

Effects of the Pesticide Diflubenzuron on Larval Horseshoe Crabs, *Limulus polyphemus*

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The trend toward the use of less broad scale pesticides has led to the use of the diflubenzuron (DFB), or Dimilin R, for insect control. This chemical is a chitin synthesis inhibitor (Post et al. 1974), and therefore is not a wide spectrum toxicant, but does have adverse effects on important nontarget organisms, the crustaceans.

Dimilin has been shown to disrupt development and reproduction in crustaceans (Costlow 1979). Larvae were found to have increased malformations and mortality at levels as low as 0.5 ug/L (Nimmo et al. 1980; Tester and Costlow 1981). Mortality was associated with the inability to cast off the exuviae at ecdysis. Adult crustaceans, though more tolerant than larvae, have been found to exhibit mortality at molt at concentrations of 100–200 ug/L (Cunningham 1976; Gulka et al. 1980), also apparently due to the inability to detach from the exuviae.

Organisms inhabiting the intertidal zone and shallow estuarine areas would be expected to be those most at risk from run-off of sprayed DFB or other pesticides. The horseshoe crab, Limulus polyphemus, is a chelicerate arthropod that is an important member of the estuarine community. Adults breed on bay beaches and bury their eggs in the sand. Larvae after hatching, move up into the water column. This study was undertaken to investigate the effects of Dimilin on molting and mortality of the larvae of L. polyphemus.

MATERIALS AND METHODS

Limulus eggs were collected from the beach at Scallop Pond, Southampton, New York, in early July and brought into the laboratory and placed in seawater to hatch. Within 12 h of hatching, larvae were placed in groups of 12 in Petri dishes with 30 mL seawater (25 o/oo S.,

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23-25 C). Three replicate groups were exposed to clean sea water, 5 ug/L DFB, or 50 ug/L DFB. The compound used was the wettable powder WP-25% (Uniroyal Corp. Naugatuck, Connecticut), which is the principal agricultural formulation. It was measured out of a stock bottle with a concentration of 0.1 mg/ml. Since this is above the solubility of the chemical, the suspension was shaken vigorously before each use. The stock bottle was refrigerated at 4 C. Concentrations that the larvae were subjected to were thus "nominal." Solutions were changed three times weekly, and crabs were fed newly hatched brine shrimp three times a week. Observations were made twice a week for mortality and molting. Crabs that molted into the tailed juvenile stage were measured for prosomal width.

RESULTS AND DISCUSSION

Molting and survival rates are seen in Figure 1. It can be seen that the crabs in the 5 ug/L group showed a slight, but significant ($P < .05$ by Chi-square) delay at 14 days, but then molted at a rate comparable to the controls. These crabs did not exhibit any significant mortality. The crabs in the 50 ug/L group, while molting at the same rate as controls, exhibited severe mortality immediately after ecdysis. In addition, the prosomal width of the crabs in this group was smaller than that of the controls and that of the group in the 5.0 ug/L Dimilin. While controls measured 5.1 ± 0.03 (S.E.) mm, crabs in the lower concentration of the pesticide measured 5.1 ± 0.05 mm, and those in the higher concentration measured only 4.5 ± 0.24 mm, which is a statistically significant difference ($P < .05$) by t-test.

While the Dimilin in this study produced effects on mortality associated with ecdysis in Limulus larvae, there are some notable differences between the effects observed here and those observed in crustacean larvae. Limulus appears to be much more resistant to Dimilin than the crustaceans that have been examined (mysids and decapod larvae, primarily), since those were affected at levels in the vicinity of 1 ug/L, while Limulus did not show significant adverse impacts at 5 ug/L, only at 50 ug/L. The mortality that we observed in the 50 ug/L groups occurred after, rather than at ecdysis, which is the stage at which crustacean larvae have been reported to die. Limulus, unlike the crustaceans, did not die in the process of shedding the old cuticle, but died after doing so. In contrast, Kas'yanov and Kostlou (1984) found that 0.1 and 1.0 mg/L DFB produced mortality at the beginning, during, and after ecdysis in Limulus. Gulka et al. (1980) reported that Dimilin accelerated ecdysis in the

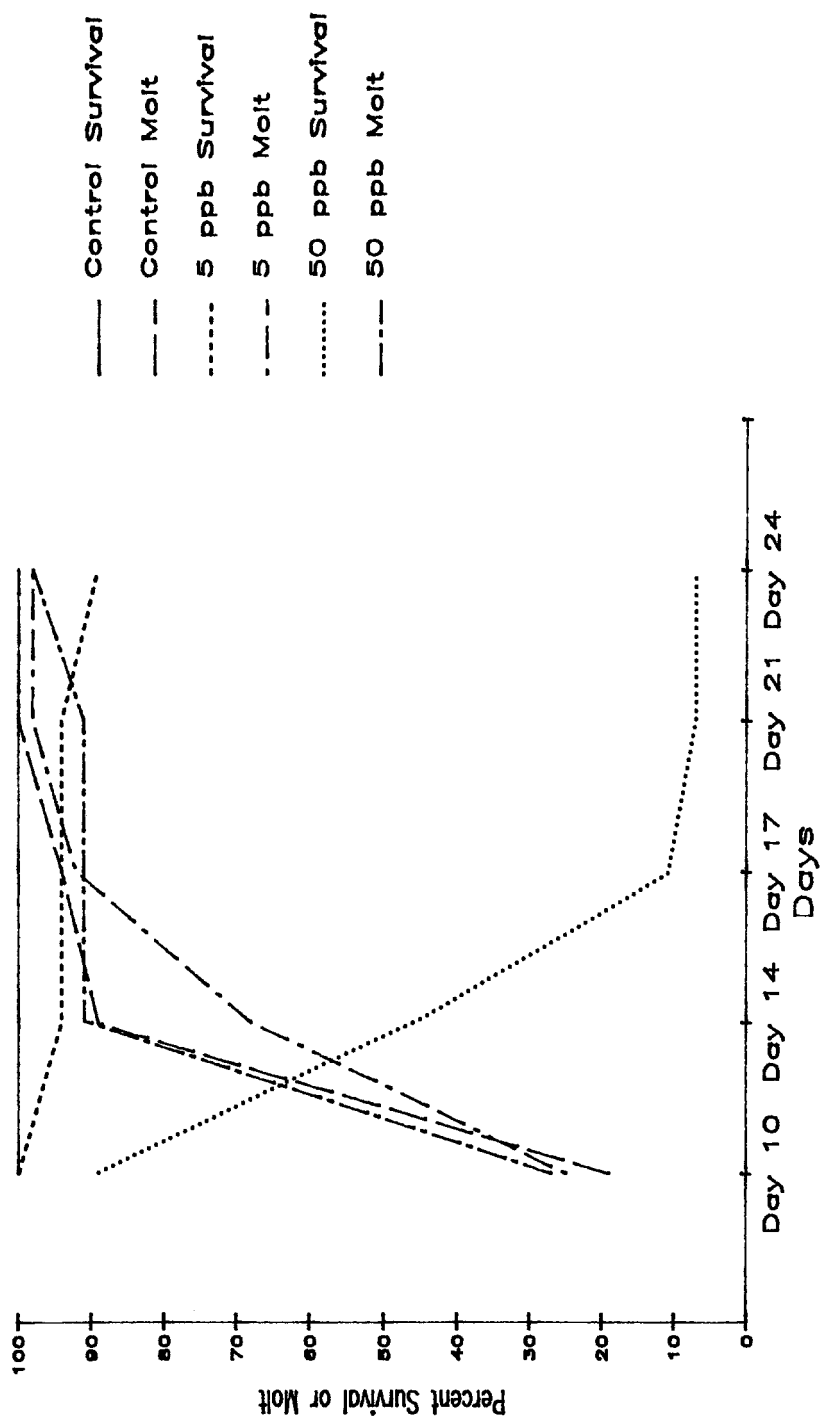


Figure 1. Survival and molt of control and DFB-treated *Limulus*

barnacle, Balanus eburneus.

Dimilin did not have a major impact on the length of the intermolt period in our study. This is in contrast to the results of Kas'Yanov and Kostlou (1984) and to the effects of Bunker C oil as observed by Strobel and Brenowitz (1981) on this species. These investigators noted a delay in molt in the oil-treated crabs. In contrast, Laughlin and Neff (1977) found that horseshoe crabs exposed to the water soluble fraction of No. 2 fuel oil did not show a significant delay in molting. The mean intermolt period for the controls in the Strobel and Brenowitz study was 12-13 days, which agrees well with our data. Their animals were collected from Long Island also. In contrast, Rudloe (1981) reports that the larvae molt into the first juvenile instar after 6-8 days. Her work was on crabs from Florida, so that the ambient temperature differences and possible population differences can account for this discrepancy. She does report, however, that the juveniles are 5 mm in prosomal width, which is in agreement with our data.

While their location on the intertidal sandy beach would appear to put Limulus larvae at risk from pesticide run-off, the larval stages have been shown to be quite resistant to Dimilin, as compared with crustacean larvae. Neff and Giam (1977), in studying the effects of PCBs and Halowax on the tailed stage of Limulus, also found them to be extremely resistant and hardy compared with other marine arthropods. It is possible that the embryos, which we did not investigate, might be more vulnerable to the effects of this and other pesticides.

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