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# Toxic Responses of Developing Fifth Instar Milkweed Bugs, *Oncopeltus fasciatus* (Hemiptera), to Aflatoxin $B_1$

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Aflatoxins (AFTs) are a group of mycotoxic metabolites produced by certain strains of fungi, A. flavus and A. parasiticus (Diener and Davis 1969). Aflatoxin $B_1$ ($AFB_1$) has been found to be the most toxic hepatocarcinogen (Butler 1965). Other aflatoxins have been used in tests with the boll weevil and include $AFB_2$, $AFG_1$, and $AFG_2$ (Moore et al. 1978).

Since most organisms tested are susceptible to aflatoxins, numerous studies have been completed on their effects in several animal systems, as well as in insects. Their toxic effects were demonstrated when an extract of "toxin" was topically applied to Epilachna vigintioctopunctata and resulted in high mortality rates (Krishnamoorthy and Sankar-Naidu 1971). Studies by Moore et al. (1978) with the boll weevil (Anthonomus grandis) and by Matsumura and Knight (1967) with the yellow-fever mosquito (Aedes aegypti) reported some insecticide-like properties associated with the aflatoxins. These effects also were reported in the various developmental stages of the life cycle of the fruit fly (Lalor et al. 1976).

Although studies on the aflatoxins have involved test systems ranging from cell cultures to laboratory animals, there appears to be a general lack of information on the ecological and economic effects of aflatoxins on insects (Lalor et al. 1976). However, this situation is gradually changing.

These studies involved the toxic responses of fifth instar milkweed bugs (Oncopeltus fasciatus) to $AFB_1$. Milkweed bugs pass through five distinct nymphal instars. In the fifth instar stage, the insect is marked with lateral spots on all of the abdominal pleurites and median spots on the fifth, sixth, seventh, eighth, and ninth dorsal abdominal tergites. The apex of the ventral abdominal surface is black and the remainder of the body is reddish-orange. Also, the adult is elongate to oval, and it is black and red in color. In addition, there are spots on the third and fourth abdominal sternites (Andre 1934). Because of this insect's ability to live and reproduce normally when provided dried sunflower seeds and water, it is a very

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desirable model to study through out the year (Andre 1934). It is thought that juvenile insect stages are more sensitive to AFT than are adults, thus the instar and its developmental and sexual responses to aflatoxins are of interest.

## MATERIALS AND METHODS

Fifth instar nymphs were used in these experiments. The colony was originally obtained from the Carolina Biological Supply Company, Burlington, NC. The nymphs were chosen at random from a stock colony and placed in one liter glass culture jars. A hole, 2.54 cm in diameter, was cut in the lid of each of these jars. Cloth gauze was positioned over the holes for ventilation. Thirty mL prescription vials were used as water bottles, and were filled with tap water or aflatoxin dissolved in tap water. A cotton wick was placed in the vial and the insects imbibed water ad libitum. One vial was placed in each culture jar. Also, 10 g of cracked, unsalted, raw sunflower seeds were placed in the bottom of each culture jar. In both the first series of experiments (20 ± 1 °C study) and the second series of experiments (25 ± °C study) three control and three experimental culture jars were utilized. All culture jars in both studies contained 10 insects. Both the first and second series of experiments utilized a total of 120 insects (30 control insects plus 30 experimental insects in each study).

The $AFB_1$ used in these studies was obtained from Calbiochem, LaJolla, CA. Initially, the $AFB_1$ (grade A, dried in situ) was dissolved in acetone (10 mg/mL), and then was added to 500 mL of previously-autoclaved tap water. This solution was stirred and heated for three hours at a temperature just high enough to allow the aflatoxin to solubilize. This stock solution was stored at 10 °C in darkness. The stock solution contained 20 ug $AFB_1$/mL of $H_2O$, and all experimental dilutions were made from this original stock solution.

Next, the stock solution and subsequent dilutions were tested for concentrations of $AFB_1$ and possible metabolites. Twenty microliter samples, along with standards (Applied Science Laboratories, State College, PA), were spotted on thin-layer chromatography plates coated with silica gel G (Horwitz et al. 1975). Quantifications were determined according to official analytical methods (AOAC Methods) and utilized a spot densitometer. Quantifications were repeated in triplicate. These toxin analyses were done by the Virginia Division of Consolidated Laboratory Services, Mycotoxin Laboratory Richmond, VA. The drinking water used in these studies, after final dilution, contained $AFB_1$ at 5 ug/mL.

All insect groups were observed for a total of 21 d. In the first series of experiments, the ambient temperature was 20 ± 1 °C, and 25 ± 1 °C in the second series of experiments. The photoperiod for both experiments was 12 hr daylight and 12 hr of darkness. The body lengths (apex of head to tip of terminal abdominal segment) of the nymphs were recorded at 48-hour intervals. Sex was recorded as the nymphs molted to adults, in

an attempt correlate the results of this study with an earlier study conducted in our laboratory (Gaston and Llewellyn, 1980). The percent mortality also was recorded at 48-hour intervals, and dead insects were removed immediately from the experiment.

## RESULTS AND DISCUSSION

Temperature differences in the two series of experiments appeared to have little overall physiological and developmental effects. Sex of the surviving insects was recorded at the end of the study, and as animals succumbed. Since insufficient numbers of insects survived to statistically determined sexual differences, the sex-related survival results in the first experimental series (low temperature, 20 °C, and $AFB_1$) were inconclusive. In the second experimental series (high temperature, 25 °C, and $AFB_1$), there were equivalent responses with respect to sex in surviving insects and overall mortality. Gaston and Llewellyn (1980) noted a predominance in surviving adult male milkweed bugs feeding on $AFB_1$.

Control animals (Figures 1 and 2) gradually increased in length and exhibited a leveling effect from 16 d to 20 d during the experimental period. There was a decrease in growth at 21 d in both series of experiments. This may have been due to the suppression of juvenile hormone possibly caused by atrophy of the corpora allata which has been demonstrated in milkweed bugs and locusts exposed to precocenes (Brooks et al. 1979). They found that early instar larvae of O. fasciatus and Locusta migratoria both simulated effects similar to those resulting from allatectomy. Also, other mycotoxins have been reported to affect growth processes. For example, Wright et al. (1980) found that Penicillium mycotoxins inhibited larval growth in Attagenus unicolor (Brahm) (megatoma (F.)), and that citrinin and rubratoxin B inhibited larval growth of Tribolium confusum and Lasioderma serricorne. In the current study, $AFB_1$-treated insects held at lower temperatures never exceeded 1 cm, whereas those at the higher temperature never exceeded 1.11 cm. Control insects had body lengths (at 21 d) that were approximately 1.17 cm in the lower-temperture study and 1.14 cm in the higher-temperature study. In both cases, $AFB_1$ appeared to retard insect growth, and in the lower-temperature study this differance in control and experimental body lengths was found to be significantly different ($p < 0.05$). Lalor et al. (1976) noted a similar response in fruitflies, as did Moore et al. (1978) with the boll weevil. It is possible that the $AFB_1$ exerts its main effect on insect juvenile hormones, and results in slower and less distinct growth responses. This seems plausible, since control insect body lengths were greater than experimentals after 4 d in both studies. Nijhout (1979) showed that fifth instar Oncopeltus must achieve a critical weight early in the instar in order to initiate the next apolysis. Also, he suggested that molting hormone (ecdysone) synthesis is triggered by stimulation of abdominal stretch receptors and that the blood acts as the transducer of growth to the stretch receptors.

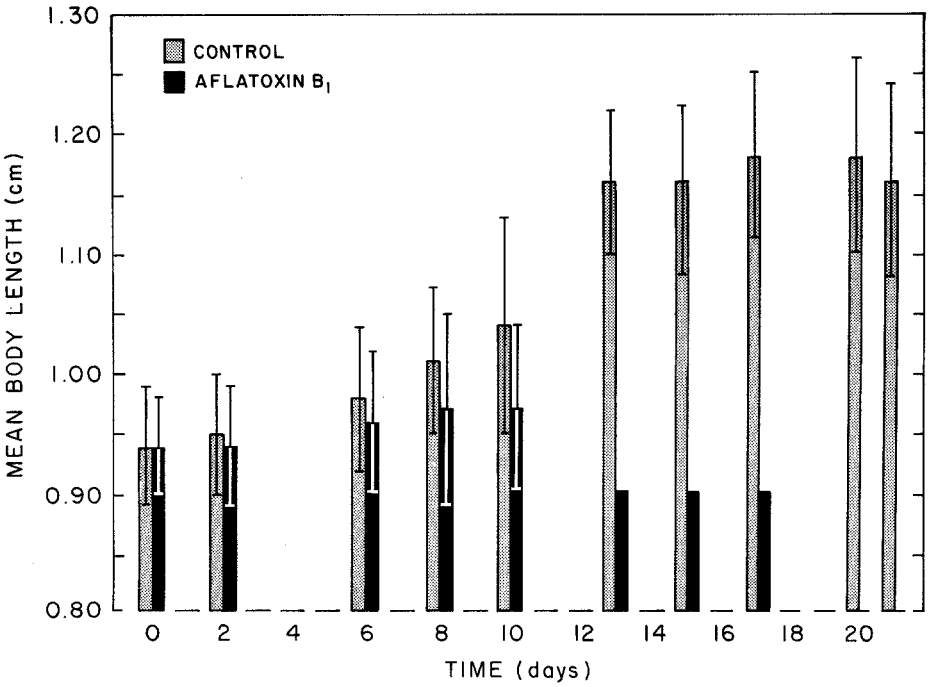


Figure 1. Oncopeltus Mean Body Length Reared at 20 ± 1 °C.

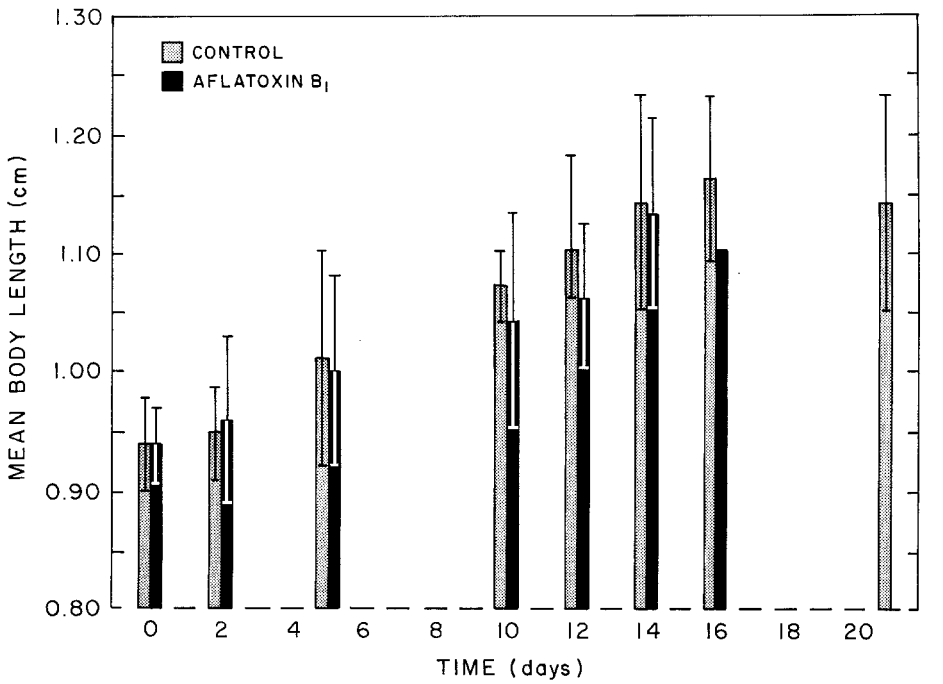


Figure 2. Oncopeltus Mean Body Length Reared at 25 ± 1 °C.

The $LT_{50}$ values in both studies appeared to have occurred prior to 13 d in the experimental insects. One hundred percent mortality had occurred in the experimentals at 20 d or 21 d (Figures 3 and 4). Wright et al. (1980) also noted a high mortality rate (99%) and an increased development time when a Penicillium purpurogenum isolate was introduced into diets fed to larvae of the confused flour bettle (Tribolium confusum Duv.). Control insects in the current study never exceeded 30% mortality at 21 d. These results suggest that $AFB_1$ increased the mortality rate in these milkweed bugs during the fifth instar stage. In comparison to another insect study in our laboratory (Llewellyn et al. 1975), it is evident that developing milkweed bugs are more susceptible to $AFB_1$ than adult cockroaches. Since several species of cockroaches (especially the American cockroach, Periplaneta americana) have varied diets, it is possible that they have developed some type of resistance to the naturally occurring AFTs that routinely occur in decaying matter, whereas the milkweed bug would be a more susceptible plant-sap feeder.

From this report and that of Gaston and Llewellyn (1980), it also is evident that fifth instar milkweed bugs show similar responses compared to those of immature fruitflies to AFT-containing diets. In both organisms, it was observed that the immature forms were more susceptible than the adults, and they showed reduced or retarded growth patterns. Also, milkweed bugs and fruitflies exhibited sex differences with respect to immatures surviving to the adult stage. It appears that neither species would serve as satisfactory bioassay organisms for AFTs, since bird eggs (Verrett et al. 1964) or fish eggs (Llewellyn et al. 1977) seem much more sensitive to the toxins. In the adult (not the immature forms), we speculate that the male and the female insects may metabolize the AFTs in different manners possibly similar to those reported in mammalian systems (Wogan and Newberne 1967), causing a difference in mortality rates. This assumption may be tied to the fact that female vitellogenesis cycles in some insects are initiated after degeneration of prothoracic glands, and that ovary production of ecdysone may be affected by these events. If aflatoxins do initiate the degeneration of prothoracid glands in Oncopeltus, as do the precocenes, female milkweed bugs may show higher mortality rates due to interruption of cyclic hormonal production (Berry 1985). Environmental temperature differences in excess of 5 °C may be required to induce noticeable mortality effects from AFT exposures. In addition, the feces and whole bodies of milkweed bugs fed AFT-containing diets merit additional study, to determine the metabolic/physiological fate of AFTs and/or their possible metabolite formation.

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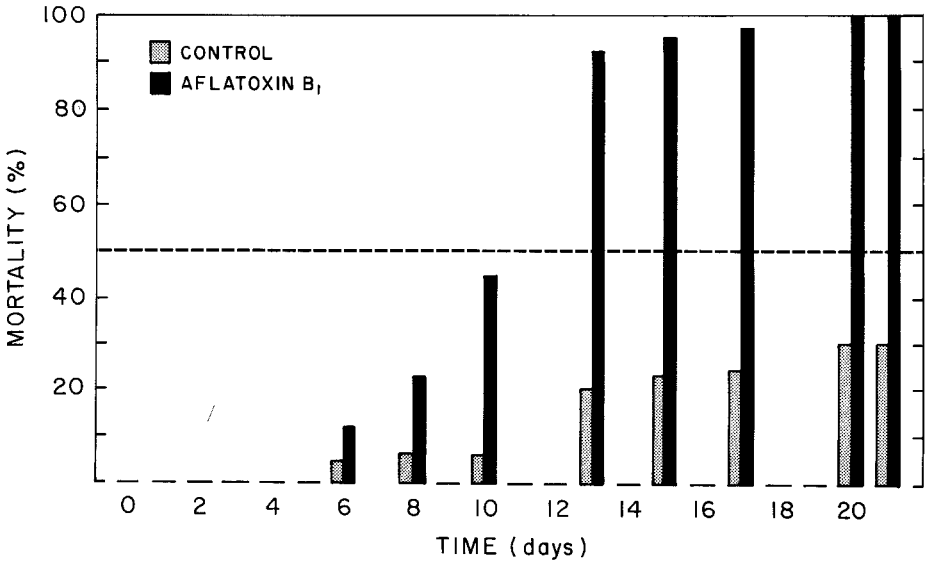


Figure 3. Oncopeltus Cumulative Mortality Reared at 20 ± 1 °C.

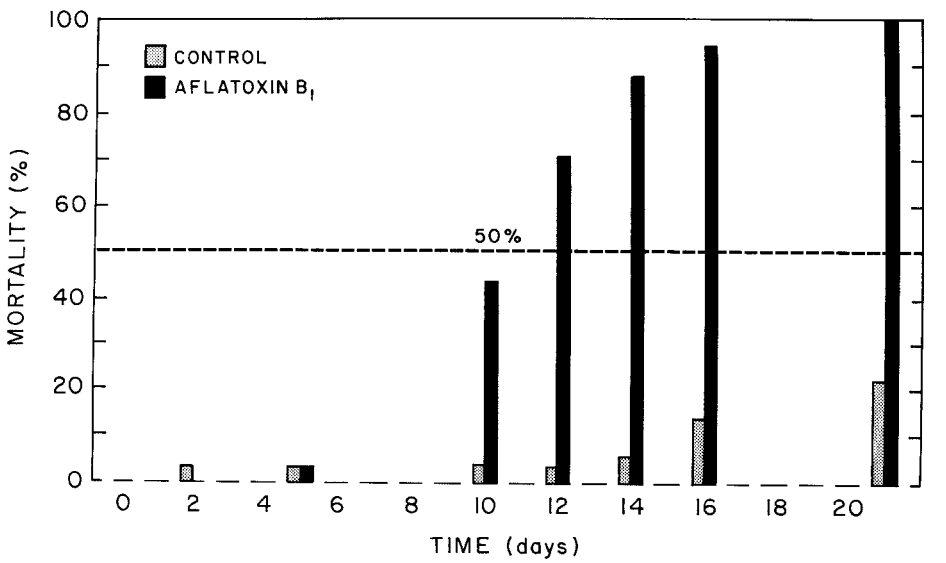


Figure 4. Oncopeltus Cumulative Mortality Reared at 25 ± 1 °C.

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