# PROTEINS, CARBOHYDRATES AND LIPIDS IN THE LARVAE, PUPAE AND ADULTS OF PECAN WEEVIL, *CURCULIO CARYAE* HORN

A. C. Thompson[1] and P. P. Sikorowski[2]

(Accepted for publication Oct. 31, 1984)

## ABSTRACT

Pecan weevil larvae, in the 4th instar, held at 20°C showed a rapid decline in fatty acids, carbohydrates and proteins with time. Regression analysis of the fatty acid data (with time) gave a best fit for a polynomial expression, whereas both protein and carbohydrate fit a logarithmic curve. The larvae at 20°C pupated in an average of 42 days. The average life span of adults fed sucrose water was 82 days.

Key Words: Pecan weevil, fatty acids, proteins, carbohydrates.

J. Entomol. Sci. 20(1): 42-46 (January 1985)

## INTRODUCTION

Pecan weevil larvae, *Curculio caryae* (Horn), exit from pecan nuts after completing four instars and enter the soil. The insect undergoes an extended life cycle emerging into adults after 2 or 3 years (Harp and Van Cleave, 1976). Understanding biochemical and physiological processes has been difficult due to the long life cycle of the insect. However, methods to shorten the long period of larval and adult inactivity have been, to some degree, successful, because pupation was recently achieved in 5 - 6 months when larvae were held at 20°C (Sikorowski et al. 1982). However, larval mortality caused by pathogens and microbial contaminants was high (42% and higher).

In this study we measured the change in lipids, proteins and carbohydrates of larvae held at 20°C. Previously, Dutcher (1982) studied the total caloric values of larvae, pupae and adults and compared them to the larval food source. He observed a caloric decrease from 466 to 161 cal/weevil during the 22 month subterranean period. Previously, we measured the lipids and fatty acids of larvae and adults collected from the soil and observed that total lipids were reduced from 41% for larvae to 6 - 8% for newly-emerged, unfed insects (Henson et al. 1973).

## MATERIALS AND METHODS

Pecan weevil larvae were collected from infested pecans gathered in October and December from Columbus, Mississippi and West Helana, Arkansas, respectively. Larvae were removed from the nuts, sterilized according to the method of Sikorowski et al. (1982) and incubated in 30-ml plastic cups containing 2% agar at 20°C. Larvae were sampled every 2 weeks (15 insects/sample) and analyzed for

[1] Boll Weevil Research Laboratory, Agriculture Research Service, U. S. Department of Agriculture, Mississippi State, Mississippi 39762-5367.

[2] Department of Entomology, Mississippi State University, Mississippi State, Mississippi 39762.

fatty acids, protein and carbohydrates. One-day-old pupae (5 insects/sample) and adults (5 insects/sample) were analyzed.

Lipids were extracted from individual insects by homogenizing them in a Potter-Elvehjem homogenizer with 20 ml of chloroform-methanol (2:1 v/v) (Thompson et al. 1972). Total lipids and fatty acids were converted to methyl esters with boron trifluoride-methanol.

Protein was analyzed by the method of Bradford (1976) as adapted by Bio-Rad Laboratories[3] (Richmond, California). Five ml of dye reagent was added to the protein sample and mixed. After standing 20 min, the absorption was measured at 595 nm. Concentratioms were determined by comparison with a standard curve of bovine plasma albumin.

Total carbohydrate was measured by the method of Dubois et al. (1956). The concentration curve was constructed by using glucose as a standard.

The study was replicated 6 times so that a total of 560 laboratory insects were used in the entire study. At 20°C, 40% of the insects pupated within 5 months. The data were analyzed by regression analysis.

## RESULTS AND DISCUSSION

Table 1 shows the concentration of protein, lipid fatty acids and carbohydrates found in the various stages of the pecan weevil held at 20°C. Laboratory emerged adults contain considerably more carbohydrates and fatty acids than field collected adults which is probably due to a shortened diapause period of the larvae. The laboratory adults were assayed within 24 h post emerged, whereas the field-trapped insects were of unknown age. The percent unsaturated fatty acids (of total fatty acids) in pupae and adults was 89%; for larvae 82%. The ratio of oleic acid (18:1) to linoleic acid (18:2) was 2:1 in all stages of the weevil and is similar to that observed by Henson et al. (1973). It is commonly known that a high concentration of unsaturated fatty acids protects the insect from decreasing temperatures by maintaining membrane function.

Table 1. Protein, fatty acids and carbohydrates in the various stages of pecan weevil.

| | $\bar{x} \pm 50$ | | | |
|---|---|---|---|---|
| Stage | Fresh wt. mg | Protein mg/insect | Carbohydrate mg/insect | Fatty acids cal/insect |
| Pupae | 102.1 ± 7.8 | 3.8 ± 0.4 | 6.4 ± 0.5 | 16.6 ± 1.6 |
| Adult (lab) | 88.1 ± 7.1 | 1.5 ± 0.1 | 3.1 ± 0.3 | 13.9 ± 1.7 |
| Adult (field) | 62.4 ± 4.5 | 4.5 ± 0.2 | 0.4 ± 0.0 | 4.8 ± 0.0 |
| Adult (field) | 63.0 ± 9.0 | 4.3 ± 0.2 | 0.4 ± 0.0 | 3.8 ± 0.0 |

The length of the larval stage from time of collection to pupation was from 7 to 11 (avg. 8.1) months at 20°C. Duration of the pupal stage varied from 20 to 57 days (avg. 41.6). The age of adults varied from 43 to 161 (avg. 81.7) days while fed on 4% sterile sucrose water and held at a temperature of 20°C. The percentage of unsaturation in the fatty acids of adults at death was 83.4%, approximating that

[3] The use of proprietary names does not necessarily imply the endorsement of these products by the U. S. Department of Agriculture.

found in the larvae (82%). The average biomass of the adult weevil at death was 61.8 mg/insect compared to 881. mg/insect for newly emerged adults (1-day-old). This is an average loss of 26.3 mg/insect (29.9%) fresh wt. over the adult life span (0.32 mg/day/insect).

Figure 1 shows the changes in carbohydrate with age of larvae. The data, when subjected to regression analysis, gave a logrithmic fit. The caloric decrease in carbohydrate over the period tested (140 days) was from 35.2 to 17.6 cal/insect (0.13 cal/day/insect).

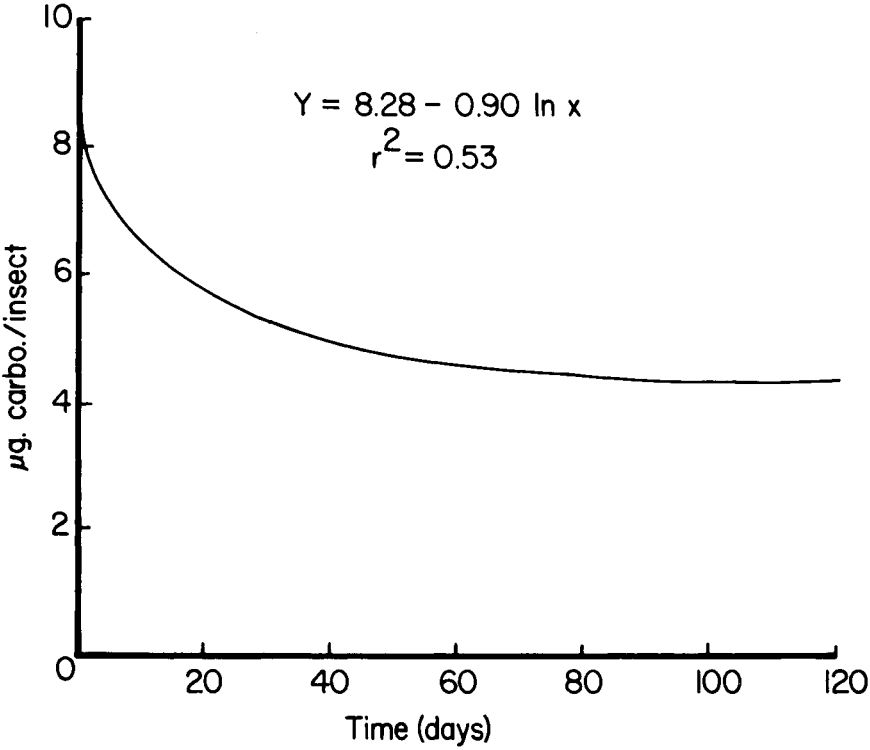


Fig. 1. Changes in carbohydrate level of pecan weevil larvae held at 20°C with time.

The protein content per insect larva (Fig. 2) is significant as related to time held at 20°C. The decrease in protein follows the same general pattern of carbohydrates with the changes following a logrithmic scale. Statistical analysis of the larval mass over the time period did not show a significant difference.

The change in fatty acid calories with time was significant at the 98% level (Fig. 3). Regression analysis shows the data to best fit a polynomial with a correlation coefficient ($r^2$) of 0.80 compared to 0.40 for the logrithmic fit.

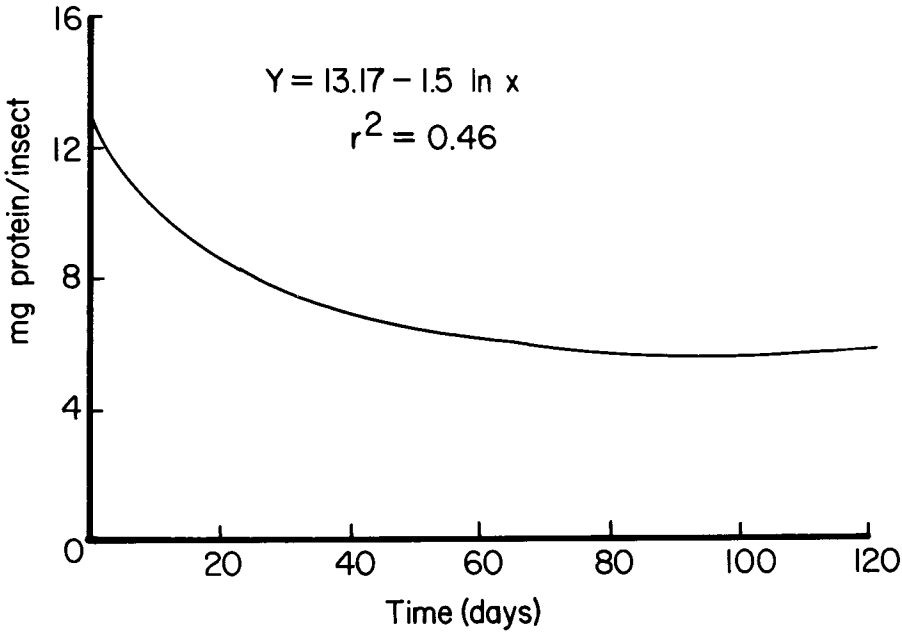


Fig. 2. Changes in protein level of pecan weevil larvae held at 20°C with time.

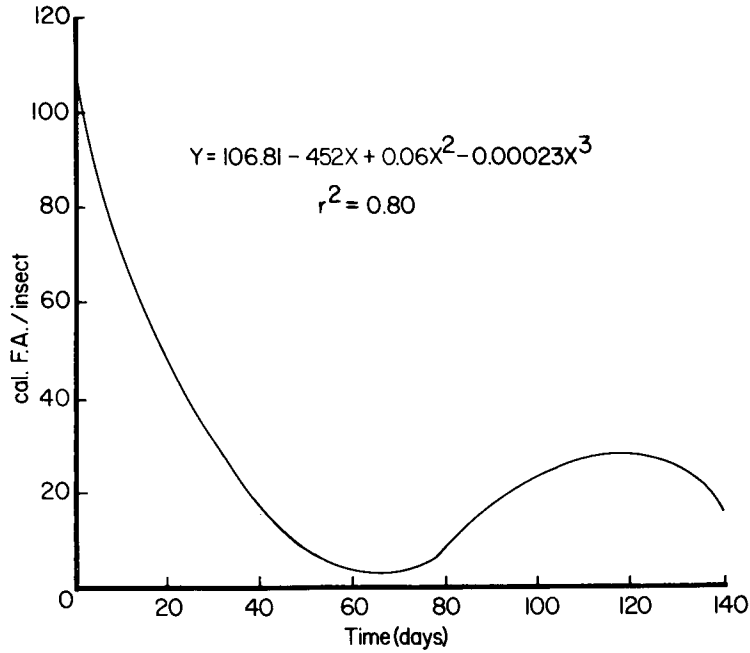


Fig. 3. Changes in fatty acids of pecan weevil larvae held at 20°C with time.

## ACKNOWLEDGMENT

The authors acknowledge the excellent technical support of M. Petty.

## LITERATURE CITED

Bradford, M. M. 1976. A rapid and senstive method for quantitation of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248-54.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colormetric method for determination of sugars and related substances. Anlyt. Chem. 28: 350-6.

Dutcher, J. D. 1983. Ecological energetics and pecan weevil bionomics. J. Georgia Entomol. Soc. 18: 208-13.

Harp, S. J., and H. W. Van Cleave. 1976. Biology of the pecan weevil. Southwestern Entomologist 1: 21-30.

Henson, R. D., A. C. Thompson, R. C. Gueldner, N. V. Mody, W. W. Neel, and P. A. Hedin. 1973. Lipid composition of the pecan weevil *Curculio caryae* (Horn). Lipids 8: 657-8.

Sikorowski, P. P., W. W. Neel, and P. A. Hedin. 1982. Effect of temperature and other factors on diapause of pecan weevil: Preliminary studies. J. Georgia Entomol. Soc. 17: 501-5.

Thompson, A. C., R. D. Henson, R. C. Gueldner, and P. A. Hedin. 1972. Constituents of the boll weevil, *Anthonomous grandis,* Boheman. III. Lipids and fatty acids of subcellular particles of pupae. Comp. Biochem. Physiol. 43B: 883-90.