Artificial Rearing of Harmonia axyridis¹ on Pulverized Drone Honey Bee Brood^{2,3}

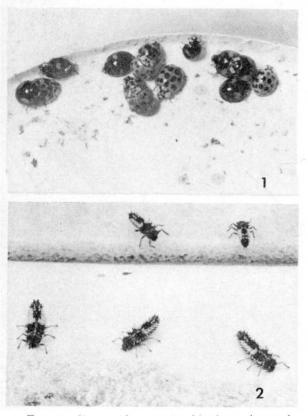
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Methods for artificially rearing aphidophagous coccinellids have been assiduously developed because of their important role in plant protection. Coccinellids are difficult to rear economically on natural foods, i.e., living aphids; thus. frozen aphids (Haug 1938) or dry foods (Smith 1965) were provided to some coccinellids. Smirnoff (1958) reared several species of coccinellids with semi-artificial food including some honey bee products as key substances. Okada (1970) found that larvae of drone honey bees, Apis mellifera L., provided sufficient nutrients for rearing Harmonia axyridis Pallas (Fig. 1).

The rearing procedure was made easier by using pulverized drone honey bee brood (Okada et al. 1973). Larvae or pupae of drone honey bees were blended and freeze-dried in vacuo before being pulverized. The lyophilized diet prepared in this way can be stored at room temperature. The diet was scattered in a plastic

¹ Coleoptera: Coccinellidae. ² Received for publication June 13, 1972. ⁸ Ralph W. Bunn endorses and communicates.



-H. axvridis reared with drone honey bee FIG. 1. powder.

FIG. 2,-Fourth instar H. axyridis feeding on pulverized drone honey bee brood.

Table 1.-Rearing H. axyridis on pulverized honey bee brood in a container.

Date (starting)	No. 1st instars used	No. adults obtained	Develop- mental period (days)	% emergence
Aug. 14 (1970)	160	40	17-22	25
Sept. 25	83	61	18-22	73
Oct. 4	128	42	21-28	33
Oct. 9	125	56	18-29	45
Oct. 10	85	6	23-32	7
Jan. 29 (1971)	79	35	17-21	44
Jan, 30	125	29	18-23	23
Jan. 31	144	46	18-25	32

container ($26 \times 32 \times 4.5$ cm), and water was supplied with a moist sponge (Fig. 2). About 100 1st instars were introduced into the container. A cardboard lid was placed over the rearing container. The containers were placed in an incubator at 25°C without special illumination. Water and food were checked every day, and diet was added or changed according to the degree of deterioration.

Table 1 summarizes portions of the results. Predaceous coccinellids ordinarily are reared individually because of cannibalism. In experiments with our diets, the rate of cannibalism was low; therefore, it was possible to rear more than one individual in a container. Percent emergence is fairly high when sufficient food is available, enabling mass production of the coccinellids.

Use of petri dishes (9 cm diam) as rearing containers gave higher rates of emergence (Okada et al. 1973). Sixteen successive generations were reared using only this diet and water. The semi-artificial food was provided for rearing other aphidophagous coccinellids (Matsuka et al. 1972) and a lacewing, Chrysopa septempunctata Wesmael (Okada and Nijima 1971), but in those cases, rearing of successive generations was restricted to some extent. Addition of certain nutrients may improve the results.

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Field Stability of the Heliothis Nucleopolyhedrosis Virus on Corn Silks^{1,2}

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The effectiveness of an insect virus, used as an insecticide, is largely dependent upon its stability after spraying. Previous studies demonstrated that the Heliothis nucleopolyhedrosis (NPV) on cotton (Bullock 1967, Allen 1968, Ignoffo and Batzer 1971) and the Trichoplusia NPV (Jaques 1968) and the Pieris granulosis virus (David et al. 1968) on crucifers are inactivated by sunlight. Use of spray additives (Jaques 1971, Ignoffo and Batzer 1971, Ignoffo et al. 1972) significantly increased the stability of these occluded viruses of the genus Baculovirus (Wildy 1971).

Our study assesses stability of the Heliothis NPV on sweet corn silks, with or without the use of activated carbon as a sunlight protectant. This study was conducted at the Biological Control of Insects Research Laboratory at Columbia, Missouri, in 1971,

Methods and Materials

Virus Preparation

A commercial preparation of the Heliothis NPV (Viron/H[®]; batch B05201)⁵ formulated at 4×10^9 PIB (polyhedral inclusion bodies) per gram was used in all experiments. The activity of this batch (weight required to produce an LD₅₀) was corroborated by bioassay against neonatal larvae of the corn earworm, Heliothis zea (Boddie). Viron/H was suspended in water and sprayed directly on corn silks at the rate of 150 g/17gal finished spray per acre. This is equivalent to $600 \times 10^{\circ}$ PIB or 100 LE (Larval Equivalents) per acre. Activated carbon (Type RB[®])⁶ when included was used at 1 lb/acre.

Virus Applications

A single application of virus was made on silks of ears in 3 separate, staggered plantings of mixed sweet corn (varieties: Hybrid 2992[®] and Dominator[®]). Treatments included application of virus with or without the activated-carbon additive. Spraying was initiated on July 19, 23, and Aug. 11 for each of the plantings when ears were in silk. Silks were collected immediately after spraying (0-day) and again 1, 3, 6, 12, and 24 days

thereafter. Approximately 6 silks were collected at each sampling date for each virus treatment. These silks were stored individually in plastic bags at -20° C until they could be bioassayed for residual viral activity using a diet-incorporation technique (Ignoffo and Batzer 1971).

Bioassay of Residual Activity

A homogenate of 3 silks/sampling date per treatment was prepared from silks stored at -20 °C. Silks were thawed, cut into 6-mm strands and homogenized (Sorval Omni-mixer®; 7500 RPM) in 75 ml of sterile, distilled water for 3 min.

The resulting homogenate was rinsed from the blender using several washes of water and brought to a final volume of 200 ml. A 20-ml sample of the homogenate was then mixed with 180 ml of a liquid, cooled, semisynthetic diet (Ignoffo 1964, 1965), excluding formalin (Ignoffo and Garcia 1966⁷), to final dilutions of 1/10, 1/100, and 1/1000. The mixed diet plus homogenate was dispersed into 50 individual 15-ml cubicles (Ignoffo and Boening 1970). Neonatal earworms were placed individually in each cubicle and incubated at $30\pm1^{\circ}$ C. Larval mortality was recorded 6 days later. An LD₅₀ was interpolated from a dose-mortality line and a standard slope for each bioassay. The LD₅₀ for each replicate was based on use of 600 larvae/posttreatment date per treatment. Each posttreatment date was replicated 3 times.

Results and Discussion

The LD₅₀ was 270 ng Viron-H/ml of diet. We therefore applied 556×10⁶ LD₃₀ units/acre or an estimated 48.4×10³ LD₅₀ units/silk (5.6 ml/silk). Approximately 30% of the theoretically applied average LD₁₀ units was recovered from silks immediately after spraying (0-day). Corn silks immediately after spraying contained an average of 34,000 LD₅₀ units (range 15,000 to 51,000).

The residual activity of carbon-protected virus was generally twice that of nonprotected virus (Table 1). Sunlight deactivation of nonprotected Heliothis NPV on corn silks was similar to that recorded on cotton (Bullock 1967, Ignoffo and Batzer 1971, Ignoffo et al. 1972). In most instances, the 1/2-life of nonprotected virus was <1 day. Addition of carbon provided significant protection and extended the 1/2-life on corn silks to ca. 3

⁹ Received for publication 18 Sept. 1972. ⁹ Mention of a commercial or proprietary product does not constitute endorsement or recommendation by the U.S. Depart-

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^{60048.} ⁹ Pittsburg Activated Carbon Co., Pittsburg, Pa. 15230.

⁷ C. M. Ignoffo and C. Garcia. 1966. Inactivation of *Heliothis* nucleopolyhedrosis virus by artificial sunlight, Quart. Rep. 11, April-June, 1966. International Minerals and Chemical Corp., p. 3-6.