Lipid of the Rice Stem Borer, *Chilo suppressalis* WALKER (Lepidoptera : Pyralidae) I. Lipid classes and Fatty acid composition in larvae reared on rice seedlings. Haruka OOUCHI, Tetsuo SAITO and Kisabu IYATOMI (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan) Received November 26, 1969. *Botyu-Kagaku* 35, 7, 1970.

2. ニカメイガの脂質に関する研究 I. イネ芽出し飼育によるニカメイガ幼虫の構成脂質と脂肪酸組成 大内 晴，斎藤哲夫，弥富喜三（名古屋大学農学部 害虫学研究室 名古屋市千種区不老町）44. 11. 26 受理

イネ芽出し苗で飼育したニカメイガの老熟幼虫の脂質を薄層クロマトグラフィー (TLC) とガスクロマトグラフィー (GLC) 法で調べた．また，飼育にもちいたイネ芽出し苗の脂肪酸を調べ，幼虫の体構成脂肪酸と比較論じた．

1) クロロホルム—メタノール (2:1) 抽出で，総脂質として幼虫生体重の約 6.3% が得られた．

2) ニカメイガ幼虫の構成脂質として以下の7つを分離同定した．トリグリセリド (81%)，ジグリセリド (3.8%)，モノグリセリド (4.9%)，コレステロール (6%)，リン脂質 (5%) 及び極少量の遊離脂肪酸と炭化水素があった．

3) 体構成主要脂肪酸は $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{20:0}$ であった．そのうち $C_{16:1}$ は全脂肪酸の約 35% であった．

4) 一方，イネ芽出し苗の $C_{16:1}$ は非常に少なく，僅か 0.4% 以下であった．

5) また幼虫のトリグリセリド中の脂肪酸の組成とリン脂質中のそれにはかなりの差異があった．

Introduction

Many workers have reported on the lipid of lepidopterous insects[1,2] but little is known about the lipids of the rice stem borer, *Chilo suppressalis* WALKER. NOGUCHI, TAMAKI and YUSHIMA[3], and KUWAHARA and ISHII[4] have observed the fatty acid composition in the rice stem borer. KUWAHARA and ISHII have found that C_{16} monoenoic acid was a characteristic fatty acid in the borer, and that the proportion of the monoenoic acid decreased in the larvae fed on synthetic medium without fatty materials.

In this point of view, the present paper mainly dealt with the composition of fatty acids in larvae of the rice stem borer in relation to that in rice seedlings as their food with special reference to C_{16} monoenoic acid.

Materials and Methods

Insect used

Egg masses of the rice stem borer, *Chilo suppressalis* WALKER used for the present experiments were collected from paddy fields at Okazaki city in Aichi prefecture. After their hatching, they were successively reared on the rice seedlings (Norin No. 18) under constant illumination for 16 hrs at 27°C and 85% in relative humidity.

Plant used

The rice seedlings, *Oriza sativa* L. used for lipid extraction were 5 days after germination at 27°C.

Lipid extraction

Seventy-five individuals of the full grown larvae which had been starved for 4 hrs were anesthetized by CO_2, and their lipids were extracted 4 times in an ice-cold glass homogenizer with 4 volumes of chloroform-methanol (2:1). The four extracts were mixed together and concentrated by a rotary evaporator under reduced pressure at 40°C. The concentrate was redissolved in petroleum ether and washed 4 times by the method of FOLCH[5]. The petroleum ether solution was dried with anhydrous sodium sulfate and the solvent was removed under reduced pressure at 40°C. The residue was redissolved in chloroform and stored at 5°C until use.

Lipid separations

Simple lipids were extracted 3 times with acetone from the chloroform stock solution, and the precipitated phospholipids were separated by centrifugation. The separation of triglycerides was carried out by thin-layer chromatography (TLC), 20×20 plates coated with 10g of silicagel

GF (Merck) activated at 110°C for 1hr. The developing solvent system was petroleum ether/ether/acetic acid (80:20:1), and the spots were detected under UV-light or heating with 25% $SbCl_3$-$CHCl_3$ and $K_2Cr_2O_7$-H_2SO_4.

Gas-lipuid chromatography (GLC)

Methyl esters of the fatty acids were prepared from the lipids by transesterification with 5% HCl in methanol under reflux at 80°C for 2 hrs[6]. The methyl esters were analysed by gas-liquid chromatography (GLC) equiped with hydrogen flame ionization detector. The operation conditions of the GLC were as follows:

Model:	Japan Electron Optics Laboratory JGC-750
Detector:	FID
Column:	Stainless steel 2m×3mm ID
	Temp. 155°C
Packing:	12% DEGS
Support:	ChromosorbW 60-80 mesh
Carrier Gas:	N_2 10kg/cm²
H_2 Flow Rate:	30ml/min.
Air Flow Rate:	0.5 l/min.
Detector Temp:	233°C
Chart Speed:	5mm/min.

The fatty acid methyl esters were identified by comparing with chromatogram of the authentic fatty acid methyl esters, and plotting the logarithm of their relative retention times against carbon chain length. Peak areas were calculated by triangulation.

The authentic samples used for TLC and GLC analysis were all reagent grade. Tripalmitin, cholesterol and cholesteryl acetate were purchased from Wako Junyaku Kogyo Co. Ltd., and monostearin and palmitic acid were from Katayama Kagaku Kogyo Co. Ltd. Fatty acid methyl esters of rice plant lipid were prepared by the same method described above. The authentic samples of fatty acid methyl esters and dipalmitin were supplied by courtsy of Sericultural Laboratory, Faculty of Agriculture, Nagoya University.

Results and Discussion

The total lipid extracted from 75 larvae with chloroform-methanol (2 : 1) was 392 mg, which comprised about 6.3% of the fresh weight.

Results of TLC of the lipids of the rice stem borer showed the following components: Hydrocarbons (H), Triglycerides (TG), Free fatty acids (FFA), Diglycerides (DG), Cholesterol (CS), Monoglycerides (MG) and Phospholipids (PL). It was shown that

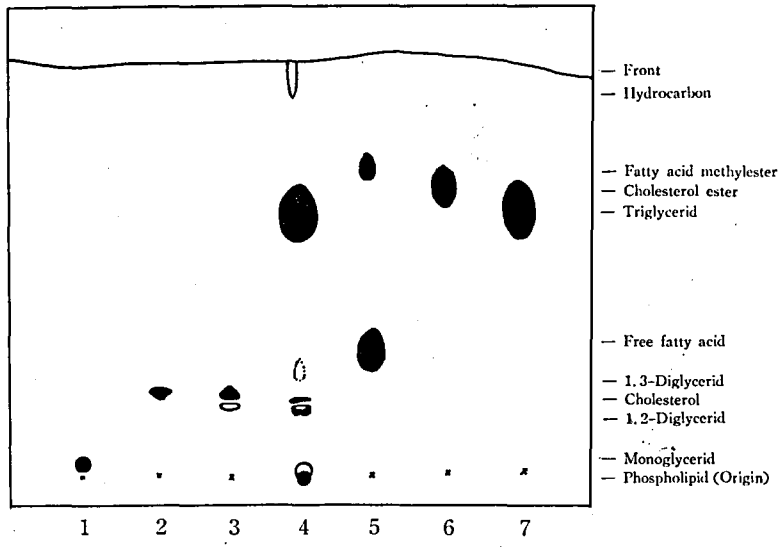

Fig. 1. TLC of the rice stem borer lipid. Solvent system: Petroleum ether/ether/acetic acid (80 : 20 : 1). 1. Monostearin; 2. Cholesterol; 3. Dipalmitin; 4. Insect lipids; 5. Palmitic acid and Fatty acid methyl esters; 6. Cholesterol acetate, and 7. Tripalmitin.

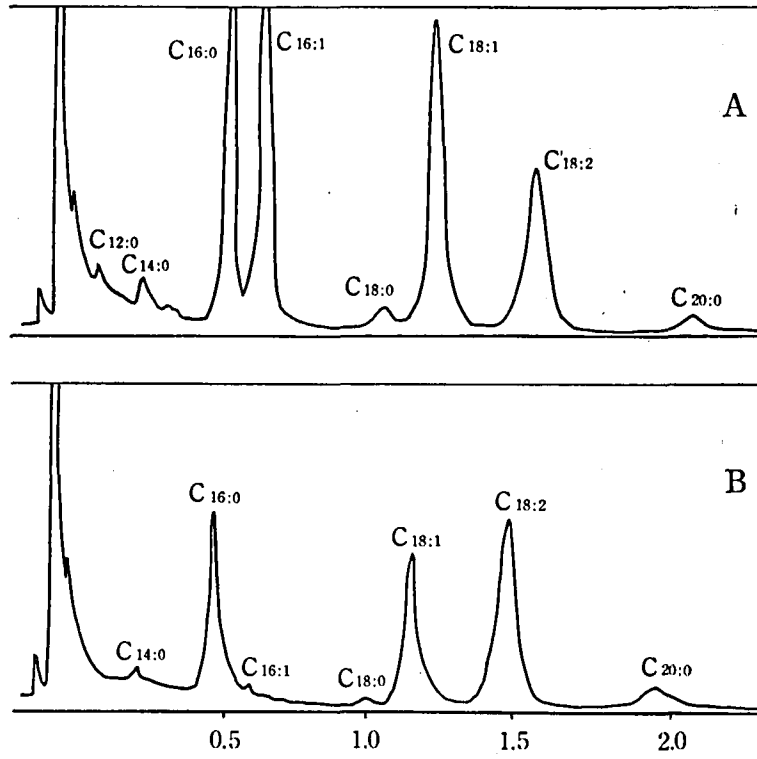

Fig. 2. GLC of fatty acid methyl esters (A) in the larvae of the rice stem borer and (B) in the rice reedlings. Relative retention time is related to $C_{18:0}$ (Stearic acid.)

TG fraction (80%) was a major glyceride of the lipids in the rice stem borer as has been demonstrated in other insects[7]. The other components were as follows: DG (3.8%), CS (6%), MG (4.9%), PL (5%) and FFA and H (traces).

The fatty acid composition in diet and larvae

The fatty acid composition of the total lipid extracts from the rice stem borer larvae was shown in comparison with that of food plant in Fig. 2.

The fatty acid composition of the borer, as has been reported previously[3,4], was quite different from that of other lepidopterous insects[1,2,8]. No requirements for lipid excepting cholesterol have been demonstrated in the growth and development of larvae of the rice stem borer[9]. It has been also known that dietary unsaturated fatty acid of C_{18} series were not required[10]. In the present results by GLC, it was found that major acids in the diet were different from those of the larvae. Especially, the proportion of palmitoleic acid was only 0.4% of the fatty acids in the rice plant, whereas it was 36% of the acids in TG of the larvae, being the highest peak.

In this connection, it is likely that rice stem borer larvae can synthesize *de novo* C_{16} monounsaturated fatty acid.

Fatty acid composition of simple lipid (SL), triglycerid (TG) and phospholipid (PL)

The fatty acid composition of each lipid class separated from the larvae of the rice stem borer lipid was shown in Fig. 3. It was evident that the percentage of fatty acids consisting SL was quite similar to that in TG. Whereas the fatty acid composition of PL did not show any resemblance to that of neutral lipid. The phospholipid contained less palmitoleic acid (6%) and more stearic (17%), linoleic (38%) and arachidic acid (9%) as shown in Fig. 3. Regarding to the fatty acid composition of PL observed, it seems that the rice stem borer is also different from other lepidopterous insect.

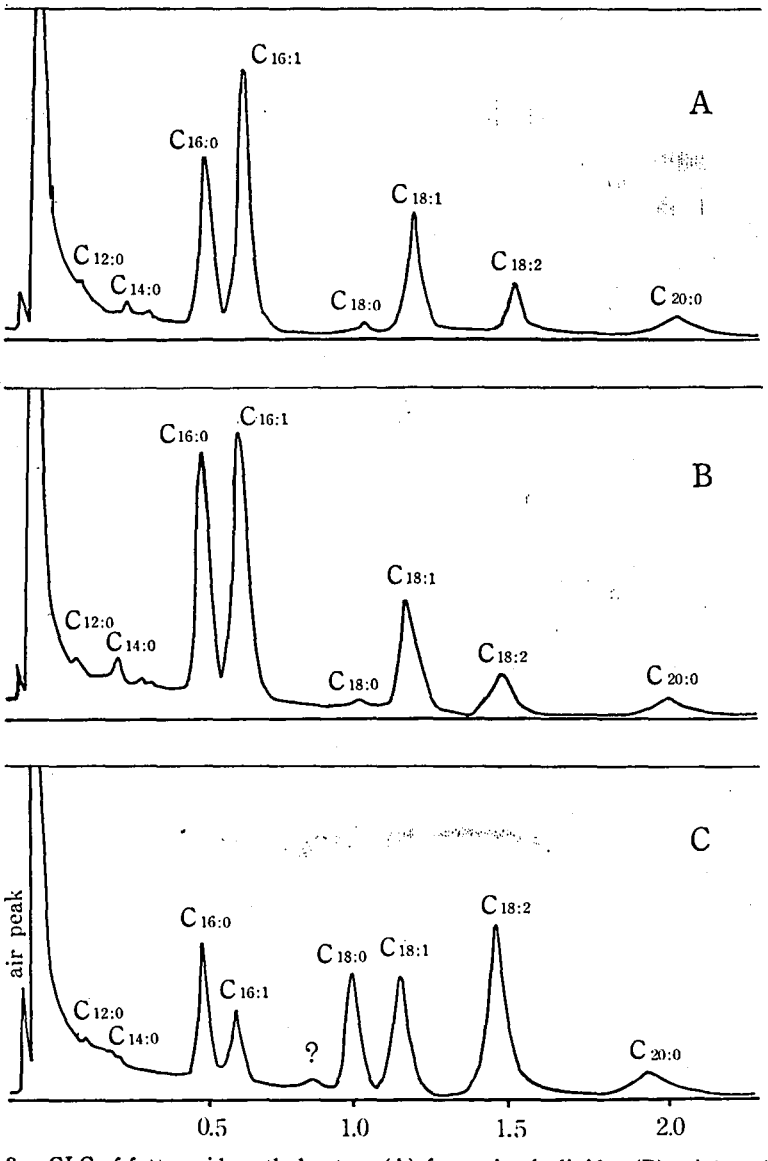

Fig. 3. GLC of fatty acid methyl esters (A) from simple lipids, (B) triglycerides and (C) phospholipids. Relative retention time is same as in Fig. 2.

Summary

Lipid classes and fatty acid composition in the larvae of the rice stem borer, *Chilo suppressalis* WALKER, were investigated by thin-layer and gas-liquid chromatography. Fatty acis composition in the rice seedlings used as diet for the larvae were also determined. Results were as follows:

1) The total lipids extracted from the larvae were separated into 7 components.

2) The major fatty acids in the rice stem borer were palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), oleic ($C_{18:1}$) and linoleic acid ($C_{18:2}$), and $C_{16:1}$ was the most dominant, being 30-36% of the total fatty acid in the neutral lipids.

3) On the other hand, a small amount of $C_{16:1}$ in the rice seedlings was observed, being less than 0.5% of the total.

4) Significant differences were found in the fatty acid composition between triglycerides and

phospholipids.

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Studies on Sex Pheromones of Pyralididae. III. The Inheritance of the Abnormal Sex Ratio Condition in a Strain of the Almond Moth, *Cadra cautella* Walker (Phycitinae). Fumiki Takahashi and Yasumasa Kuwahara (College of Agriculture, Kyoto University, Kyoto) Received Jan. 10, 1970, *Botyu-Kagaku* 35, 11, 1970 (with English Summary 21).

3. メイガ科の性誘引物質に関する研究（第3報） スジマダラメイガの異常性比の遺伝様式*

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30°Cで飼育すると正常な性比（♀:♂=1:1）を示すが，低温（20°C）で飼育するとほとんど雌のみが羽化し，雄は羽化しても交尾器に異常がみられるというスジマダラメイガFT系統の性質を遺伝学的に検討した．野外採集または継代飼育された5系統について，低温感受性をしらべたところ，3系統に世代を通じて遺伝する性比異常がみられた．FT系統の異常性比の性質は母親を通じて細胞質遺伝する傾向の強いことが示された．異常性比は幼虫期と蛹期での雄の高い死亡率に基づいている．FT系統幼虫の体液を性比の正常なスジマダラメイガの他系統の幼虫，およびスジコナマダラメイガとノシメマダラメイガの幼虫に注射したが，異常性比の性質を発現させることはできなかった．また細菌性のものの存在を体液中に確認することができなかった．スジマダラメイガの異常性比の性質を *Drosophila* や *Aedes* での場合と比較して考察した．

マダラメイガ亜科 Phycitinae に属する数種の蛾の性誘引物質の化学的研究と，それらの種間の相互関係の検討が筆者らによって進められている．性誘引物質の単離精製には大量の材料を必要とする．スジマダラメイガ *Cadra cautella* Walker の未交尾雌成虫の大量生産については，桑原ら[10]および高橋ら[23]によって報告されたように，その1系統のもつ性比に関した特別な性質が利用された．すなわち，この系統を 30°C で飼育すると雌雄がほぼ同数羽化するが，低温（20°C）で飼育するとほとんど雌のみが羽化し，雄は羽化しても，その交尾器には多くの異常がみられる[22]という性質である．

飼育条件によってほとんど雌のみを生ずるという1系統の性質が，他の近縁種にもみられる一般的な性質であるならば，その性質は近縁種の性誘引物質の抽出材料の生産を非常に容易にすると考えられた．そのため，その性質を示すFT系統の基礎的特性，とくにその遺伝様式についてしらべた．さらにその性質が細胞質遺伝をする可能性の高いことなどがわかったので，その性質の同種異系統および近縁の他種への伝達の可能性についても検討を加えたので，それらの結果を報告する．

実験材料と飼育方法

用いた材料は京都大学農学部昆虫学研究室において

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