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Preliminary Study of Synergism of Acid Rain and Diflubenzuron

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Diflubenzuron[1] (Dimilin®) was used on over 7 million acres in the U.S. in 1990 to control forest pests, particularly the gypsy moth. This chitin synthesis inhibitor affects insects and other arthropods. It is a restricted use pesticide due to its nontarget effects on aquatic macroinvertebrates (U.S. EPA 1985). The effects of a single aerial application on nontarget aquatic macroinvertebrate communities were reviewed by Eisler (1992). Crustacea and immature insects (especially the true flies, mosquitoes, midges and black flies) are the most sensitive nontarget aquatic organisms to diflubenzuron.

Diflubenzuron, N-[[4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, is not the only mortality factor aquatic organisms face from human pollution. Acid deposition is a frequent stress factor in freshwater habitats in the Northeast USA. Acidic pulses can drop vernal pools (e.g., temporary, springtime, snowmelt pools) to pH levels below 3.0 (Jackson 1990). Aquatic invertebrates vary in their tolerance to acidification (Zischke et al. 1983). Reduced pH completely eliminates some species (Hall et al. 1980). A combination of stress factors could lead to synergistic effects, over and above the impact seen with a single stressor. The purpose of this study was to determine if there are synergist effects of diflubenzuron and lowered pH on the mortality of a nontarget aquatic organism.

MATERIALS AND METHODS

Yellow fever mosquito larvae, *Aedes aegypti* (Rockefellar strain), from colony reared for >100 generations, were used as the study organisms. Twenty, 4-d-old larvae were placed in a crystalizing dish with 400 mL reconstituted soft fresh water (12 mg/L $NaHCO_3$, 7.5 mg/L $CaSO_4 \cdot 2\ H_2O$, 7.5 mg/L $MgSO_4$, 0.5 mg/L KCl; hardness = 10-13 mg $CaCO_3$/L, APHA 1985). The

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pH was adjusted to 4.5 or 6.6 with an artificial acid rain solution (6.5% and 3% sulfuric and nitric acids, respectively). Mortality levels of control larvae were excessive when pH levels below 4.3 were maintained. Larvae were reared for 4 d in water at the treatment pH before their experimental use. Larvae at this age are 4-6 days from pupation. Four mL of diflubenzuron in 95% ethanol or, for the control, four mL of 95% ethanol alone, was added to each dish. Diflubenzuron concentrations ranged from 0.05 n*M* to 50 µ*M*. Each dose was replicated at least 5 times. The dishes were stacked upon one another and placed in an incubator at 27 ^{o}C. After 96 hr, the numbers of live larvae were counted. POLO-PC (Le-Ora Software, Berkeley, California) was used to analyze the significance of the fit of the data to the probit model, to calculate the LC_{50} values, and to determine if the mortality curves were significantly different between pH treatments.

RESULTS AND DISCUSSION

Increased acidity increased mortality 100 times (Fig. 1). The LC_{50} was 5 n*M* (1.7 µg/L) at pH 4.5 and 500 n*M* (173.6 µg/L) at pH 6.6. Figure 1 presents the probit mortality curves; dotted lines indicate the 95% confidence interval for each acid treatment. The R^2 values are 0.8235 and 0.8677 for pH 4.5 and pH 6.6, respectively. The mortality curves are significantly different in y intercept (α=0.05) and are parallel.

The synergistic action of diflubenzuron with lowered pH has implications for the prediction of the impact of diflubenzuron on nontarget arthropods. Field data obtained in habitats not exposed to acid precipitation may underestimate nontarget mortality in habitats that experience acidic precipitation. The timing of diflubenzuron application in relation to annual acidic pulses could greatly alter nontarget aquatic mortality.

One possible physiological explanation of the effect we have shown is that insect cuticle is more than a simple chitin coat. Cuticle is composed primarily of protein, with $\leq$50% chitin. In immature aquatic insects with nonsclerotized cuticles, most of the protein is noncovalently bound to the chitin (Andersen 1979). The protein is believed to wrap around chitin microfibrils, providing additional structural support (Kramer et al. 1985). Different cuticular proteins have differing isoelectric points, ranging from pH 3-6 (Andersen 1979) and protein structural integrity is best near its isoelectric point. It is possible that an insect whose cuticle is weakened by the reduced chitin content due to diflubenzuron could still survive since its cuticular proteins continue to provide some support. Diflubenzuron has not been shown to effect cuticular proteins (Grosscurt and Jongsma 1987). However, if protein structure was

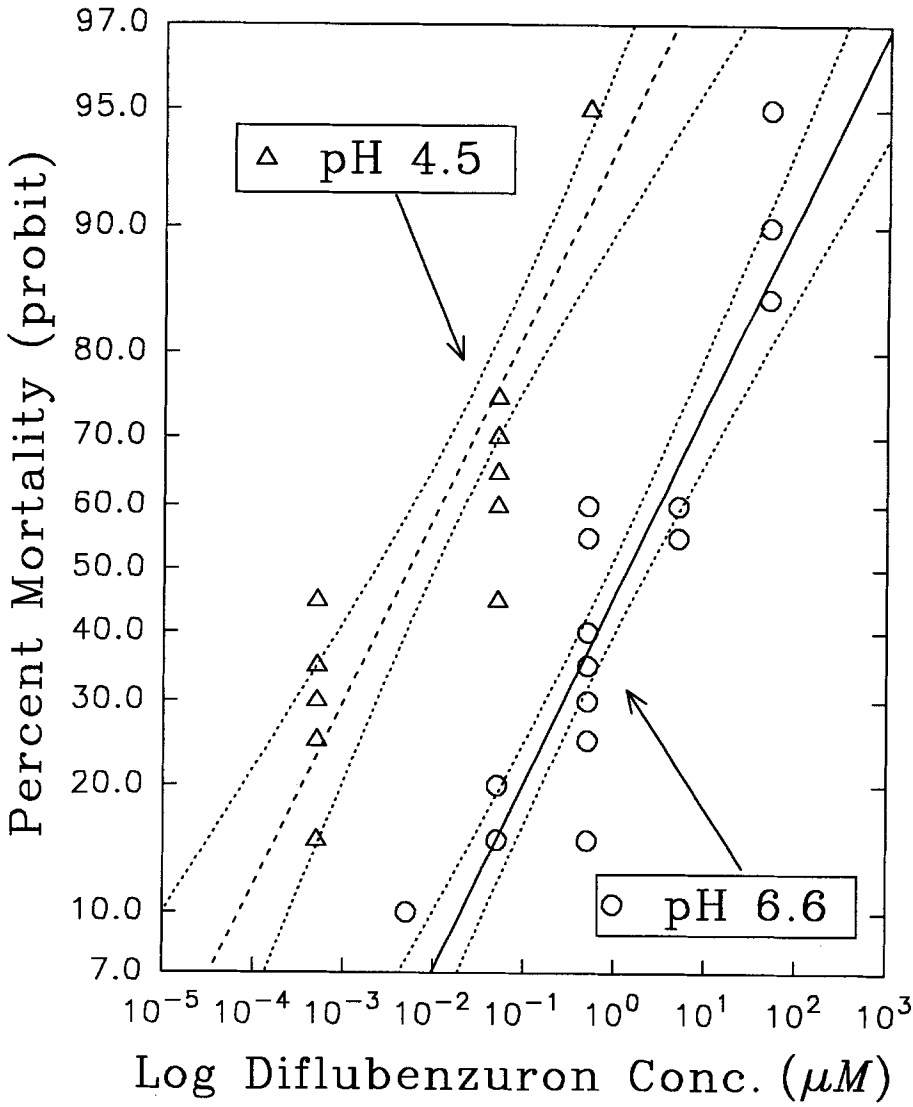

Figure 1. Log dose -- probit response curve to diflubenzuron dose, at pH 4.5 and pH 6.6 for 4 d old *Aedes aegypti* larvae.

weakened by a change in pH, then increased mortality would be the expected result.

Another explanation is that ion availability is lowered by lowered pH, and aquatic insects must continuously take-up salts (e.g., Na^{2+}, Ca^{2+}, K^{+}) to survive (Sutcliffe and Hildrew 1986). Ion uptake in *Aedes aegypti* occurs through the anal papillae (Clements 1992). Reduction in chitin content due to diflubenzuron in the papillae may reduce their efficiency. This combined with reduced ion availability could result in mortality not seen at reduced acidity.

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