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ASSIMILATION OF CAROTENOIDS WITH FOOD BY THE BEETLE, *LEPTINOTARSA DECEMLINEATA*

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Abstract—The author investigated by means of columnar and thin-layer chromatography the carotenoids in the leaves of *Solanum tuberosum* L. and in larvae and adults of *Leptinotarsa decemlineata.*

The following carotenoids were identified: (a) in the leaves of *Solanum tuberosum*: β-carotene, γ-carotene, lutein (free and ester), and neoxanthin; (b) in larvae of *Leptinotarsa*: α-carotene, β-carotene, γ-carotene, lutein (free and ester), zeaxanthin, and neoxanthin; (c) in adults of *Leptinotarsa*: β-carotene, echinenone, isocryptoxanthin, canthaxanthin lutein (free and ester), and neoxanthin.

The results are discussed in the light of other studies on carotenoid distribution in insects and assimilation of carotenoids with food by *Leptinotarsa.*

INTRODUCTION

IT IS generally accepted that in the water species of animals, carotenoids are introduced into the organism with the food and are then deposited in the various organs (KARRER and JUCKER, 1948; GOODWIN, 1960). The studies made by GOODWIN and SRISUKH (1949), however, showed that locusts are capable of synthetizing astaxanthin. It was in this context that we decided to carry out investigations on the problem of which of the carotenoids consumed with food are assimilated by larval and adult *Leptinotarsa decemlineata* Say., a serious threat to potato crops.

Studies on the carotenoids in *L. decemlineata* have been carried out by PALMER and KNIGHT (1924), MANUNTA and SOLINAS (1952), KARPUNINA (1961), and LEUENBERGER and THOMMEN (1970). These authors, however, reported the presence of β-carotene in the species but did not take into consideration other carotenoids.

MATERIALS AND METHODS

Larvae and adults of *L. decemlineata* were collected in August 1970 from potato fields. The specimens were placed into test-tubes to which acetone was immediately added. Samples of the potato leaves which the specimens had been feeding on were taken at the same time. The material was kept in a refrigerator in a nitrogen atmosphere until the carotenoids contained in it were determined.

Pigment extraction and separation

Columnar and thin-layer chromatography was used for the determination of the carotenoids according to the method previously described (CZECZUGA and

CZERPAK, 1968). The separation of the carotenoids was begun, after the preliminary analysis, by means of column chromatography with activated aluminium oxide (Al_2O_3). The analysis was carried out chiefly on the basis of the method of GREEN (1957) and partly on the basis of the methods of GILCHRIST and GREEN (1960) and IWATA *et al.* (1961).

For the experiments a glass column 1 cm in diameter and 15 to 20 cm long was used. The carotenoid extract freshly prepared in petroleum ether was transferred to the column with Al_2O_3 moistened previously with pure petroleum ether at boiling point 60 to 80°C.

Hydrolysis of the ester compounds of the carotenoids was carried out with 10% potassium hydroxide in methanol, under nitrogen in the dark, at room temperature for 12 hr.

Thin-layer chromatography on silica gel-6 plates was also used to separate and identify the pigments of carotenoids (Merck) according to Ståhl. The carotenoids were saponified with 10% KOH in methanol before separation.

Pigment identification

The pigments were identified by the following methods: (1) Absorption spectra of pigments in various solvents were recorded by a Beckman spectrophotometer, model 2400 DU. (2) Behaviour on column chromatography. (3) Comparison of R_f on thin-layer chromatography with authentic β-carotene and lutein. (4) The partition characteristics of the carotenoid between hexane and 95% methanol using the method of PETRACEK and ZECHMEISTER (1956).

RESULTS

Carotenoids in the leaves of Solanum tuberosum (*Tables* 1, 2)

The acetone extract from *S. tuberosum* was first divided into two layers one of which contained chlorophylls and the other carotenoids. The latter layer was separated into seven fractions by means of columnar chromatography; most of these were identified.

First fraction. This fraction was eluted with pure petroleum ether. Two subfractions were obtained, one yellow and the other pink. Both of these subfractions in a petroleum ether solvent gave absorption maxima at 448 and 475 nm, maxima indicating the presence of β-carotene in the *Solanum* leaves. In view of the data given by GOODWIN (1965) it can be assumed that the extract of the first fraction also contained a negligible amount of α-carotene.

Second fraction. This fraction was eluted from the absorbent with 10% acetone in petroleum ether. This fraction in a hexane solvent gave maxima at 437, 464, and 494 nm, which is, as we know, characteristic of γ-carotene. The partition ratio is 100 : 0.

Third fraction. This fraction was eluted with 30% acetone in petroleum ether. Two maxima were obtained in a hexane solvent, one at 442 nm and the other at 470 nm. According to reported data (WOLFE and CORNWELL, 1965; LEE, 1966) these maxima correspond to those of lutein ester.