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Lipid in the Brown Planthopper, *Nilaparvata lugens* STÅL (Hemiptera: Delphacidae) I. Lipid Contents and Composition in Two Wing-Forms, Brachypterous and Macropterous

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Total lipid content and its constituent in the brown planthopper, *Nilaparvata lugens*, were analyzed by thin layer chromatography (TLC) and gas liquid chromatography (GLC). The planthoppers analyzed were newly emerged adults of both sexes of two wing-forms, brachypterous and macropterous. All of them were reared throughout their nymphal stage with young rice seedlings at 25°C, 14 hr illumination. Total lipid was extracted with 2 : 1 chloroform-methanol (v/v). Total lipid contents on a dry weight basis in a brachypterous male, macropterous male, brachypterous female and macropterous female were 23.5%, 30.4%, 21.4% and 30.9%, respectively. The lipids were separated to neutral lipids (NL) and polar lipids (PL), and their constituents were determined by TLC. Hydrocarbon, triglyceride, free fatty acid, sterol, diglyceride and monoglyceride were found in NL, phosphatidylethanolamine, phosphatidylcholine, lysophosphatidyl-ethanolamine, sphingomyeline and lysophosphatidylcholine in PL. The fatty acid composition in NL, PL and triglyceride was analyzed by GLC. The main fatty acids were $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ in all three classes of lipids. About 80% was occupied by $C_{16:0}$ and $C_{18:1}$ in NL and triglyceride, while more than 60% by only $C_{18:2}$ in PL. Triglyceride was analyzed by GLC, and the carbon numbers of acyl moieties were determined to be 46, 48, 50, 52, 54 and 56.

INTRODUCTION

The phenomenon of mass flight by planthoppers has long been known in Japan. This phenomenon can be characterized as follows. A large number of macropterous planthoppers are captured sometimes in light traps, especially from June to July, and the phenomenon has been usually conspicuous in the western area of Kyushu Island. Most planthoppers captured are the brown planthopper, *Nilaparvata lugens* and the white-back planthopper, *Sogatella furcifera* which are both rice plant pests.

Since a great number of flying planthoppers were observed on a meteorological research vessel located at least 450 km away from the mainland of Japan in June, 1967 (MIYATA et al., 1967), a survey for planthopper on the East China Sea was commenced, and many flying planthoppers were collected from June to July (KISHIMOTO, 1971).

Although the origin of the flying planthoppers over ocean and the relationship between mass flights on land and over the ocean is still not clear, it must be a fact that many planthoppers fly over the ocean at least from June to July.

As the energy source for flight, Dipterous and Hymenopterous insects consume carbohydrates, while fat is an important energy source for flight in other orders of insects (JOHNSON, 1969). For example, components ascertained as the energy source were glycogen in *Drosophila*, some glycogen and lipid in the desert locust, *Schistocerca gregaria* (WEIS-FOGH, 1952) and lipid in the bean aphid, *Aphis fabae* (COCKBAIN, 1961).

In the present study, we focussed on the lipid of the brown planthopper belonging to the same order with aphids. The lipid contents and composition were analyzed and compared between adults of both sexes of markedly different wing-forms, brachypterous (unable to fly) and macropterous (able to fly).

MATERIALS AND METHODS

Insects: The brown planthoppers, *Nilaparvata lugens*, collected in September, 1969 at a paddy field in Kyushu Agricultural Experimental Station, Fukuoka Prefecture, have been reared successively in rearing bottles (480 cm^3 in volume) at 25°C, 14 hr illumination in our laboratory. Rice seeds of the variety, Kinmaze, were germinated in the bottles, and served as food and also oviposition site. All the insects used for analyses were two wing-forms, brachypterous and macropterous, of both sexes within one day after emergence.

Lipid extraction: Extraction of lipid from the insect bodies was performed according to FOLCH et al. (1957). Total lipid was extracted from whole insect bodies three times with 2 : 1 chloroform-methanol (v/v) (CM) in an ice-cold glass homogenizer. The three extracts were combined and concentrated under reduced pressure at 40°C. The concentrate was dissolved in CM, and extracted with water to remove soluble substances.

Lipid separations: The total lipid was separated by silicic acid column chromatography. Silicic acid (100 mesh, Malinckrodt) was washed five times with water and twice with methanol, and then activated at 110°C for 6 hr. One gram of the activated silicic acid was packed with chloroform in a column of 5 mm in diameter and 10 cm in height. Neutral lipids (NL) were first eluted with chloroform (40 ml), and then polar lipids (PL) with methanol (20 ml).

Thin layer chromatography (TLC): Components of NL and PL were separated by TLC. Glass plates coated with 250 μ thin layer of Kieselgel G (Merck) were activated at 120°C for 6 hr. Solvent systems for developing were petroleum ether/ether /acetic acid (80 : 30 : 1) for NL, and chloroform/methanol/water (65 : 25 : 4) for PL. Separated spots were detected by color reaction with 50% sulfuric acid for all lipids, ZINZADE's reagent for phospholipids, ninhydrin solution for amines, DRAGENDROF's reagent for cholines, anthron solution for glycolipids and antimony trichloride solution for steroids, respectively. Rf values of the spots were compared with those of authentic samples. The relative amount of each spot of NL and PL was determined by a photodensitometer (photoelectric microphotometer, Shimadzu). Spots of TG, faintly colored with iodine vapor, were scraped off, and extracted with chloroform.

Preparation of methyl esters of fatty acids: NL, PL and TG fractions were converted to their methyl esters by refluxing in 5% HCl in anhydrous methanol at 90°C for 2 hr. After the reaction was completed, 1.5 ml of water was added to the reaction mixture.

The methyl esters were then extracted three times with petroleum ether and washed seven times with water, and then dried with anhydrous sodium sulfate.

Gas liquid chromatography (GLC): Fatty acid composition, in NL, PL and TG was determined by the GLC, YANAGIMOTO's Model G–80, equipped with a hydrogen flame ionization detector. The column used was 75 cm stainless steel tube packed with 30% DEGS coated on 60—80 mesh chromosorb W. The column temperature was progressed from 60°C to 180°C at the rate of 6°C/min. The flow rates of the carrier gas (helium), hydrogen and air were 35 ml/min, 0.35 kg/cm^2 and 0.9 l/min, respectively. Each peak was identified by comparing with the chromatograms of the authentic methyl esters of fatty acids, and using the linear relationship between relative retention time and carbon chain length (HORNING et al., 1963). Peak areas were calculated by triangulation. The TG fraction was also analyzed by the GLC, YANAGIMOTO's Model G–8 equipped with a hydrogen flame ionization detector. The column was 35 cm stainless steel tube packed with 2% OV–17 coated on 60—80 mesh chromosorb W. The column temperature was progressed from 200°C to 350°C at the rate of 4°C/min. The flow rates of the carrier gas (helium), hydrogen and air were 35 ml/min, 0.35 kg/cm^2 and 1.1 l/min, respectively. Each peak was identified by comparing with the chromatograms of the authentic samples.

RESULTS AND DISCUSSION

Total lipid content on a dry weight basis for the macropterous form was higher than that of the brachypterous form by about 7% in male and by about 9% in female (Table 1). Such an intraspecific difference in lipid content has been known in other insect species. MATTHÉE (1945) recognized that the lipid content of the gregarious phase was higher than that of the solitary phase in two species of locusts, *Locusta migratoria* and *Locustana pardalina*, and two species of moths, *Laphigma exempta* and *Spodoptera abyssinia*. UTIDA and TAKAHASHI (1958) also found that the fly form of the cowpea weevil, *Callosobruchus quadrimaculatus* contained higher amounts of lipid than the non-fly form.

The amount of PL in the macropterous form was very close to that in the brachypterous one, and the difference of the total lipid content between the two wing-forms was due to the highly cumulated NL in the macropterous one (Table 1).

The following eight components were detected in NL by TLC: hydrocarbon

Table 1. TOTAL LIPID, NEUTRAL LIPID AND POLAR LIPID CONTENTS OF *N. lugens*

	Male		Female	
	B[b]	M[c]	B[b]	M[c]
Number of insects	317	321	194	203
Fresh weight (mg)	300.5	305.5	299.9	304.5
Total lipid content (%)[a]	23.46	30.38	21.39	30.43
Neutral lipid content (%)[a]	15.90	23.59	14.46	24.62
Polar lipid content (%)[a]	7.55	6.78	6.93	5.81

[a] Dry weight basis.
[b] Brachypterous form.
[c] Macropterous form.

(H), triglyceride (TG), unknown compound (X_1), free fatty acid (FFA), sterol (S), diglyceride (DG) and monoglyceride (MG) (Fig. 1a). In every case the relative amount of TG was more than 73% of NL, while all other components constituted less than 12% (Table 2).

The results of color reaction against four spraying reagents revealed that all the components of PL were phospholipids (Table 3). The components detected were as follows: unknown compound (X_2), phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylethanolamine (LPE), sphingomyeline (SPM) and lysophosphatidylcholine (LPC) (Fig. 1b). PC (38—49%) and PE (20—26%)

Table 2. Percentage of Each Fraction in Neutral Lipid of *N. lugens*

		H	TG	X_1	FFA	S	DG	MG[a]
Male	B	3.8	81.0	1.2	2.6	3.5	5.9	1.9
	M	9.0	76.4	0.7	4.2	1.0	7.3	1.2
Female	B	11.6	74.1	1.2	3.4	1.5	7.9	0.3
	M	11.7	73.2	2.0	3.8	2.7	4.7	1.8

[a] See Fig. 1 as regards the abbreviations in the table.

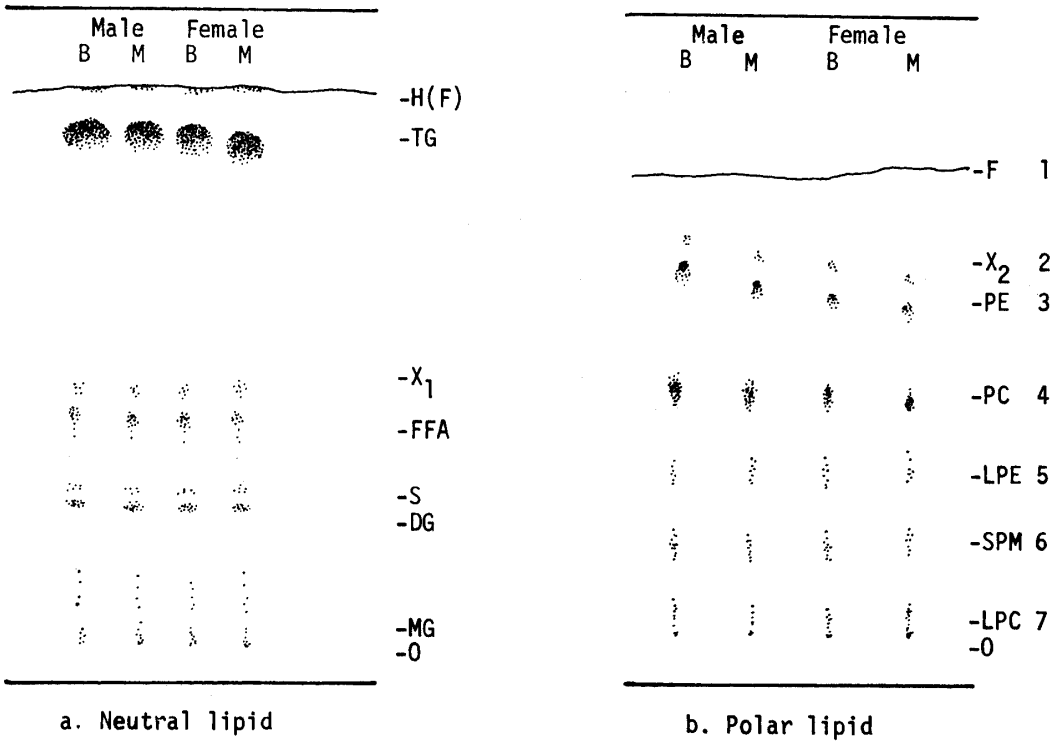

Fig. 1. Thin layer chromatograms of neutral lipid and polar lipid of *N. lugens*.
NL: 0-origin, MG-monoglyceride, DG-diglyceride, S-sterol, FFA-free fatty acid, X_1-unknown, TG-triglyceride, H-hydrocarbon, F-solvent front. PL: 0-origin, LPC-lysophosphatidylcholine, SPM-sphingomyeline, LPE-lysophosphatidylethanolamine, PC-phosphatidylcholine, PE-phosphatidylethanolamine, X_2-unknown, F-solvent front.

Table 3. COLOR REACTIONS OF POLAR LIPID AS DETECTED BY FOUR REAGENTS[a]

Spot number[b]	ZINZADE's reagent	Ninhydrin reagent	DRAGENDORFF's reagent	Anthrone reagent
1	−	−	−	−
2	+	−	−	−
3	+	+	+	−
4	+	−	+	−
5	+	+	−	−
6	+	−	±	−
7	+	−	±	−

[a] + : Positive, − : Negative.
[b] Refer to Fig. 1.

Table 4. PERCENTAGE OF EACH FRACTION IN POLAR LIPID OF *N. lugens*

		X_2	PE	PC	LPE	SPM	LPC[a]
Male	B	4.1	21.7	48.5	4.7	12.1	8.9
	M	5.8	23.7	38.5	6.1	14.0	11.9
Female	B	4.7	25.7	44.2	4.7	13.0	7.6
	M	6.2	20.3	45.7	9.0	11.3	7.4

[a] See Fig. 1 as regards the abbreviations in the table.

Table 5. FATTY ACID COMPOSITION (%) IN TRIGLYCERIDE, NEUTRAL LIPID AND POLAR LIPID OF *N. lugens*

Fatty[a] acid	TG				NL				PL			
	Male		Female		Male		Female		Male		Female	
	B	M	B	M	B	M	B	M	B	M	B	M
C 16:0	35.3	36.8	33.8	34.9	35.9	35.7	43.7	41.0	9.4	9.4	8.5	9.0
C 16:1	4.2	2.9	4.0	2.8	1.4	1.9	2.2	1.8	4.9	2.0	2.7	2.1
C 18:0	5.7	5.5	5.0	7.2	5.6	5.3	7.5	4.9	7.9	10.6	11.0	10.0
C 18:1	44.2	44.7	45.2	43.7	45.7	47.0	37.3	45.9	15.7	14.9	13.8	13.7
C 18:2	10.6	10.0	12.0	11.5	11.4	10.2	9.3	6.4	62.2	63.0	64.0	65.3

[a] Fatty acids having carbon chain length less than 16 and those more than 18 were omitted because of their little amounts.

were dominant, and SPM (11—14%) was the next dominant (Table 4). These identified components in NL and PL were similar to those found in the rice stem borer, *Chilo suppressalis* (OOUCHI et al., 1970; OOUCHI and ITO, 1970).

Sixteen kinds of fatty acids were detected in NL, PL and TG, respectively: i. e., $C_{6:0}$, $C_{6:1}$, $C_{8:0}$, $C_{8:1}$, $C_{10:0}$, $C_{10:1}$, $C_{12:0}$, $C_{12:1}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{20:0}$. Among them the main fatty acids were $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$, while the others were mostly less than 1%. In the pattern of fatty acid composition TG was similar to that of NL, and $C_{16:0}$ and $C_{18:1}$ were the main components (Table 5). The pattern of fatty acid composition of NL in the brown planthopper resembled those of seven species of leafhoppers (NOGUCHI et al., 1968; STRONG, 1963). On the

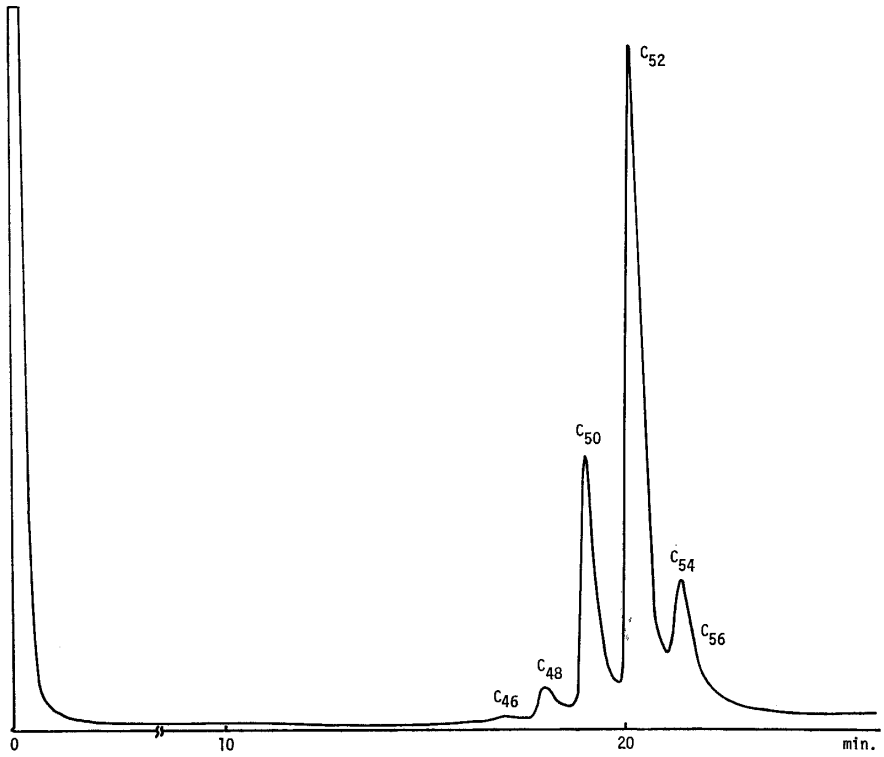

Fig. 2. A chromatogram of triglyceride in the macropterous female of *N. lugens*.

Table 6. Triglyceride Composition (%) of *N. lugens*

		Carbon numbers of acyl moiety					
		46	48	50	52	54	56
Male	B	0.8	4.0	26.4	51.3	17.6	trace
	M	1.2	4.2	23.3	55.6	15.6	trace
Female	B	0.6	3.8	23.2	55.2	17.1	trace
	M	0.4	4.4	21.8	48.7	24.8	trace

other hand, the pattern is quite different from 21 species of aphids having C_{14} as the main component (Strong, 1963), and from scale insects having C_{11}, C_{12} or C_{14} as the main component (Hashimoto et al., 1967, 1968; Tamaki and Kawai, 1967). The content of $C_{16:1}$, which was very large in amount in *C. suppressalis* (Kuwahara and Ishii, 1968; Oouchi et al., 1970), was less than 5% in the brown planthopper. In PL, fatty acids shorter than 16 in carbon chain length and those longer than 18 were very little in amount like those in NL and TG. But a point of difference in fatty acid composition of PL from NL and TG was that more than 60% was $C_{18:2}$, and $C_{16:1}$ and $C_{18:1}$ were considerably less than those in NL or TG (Table 5). $C_{20:0}$ was the longest in fatty acids of NL, TG and PL in the brown planthopper, and fatty acids from C_{22} to C_{26} detected in *C. suppressalis* (Oouchi and Ito, 1970) were not detected at all.

One example of the gas liquid chromatogram of TG in the brown planthopper is shown in Fig. 2. Carbon numbers of acyl moieties in TG were 46, 48, 50, 52, 54 and

56. Among them total amounts of 50, 52 and 54 were more than 92% (Table 6). It is inferred from this result that triglycerides are mainly consisted of fatty acids having C_{16}, C_{16} and C_{18}, those having C_{16}, C_{18} and C_{18}, and those having C_{18}, C_{18} and C_{18}.

It is concluded from our present study that no systematic quantitative or qualitative change related to wing-form or sex was detected in the constituents of NL and PL, fatty acid composition of NL, PL and TG, and carbon number of acyl moieties in TG. However, the highly cumulated NL mainly consisted of TG in the macropterous form of both sexes might have a significant meaning for their flightactivity.

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