

Effect of Alfalfa Looper Nuclear Polyhedrosis Virus on Honeybees

The alfalfa looper nuclear polyhedrosis virus (ACNPV) produced *in vivo* or *in vitro* has been reported to have a broad host range (P. V. Vail, D. L. Jay, and D. K. Hunter, *J. Invertebr. Pathol.* 21, 16-23, 1973; P. V. Vail, D. L. Jay, and D. K. Hunter, *Proc. IVth Int. Colloq. Insect Pathol.* pp. 297-304, 1971). The research reported was undertaken to determine the effect of ACNPV fed *ad libitum* to caged honeybees, *Apis mellifera*, and to colonies of honeybees.

The methods used to study honeybee longevity have been previously described (H. L. Morton, J. O. Moffett, and R. MacDonald, *Environ. Entomol.* 1, 102-104, 1972) and were briefly as follows: Ten grams (ca. 100) of newly emerged worker honeybees less than 24 hr old were placed in each of ten screened cages 2 × 6 × 6 in. Five grams of maintenance diet were placed in each cage at the beginning of the feeding trial. Bees obtained distilled water and 60% sucrose solution from two 5-dram plastic vials placed on top of each cage. The sucrose solution, diet, and water were fed *ad libitum* to the bees in five cages. The five other cages were provided similarly but with 7.5 × 10⁴ polyinclusion bodies (PIB)/g of sucrose solution. The PIB's were obtained by passage of the virus through the larvae of *Autographa californica*, then partially purified by differential centrifugation, and freeze-drying the inoculum in maltose (P. V. Vail, C. F. Soo Hoo, R. S. Seay, R. G. Killinen, and W. W. Wolf, *Environ. Entomol.* 1, 780-785). The feeding period was 60 days. Dead bees were collected daily. All dead bees from each 5-day period were stored together as a single sample and frozen. Smears of the midgut and fat body of 90 bees fed the virus were examined with the light microscope.

No significant difference at the 5% level of probability was apparent between the half-life (the number of days required for one-half of the bees in a cage to die) of check bees (35

days) and the half-life of virus-fed bees (33 days). There was no observable difference in the behavior of caged bees during this test. Smears of the fat body did not contain PIB's. Low numbers of PIB's were found in the midgut contents of five bees; however, no PIB's were found in the fat body.

The method used to evaluate the effects of the virus on brood production was the same as that used to evaluate the effects of herbicides (H. L. Morton and J. O. Moffett, *Environ. Entomol.* 1, 611-614, 1972). Briefly, the method consisted of placing 3 lb of honeybees and a laying queen on beeswax frames in a hive. Five colonies received uncontaminated 60% sucrose solution, and five colonies received 7.5 × 10⁵ PIB/g of 60% sucrose. The sucrose solution and maintenance diet were placed directly above the frame containing the brood and were replaced as needed. Each colony was placed in a 12 × 12 × 9-ft saran mesh cage. The feeding period was 30 days.

The colonies were observed daily for the first 3 days and twice weekly following the start of egg-laying (for abnormal behavior patterns). Larvae were removed from the colonies 4 days after the eggs were observed and then at weekly intervals and frozen. The area of capped brood was measured at 18 and 25 days after the eggs were first observed in the colonies.

Eggs were first observed 2 days after the queens were released in the colonies. Four days after oviposition, it appeared that the larvae in virus-fed colonies were positioned abnormally. However, subsequent examination of the colonies did not support this observation. Two colonies that received the virus were queenless for a short period during the test, but normal brood production resumed after new queens were introduced.

The amount of brood produced in both check and treated colonies was relatively

low, perhaps because of "cage effect" or other unknown factors. No significant differences in brood production were observed, and no abnormal behavior patterns were detected in colonies receiving the virus. Microscopical examination of 40 larvae did not reveal PIB in the gut or fat body.

Data from these longevity and reproduction tests suggest that the alfalfa looper nuclear polyhedrosis virus has minimum or no effect on honeybee longevity, egg laying by the queen, egg hatch, care of larvae by workers, growth of larvae, pupation, or development and emergence of adult workers.

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