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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 Yamami (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan) Received November 26, 1969. Botyu-Kagaku 35, 7, 1970.

2. Ninkameiga no seibutsu ni kansuru kenkyuu

Introduction

Many workers have reported on the lipid of lepidopterous insects1,2 but little is known about the lipids of the rice stem borer, Chilo suppressalis Walker. Noguchi, Tamaki and Yushima3, and Kuwahara and Ishii4 have observed the fatty acid composition in the rice stem borer. Kuwahara and Ishii have found that C18 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 C18 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, Oryza 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 CO2 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 Folch5. 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 1 hr. 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% SbCl₅-CHCl₃ and K₂Cr₂O₇-H₂SO₄.

Gas-liquid 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. The methyl esters were analysed by gas-liquid chromatography (GLC) equipped 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: N₂ 10kg/cm²
- H₂ Flow Rate: 30ml/min.
- Air Flow Rate: 0.5l/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 courtesy of Sericultural Laboratory, Faculty of Agriculture, Nagoya University.

Results and Discussion

The total lipid extracted from 75 larvae with chloroform-methanol (2:1) was 392mg, 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...
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 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\(^6\)\(^9\), 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\(^8\). 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\(_{16}\) mono-unsaturated fatty acid.

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.
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 acid 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_{18:1} 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_{18:1} 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...
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Condition in Cadra cautella

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References

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

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層に異常がみられるというシンプラメイガ FT系の性質を遺伝学的に検討した。野外採集または雛代飼育された5系統について、低温感受性を示したところ、3系統に世代を通じて異常性比異常がみられた。 FT系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もとが示された。異常性比の雌と雄における高飼育で死亡率が低くなった。 FT系異常性比の雌を正常なシンプラメイガの雄と、およびシンプラメイガの雌に注射したが、異常性比の性質を飼育させることはできなかった。 また飼育条件の存在を体験中に確認することができなかった。シンプラメイガの異常性比の性質を Drosothila や Aedes での場合と比較して考察した。


飼育条件によってほとんどの雌のみを生ずるという1系統の性質が、他の近縁種に見られる一般的な性質であるならば、その性質は近縁の性誘引物質の抽出材料の選択非常に容易に考えられた。それが、その性質を示す FT系の基礎的特性、とくにその遺伝様式について知ることは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、その性質の同系異系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

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