μl glass capillary tube (Microcaps®, Drummond Scientific Co., U.S.A.). One to 3 μl of the sap was used for analysis as follows. The sap was transferred to a small glass vial containing 100 μl of 80% ethyl alcohol. After the ethyl alcohol was evaporated at 40°C, the sap was stored at −20°C. Amino acids were analyzed with an amino acid analyzer (Jeol, JLC-300). Sap was dissolved in 150 μl of lithium citric acid buffer (pH 2.2), and 50–100 μl of the solution was injected into the analyzer. The combined contents of cysteine and cystine in some samples were determined as cysteic acid by oxidation with performic acid (SCHRAM et al., 1954). The concentrations of amino acids were averaged for 5 to 6 potted rice plants in each of the 3 treatments mentioned in the previous section.

Survival rate of planthopper nymphs on the potted rice plants: Each pot contained 3 rice plants, and was caged with nylon gauze. Eight to 10 potted rice plants from 2 treatments (the chemical and organic cultivations) were used to examine the survival rate of planthopper nymphs. Twenty nymphs of WBPH or BPH within 24 h after hatching were introduced into each cage on July 10 and 18, 1993, respectively. The cages were kept at 25°C and 16L–8D conditions. The number of adults and wing form were examined after emergence.

RESULTS

Total nitrogen content in the leaves and stems of the rice plants in the fields

Figure 1 shows changes of the nitrogen content in leaves and stems of the rice plants in the chemically fertilized, poultry-manured, non-fertilized and organic fields. The nitrogen contents of the rice plants were lowest in the organic field from July to September, except in early July when they were almost the same as those of the non-fertilized field. The nitrogen content in the chemically fertilized field was highest from July to September.

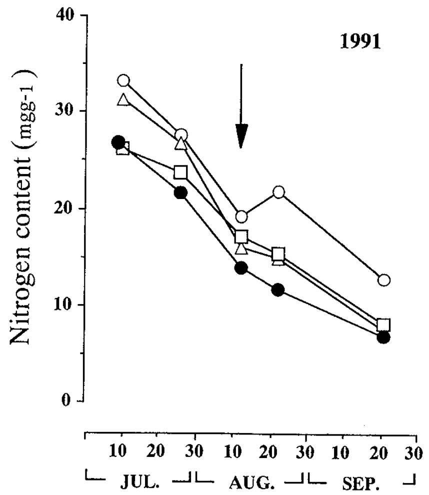

Fig. 1. Changes of nitrogen content in the rice leaves and stems. Arrows indicate the time of top dressing at ear formation in the chemically fertilized field. ○: Chemically fertilized field, △: poultry-manured field, □: non-fertilized field, ●: organically farmed field.

Table 1. Amino acid content in the rice phloem sap of potted rice plants (1993)

Amino acid	Amino acid content (mM)					
	Chemical cultivation		Organic cultivation		Chemical-organic cultivation	
	($x \pm$ SE)	(%)	($x \pm$ SE)	(%)	($x \pm$ SE)	(%)
ASP	25.1±1.0	(16.7)	23.0±1.9	(17.6)	27.5±2.4	(16.1)
THR	7.4±1.0	(4.9)	5.8±0.9	(4.4)	5.6±0.3	(3.3)
SER	20.1±3.1	(13.4)	20.0±1.6	(15.3)	24.4±1.6	(14.3)
ASN[a]	8.0±2.0 c	(5.3)	2.1±0.4 d	(1.6)	7.3±3.2 cd	(4.3)
GLU	24.3±1.9	(16.2)	20.5±2.6	(15.7)	25.6±3.1	(15.0)
GLN	18.8±4.4	(12.5)	13.0±0.6	(10.0)	20.6±3.3	(12.0)
PRO	2.2±0.4	(1.5)	3.0±0.4	(2.3)	2.9±0.2	(1.7)
GLY	0.3±0.1	(0.2)	0.4±0.03	(0.3)	0.7±0.02	(0.4)
ALA	11.5±3.7	(7.7)	7.6±0.9	(5.8)	15.4±1.6	(9.0)
VAL	6.2±0.9	(4.1)	7.1±0.9	(5.4)	5.4±0.5	(3.2)
CYSH[b]	2.0±0.3	(1.3)	1.6±0.2	(1.2)	1.8±0.3	(1.1)
MET	0.5±0.1	(0.3)	0.7±0.03	(0.5)	0.7±0.06	(0.4)
ILE	3.6±0.6	(2.4)	4.3±0.6	(3.3)	3.0±0.4	(1.8)
LEU	3.7±0.7	(2.5)	4.5±0.6	(3.4)	3.0±0.4	(1.8)
TYR	2.1±0.4	(1.4)	3.1±0.2	(2.4)	3.2±0.2	(1.9)
PHE	1.6±0.2	(1.1)	1.9±0.3	(1.5)	1.7±0.1	(1.0)
HIS	2.0±0.2	(1.3)	1.8±0.3	(1.4)	1.5±0.1	(0.9)
LYS	5.3±0.7	(3.5)	5.7±0.7	(4.4)	5.2±0.4	(3.0)
TRP	1.1±0.2	(0.7)	1.0±0.2	(0.8)	0.6±0.1	(0.4)
ARG	5.3±0.8	(6.1)	4.3±0.4	(4.4)	4.3±0.3	(4.0)
Total	150.1±17.4	(100)	130.6±8.4	(100)	171.1±11.7	(100)

[a] Means followed by the same letter are not significantly different at $p = 0.05$ by KRUSKAL-WALIS test.
[b] Combined content of cystaine and cystine was measured as cysteic acid (see Methods).

Amino acid content in the phloem sap of the potted rice plants

Table 1 shows the amino acid content and its composition in the phloem sap of the potted rice plants in the chemical, organic and chemical-organic cultivations. The amount of total amino acids in the organic cultivation was the smallest among the 3 treatments, although the difference was not statistically significant. The content of asparagine was significantly lower in the organic cultivation than in the chemical one ($p < 0.05$, KRUSKAL-WALIS test). The percentage of asparagine also tended to be lower in the organic cultivation. Contents of some other amino acids, e.g., glutamine, glutamic acid and alanine, in the organic cultivation tended to be lower than those in the chemical one, although the differences were not statistically significant. The amount of total amino acids and asparagine content in the chemical-organic cultivation tended to be larger than that in the organic one.

Survival rate of planthopper nymphs on potted rice plants

The survival rate of nymphs and the percentage of the brachypterous female adults of WBPH and BPH on the potted rice plants were compared between chemical and organic cultivations (Table 2). The survival rate was significantly lower on the organic than on the chemical cultivation ($p < 0.01$ for WBPH; $p < 0.05$ for BPH, Z-test). Furthermore, the percentages of brachypterous females of both WBPH and BPH tended to be lower on the

Table 2. Survival rate during nymphal period and percentage of emerged brachypterous female WBPH and BPH adults reared on chemically and organically cultivated potted rice plants (1993)

Planthopper	Treatment	Survival rate (%)		N	Percentage of emerged brachypterous female adults		N
WBPN	Chemical cultivation	64.5	a	200	53.8	n.s.	80
	Organic cultivation	48.5		200	44.7		47
BPN	Chemical cultivation	72.5	b	160	60.6	n.s.	71
	Organic cultivation	61.0		200	50.9		57

a: $p < 0.01$, b: $p < 0.05$, n.s.: not significantly different at $p = 0.05$ by Z-test.

organic than on the chemical cultivation, although the difference was not statistically significant (Table 2).

DISCUSSION

KAJIMURA et al. (1993) suggested that the extremely low density of BPH and WBPH in the organically farmed field may not be due to the actions of natural enemies, but to other factors such as the nutritional conditions of the rice plants. The objective of our study was to identify the nutritional conditions that differ between the organic and chemically-fertilized fields. Furthermore, if there are differences in nutritional conditions, it is important to know how they affect the biotic performance of the planthoppers.

Our study indicated that the nitrogen content of the rice plants was lower in the organically farmed field than that of the chemically fertilized, poultry-manured and non-fertilized fields (Fig. 1). It has been reported that the amount of nitrogen applied to host plants influenced various biotic performances of planthoppers, for example, the reproduction, fecundity, host selection, survival and feeding rates (FUJIWARA and NODA, 1968; SUGIMOTO and YAMAZAKI, 1969; KANNO et al., 1977; SOGAWA, 1970 a). The reproductive rate of WBPH was lower in the organic field (KAJIMURA et al., 1993), and this may be due to a lower level of the nitrogen content of organically grown rice plants. In our study the survival rate of the nymphs was lower on the potted organic rice plants than on those from the chemical cultivation (Table 2). In the preliminary experiment, we found that the fecundity of a WBPH female was lower on the potted rice plants from the organic cultivation (KAJIMURA, unpublished).

The amino acid content in the phloem sap of the potted rice plants was compared among the chemical, organic and chemical-organic cultivations. Asparagine content was significantly lower in the organic cultivation than in the chemical cultivation. SOGAWA and PATHAK (1970) reported that Mudgo, a rice variety resistant to BPH, contained a smaller amount of amino acids and a lower level of asparagine. Tadakan, another rice variety resistant to the green leafhopper *Nephotettix cincticepts,* contained a smaller amount of total amino acids and almost no asparagine (CHINO et al., 1987). It is reported that asparagine promotes feeding activity of BPH (SOGAWA, 1970 b, 1972). KENNEDY (1958) pointed out that nutritional elements of plants affect aphids not as a direct form of nutrition but as a sensory stimuli for feeding. SOGAWA (1970 a) reported that the feeding rate of BPH was extremely low on rice plants with a low nitrogen content, and suggested that this was caused not by a direct effect of low nutrition but by an inhibitory effect on feeding. The lower survival rate of nymphs on potted rice plants of organic cultivation may be caused by a lower feeding rate by planthoppers.

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