THE EFFECT OF X-IRRADIATION ON THE LARVAE OF *PAPILIO POLYXENES ASTERIUS* (PAPILIONIDAE)

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The effects of X-irradiation on the larvae of insects has been studied with many species, but few studies on the effect of X-irradiation on the larvae of Lepidoptera have been reported. The purpose of this report is to show an unusual degree of sensitivity of young larvae of *Papilio polyxenes asterius* Stoll to X-rays. Whereas *Drosophila* larvae exposed to 4000r and 6925r of X-rays were able to pupate (Villee, 1946), the larvae of *P. polyxenes asterius* exposed to single doses of 1500r, 3000r, 4500r, and 6000r failed to pupate and died within 17 days. Seventy-nine percent of the larvae used as a control in this experiment survived and pupated.

Materials and Experimental Methods

One hundred and ten ova were obtained from Mr. Robert A. Colborne of Columbus, Ohio. These ova had been deposited on *Anethum graveolens* L. by a female on August 29, 1966. One hundred and three larvae emerged on September 2. Two more larvae emerged on September 3, and the remaining five ova did not hatch. Clear plastic boxes, measuring 5″ × 5″ × 1½″, were used to rear the larvae. Air holes were drilled in each box, which housed 20 of the newly emerged larvae. They were reared on *Daucus carota* L. The 103 young larvae were allowed to mature at their normal rate until September 10. At this time, the eight-day-old larvae were divided into five groups: four groups of 20 to be radiated and one group of 23 larvae served as a control. All the larvae were in their second instar at the time of irradiation. On the eighth day of life, the four groups of larvae were irradiated at dosages of 1500r, 3000r, 4500r, and 6000r. Irradiation factors were 250 Kv, 15 MA, no filter, dosage rate = 233r/10sec., target distance = 17 cm. Radiation was carried out with a Phillips machine and at a temperature of 72°F. Since the irradiation rate was 233r/10sec., the time required to administer 1500r was 65 sec., 3000r in 2 min. 10 sec., 4500r in 3 min. 15 sec., and 6000r in 4 min. 20 sec. Precautions were taken to avoid "backscatter." The larvae were irradiated in the open, clear plastic boxes and afterwards transferred to new boxes and fresh foodplant.

Results

None of the irradiated larvae of the four groups survived to pupate. The greatest losses were suffered by the four irradiated groups the day immediately following irradiation. From this point, the individual groups suffered their losses at different rates. All larvae were dead by the 17th day after irradiation and all died in their second or third instar. The majority of the larvae that lived past the first day died while in the process of moulting. The larvae were observed periodically with a dissecting microscope at 30× for external effects of the X-irradiation. No gross visible signs of radiation burns were observed on any of the larvae. The body length was periodically measured. For the first seven days after irradiation, the average length of the larvae of all five groups was as close as ± 2 mm. But after the seventh day following irradiation, the larvae of the irradiated groups averaged 3–8 mm. less than the length of the control larvae. The rates of survival of the 3000r, 6000r and control groups are graphed in Figure I. No true regularity among the five groups was observed.

DISCUSSION

The effects of X-irradiation on the larvae of Lepidoptera has been reported in the available literature only once, by Whiting (1950). However, considerable research has been carried out and reported on the effects of X-irradiation on the larvae of other insects.

Villee observed that the larvae of *Drosophila* could tolerate 6925r of X-rays and pupate. After the puparium was formed, only minor malformations developed in the adult *Drosophila*. In *Habrobracon* females, Clark (1961) observed that 3000r delivered to the larvae did not interfere with survival and pupation, but did reduce the life span of the subsequent adults from a normal of 25–29 days to six days.

Whiting (1950) showed that a dose of 40,000r of X-rays prevented pupation of the flour moth *Anagasta* (= *Ephestia*) *kühniella* (Z.), many individuals of which continued to live in the larval form for up to 40 days, *i.e.* 37 days after the control larvae pupated. In contrast, the larvae of *Papilio polyxenes asterius* in our experiment failed to survive even the lowest dose of 1500r X-rays. All the larvae of the four irradiated groups (1500r, 3000r, 4500r, and 6000r) expired by the 17th day after irradiation in the second or third instar. It would be plausible to assume that even a much smaller total dose of X-rays, in the vicinity of 750–1000r would be lethal for the larvae of *Papilio polyxenes asterius*, since the larval time period for development to pupation is on the average 28–30 days.

The radiation responses which we have observed in our experiment appear to be of two types, an early and a latent. The early responses appeared immediately, within one day after irradiation, and the latent effects developed when the moulting stage began. The explanation for

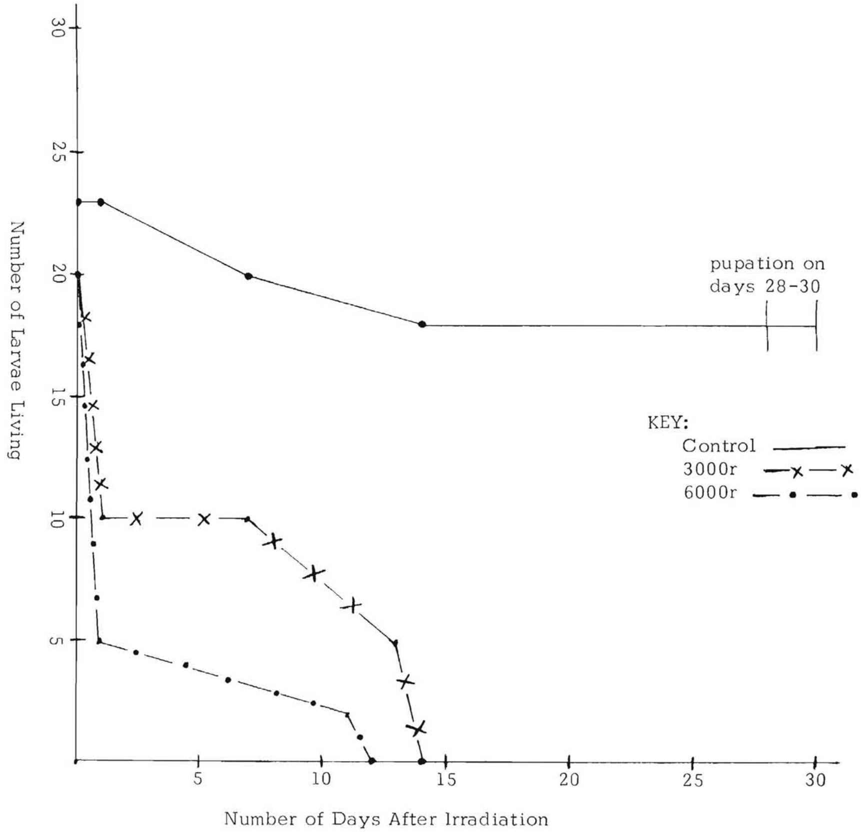

Fig. I. Rates of survival of irradiated larvae of *Papilio polyxenes asterius* Stoll.

the latent damage and death may rest upon the observation that "when insects pass from one instar to another by going through a moulting process, there is a brief period of intense mitotic activity, and if the insect is irradiated prior to moulting, visible damage and death may not show up until moulting occurs" (Baldwin and Salthouse, 1959a,b, 1961). In our observations, the majority of the larvae which survived the first day after irradiation died while in the process of moulting. It has been proven that the precise stage which suffered the most radiation damage was the metaphase (one of the stages of mitosis); at dosages which prevented moulting, division proceeded normally until metaphase and then ceased (Baldwin and Salthouse, 1959c). Joly and Biellman (1958) using *Locusta migratoria* L. found that the timing of irradiation could interfere with moulting. They found that if the insects were irradiated before moulting, then that particular moult succeeded but further moults were prevented. However, if the irradiation preceded a moult by a sufficient time, then that moult was blocked. The studies of Bergonie and Tribondeau (1906) help to explain the latter phenomenon. They found that (1) the sensitivity of cells to irradiation is directly proportional to their reproduction activity and inversely proportional to their degree of differentiation; and (2) after insects hatch from the egg, very little cell division occurs during larval life. The cell division and differentiation of tissues occur instead during the embryonic development of the egg, so that in larval life, growth occurs primarily by enlargement of cell volume without an increase in cell number. Short bursts of mitotic activity occur just before moulting and in later stages of pupation (when pupal forms occur).

The explanation for the unusual degree of radiation sensitivity of the larvae of *Papilio polyxenes asterius* is under study. This degree of sensitivity does not hold for all species of butterflies since our comparative studies with *Colias* using comparable single dosages of X-rays show that the larvae are much more resistant to X-irradiation than the *Papilio polyxenes asterius* larvae. Many larvae of the *Colias* survived the exposures to X-rays, pupated, and formed adults with a variety of abnormalities and a shortened life span.

SUMMARY

An unusual degree of sensitivity to moderate doses of X-rays occurs in the larvae of *Papilio polyxenes asterius*. By contrast, the larvae of *Drosophila, Habrobracon, Locusta, Anagasta,* and *Colias* are much more resistant to X-irradiation. Therefore, the general statement that larvae of all insects are quite resistant to X-irradiation does not hold true.

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APPARENT PARTIAL COURTSHIP BETWEEN *MEGATHYMUS YUCCAE COLORADENSIS* AND *M. STRECKERI* (MEGATHYMIDAE)

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On April 23, 1966, while collecting on the west slope of South Sandia Peak at an elevation of approximately 6,200 feet, about three miles south of Embudito Canyon, Bernalillo County, New Mexico, I saw two *Megathymus* apparently engaged in a courtship flight. As I approached in the hopes of capturing them, the larger one lit on a bare patch of soil and the smaller one immediately lit beside it, about one inch away. I had approached close enough to identify them both, and was surprised to discover that the large *Megathymus* was a female *Megathymus streckeri* (Skinner) and the small one was a male *Megathymus yuccae coloradensis* Riley. An attempt to capture them was delayed to see what would happen. The female *M. streckeri* remained passive with her wings tightly closed. The male *M. y. coloradensis* fluttered its wings for a moment, then curved its abdomen so that the tip touched the tip of the female *M. streckeri's* abdomen. The female responded by flying up with the male in close pursuit. At this point I captured them.

The behavior of these two individuals is similar to the mating behavior described for *Agathymus* in Arizona by Roever (1965), but differs in several aspects. In the mating procedure of *Agathymus polingi* (Skinner), the female fluttered her wings while the male was passive. This is the opposite of the behavior of the *M. streckeri* and the *M. y. coloradensis*. There is the possibility that the male or the female discovered that the other individual was not of their species and responded ac-