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Implications for the Use of Diflubenzuron to Reduce Arthropod Populations Inhabiting Evaporation Ponds of the San Joaquin Valley, California

C. S. McCasland,[1,*] R. J. Cooper,[1,†] D. A. Barnum[2]

[1] Department of Biology, The University of Memphis, Memphis, TN 38152, USA
[2] USGS, Biological Resources Division, Kern Field Station, Delano, CA 93216, USA

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Diflubenzuron (DFB) is an arthropod growth regulator whose registered uses include control of gypsy moths (Lymantria dispar), mosquitoes, and cotton boll weevils (Anthonomus grandis). DFB acts as a toxin that interferes with the molting process, the toxic effects are delayed, and do not appear until the initiation of a molt. During molting, the cuticle of treated juveniles is only partially formed and is improperly attached to the epidermis. The new cuticle cannot withstand the increased pressure during ecdysis and/or give sufficient support to the muscles involved in the molting process. This results in the inability of affected individuals to cast their exuviae, which leads to the characteristic appearance of DFB killed organisms, and they die of a rupture of the newly formed cuticle or from starvation (Farlow 1976). One of the major reasons that DFB is used commercially is that the potential for bioaccumulation is quite low in terrestrial ecosystems; it only affects immature phytophagous arthropods, and has been shown to have few adverse toxicological effects in terrestrial systems. This pesticide is also used frequently because it is not persistent and degrades rapidly, and sub-chronic effects do not appear to occur.

DFB does not appear to affect vertebrates directly. Once DFB is ingested by vertebrates, it passes through the gut and is detoxified without causing adverse effects, then passes out in urine and feces. Indirect effects on birds have been observed resulting from reduction of invertebrate prey numbers. Cooper et al. (1990) observed a significant difference in foraging territory size of insectivorous birds between DFB treated and untreated plots. In addition, the diets of insectivorous birds were significantly different between DFB treated plots and control plots (Sample et al. 1993) which may have contributed to decreases in fat reserves (Whitmore et al. 1993). The differences in both territory size and diet were attributed to a decrease in lepidopteran larvae, an important prey source that was found to be severely affected by DFB applications (Cooper et al. 1990, Sample et al. 1993). It is likely that a reduction in prey caused by an application of DFB will not only affect the diet and territory size of insectivorous birds but also nesting success as well. Rodenhouse and Holmes (1992) found that an aerial application of Bacillus thuringiensis also created a reduction in food resources. They further found that fewer nesting attempts were made due

**Present address:* U. S. Fish and Wildlife Service, Coast Bay Delta Branch, 3310 El Camino Avenue, Suite 130, Sacramento, CA 95821, USA

†*Present address:* Warnell School of Forest Resources, University of Georgia, Athens, GA 30602, USA

Correspondence to: C. S. McCasland

to the pesticide application. Because of the potential for decreased reproductive output of vertebrate predators, due to a reduction in prey resources, this pesticide may be beneficial as a short-term technique to mitigate the effects of selenium contamination in the San Joaquin Valley of California and elsewhere. High concentrations of selenium in irrigation drainwater have been identified as the source of reproductive abnormalities in aquatic birds at Kesterson National Wildlife Refuge (Ohlendorf et al. 1986) and sub-surface agricultural evaporation ponds in the Tulare Lake Basin (Skorupa and Ohlendorf 1991, Ohlendorf et al. 1993). Specifically, breeding females acquired and transferred high concentrations of accumulated selenium from aquatic plants, invertebrates, and fish to their eggs resulting in the observed abnormalities (Presser and Ohlendorf 1987, Ohlendorf 1989). The objective of this study was to determine the effects of DFB on the survival of immature (Trichocorixa reticulata) (Hemiptera:Corixidae), an important food source for nesting birds utilizing sub-surface agricultural evaporation basins found in the San Joaquin Valley of California. DFB has been shown to be extremely toxic to several other arthropods that are abundant within these evaporation ponds, such as brine shrimp (Artemia salina) (Cunningham 1976) water fleas (Daphnia spp.) (Julin and Sanders 1978, Johnson and Finley 1980, Hansen and Garton 1982) and midges (Chironomidae) (Julin and Sanders 1978, Ali and Lord 1980, Johnson and Finley 1980). Because T. reticulata are hemi- metabolous, we believed it was likely that they would not be as greatly affected as arthropods undergoing complete metamorphosis.

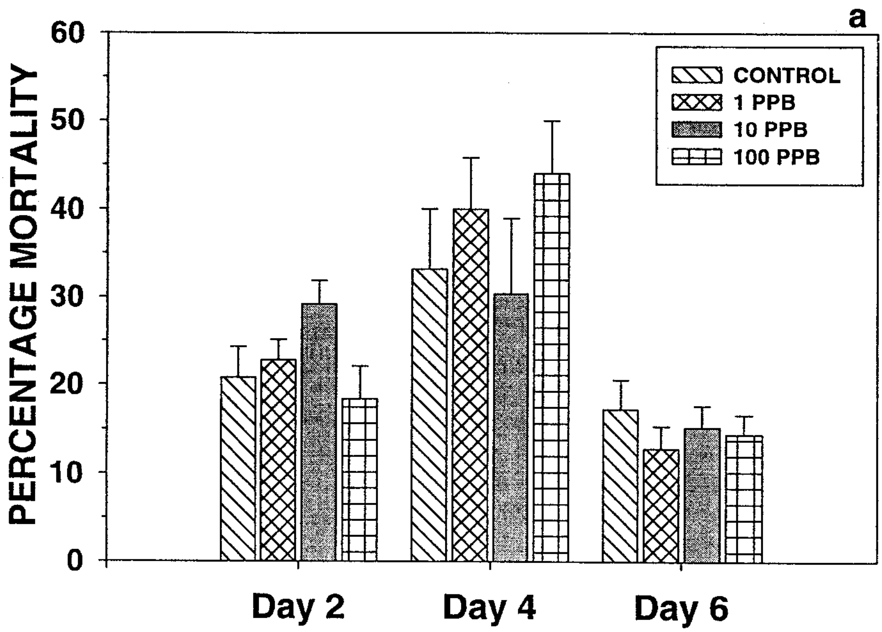
MATERIALS AND METHODS

Immature stages of T. reticulata (water boatman) were collected from agricultural drainage evaporation ponds to test the effects of DFB on these nymphs. Immature water boatmen were collected from cell Al of Tulare Lake Drainage District's Hacienda Facility, Kings County, California, and were taken back to Kern National Wildlife Refuge and separated by age and size. Thirty-five individuals in the third and fourth instar age groups were placed into forty, two-liter tubs (a total of 1400 individuals). Each tub contained 500 ml of pond water that contained one of four concentrations of DFB (25 percent wettable powder); 1 part per billion (ppb) DFB, 10 ppb, 100 ppb, or a control containing no DFB. Each treatment had ten replications. The number of dead individuals found in each tub was recorded every other day for six days. Because DFB is rapidly broken down into nontoxic compounds, this experiment only ran for six days. The response to the application of DFB was measured as percent mortality, which was calculated as the number of dead individuals divided by the number of individuals alive at the previous sampling interval. A one-way analysis of variance (ANOVA) was used to test the overall differences in mortality among the four treatments. If mortality differed between treatments, A Tukey's Honestly Significant Difference test was used to determine which treatments were significantly different from one another. The variable percent mortality was arc-sin transformed for the ANOVA tests but is presented as a percentage in the following figures. A second experiment similar to the one described above was also conducted, but with three differences; 1) first and second instars were used instead of third and fourth instars, 2) 0.10 grams of powdered *Spirulina* were added to the stock solution of DFB and the control, and 3) 40 individuals were used per tub instead of thirty-five (a total of 1600 individuals). *Spirulina* was added to the stock solution and the control to determine if DFB might be binding to particles in the extremely turbid pond water. We added our own binding agent because very little pond water is needed to acquire this concentration, thus assuring that most of the organic matter binding to the pesticide was a food source for these insects. This experiment also ran for six days but mortality data were only collected twice.

RESULTS AND DISCUSSION

No significant differences were found among the four treatments during the first experiment (July 23 $F_{3,36} = 2.19$, $p = 0.1065$, July 25 $F_{3,36} = 0.59$, $p = 0.6231$, and July 27 $F_{3,36} = 0.49$, $p = 0.6899$) (Figure 1). There were significant differences among treatments during the second experiment for both dates (July 26 $F_{3,36} = 4.04$, $p = 0.0141$, July29 $F_{3,36} = 5.00$, $p = 0.0053$). After the first collection date, mortality in the 108 ppb treatment was significantly greater than in both the control and 1 ppb treatments (Figure 1). During the second collection, percent mortality in the 10 ppb treatment was greater than mortality in the 100 ppb treatment, which was likely related to the high mortality observed for the 100 ppb treatment during the first collection period. Because DFB affects arthropods during the molting process, it is possible that individuals in the 10 ppb treatment succumbed to chronic effects of the DFB application while the majority of individuals affected by DFB in the 100 ppb treatment were killed quickly due to the higher concentration of DFB.

There are two possible explanations as to why DFB was more effective in causing mortality in first and second instars than third and fourth instars; DFB may simply be more effective on earlier stages of developing *T. reticulata*, or the addition of *Spirulina* in the stock solution allowed the DFB to bind to a source of organic matter that was ultimately used as food. DFB has been found to be more effective on earlier stages of developing arthropods (Eisler 1992), because these life stages are usually shorter than later stages (Cunningham 1986). It is possible that DFB was broken down within the nymphs before a molting event occurred in third and fourth instars. However, it is also possible that the addition of *Spirulina* to the stock and control solutions provided an organic matter substrate to which DFB could bind, and that the reduced survivorship of groups that were given DFB was related to the use of this substrate as a food source. The best explanation for the increase in mortality of groups treated with DFB in the second experiment is likely related to both factors. The availability of DFB-tainted food was likely increased during the second experiment through the addition of *Spirulina*. The fact that younger nymphal stages molt more quickly also accounted for the increase in observed mortality.



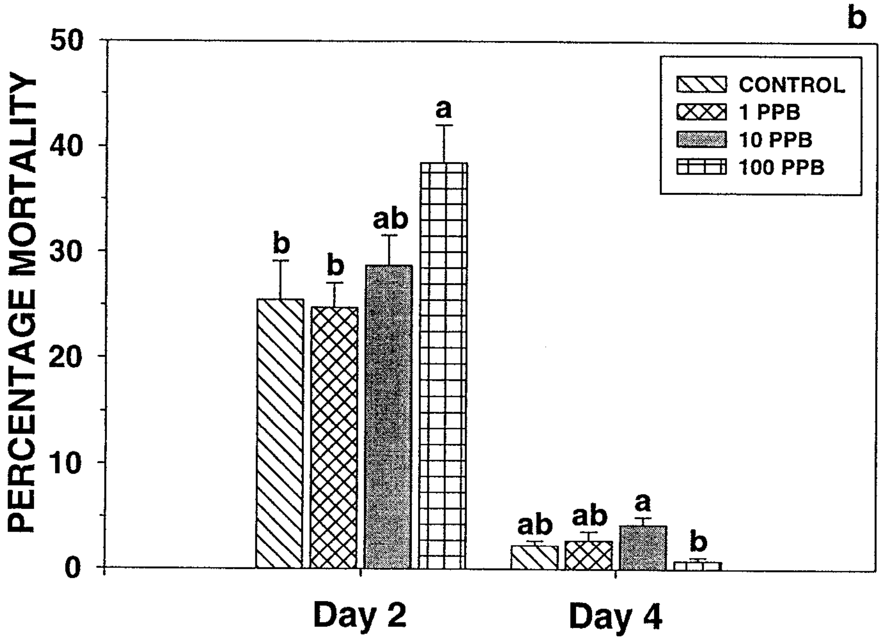


Figure 1. Mean number (se) of dead corixids per treatment for trial 1 (a), and trial 2 (b). Bars with different letters represent significant differences.

DFB was shown to decrease the survivorship of the early nymphal stages of T. reticulata, and thus could affect the overall population size of this abundant and important prey source. Although DFB was shown to be effective in a laboratory situation, some caution is needed in its use on the subsurface agricultural drainage basins found in the southern San Joaquin Valley. DFB rapidly breaks down in aquatic systems, especially in aquatic environments characterized by high temperature and alkalinity (Cunningham 1986, lvie et al. 1980) which are common traits for almost all of the evaporation basins found in the San Joaquin Valley (Parker and Knight 1989). Also, DFB rapidly bonds with organic matter (Schaefer and Dupras 1977), and therefore may not actually reach the bottom of the ponds, due to the frequent high winds that occur in this area that serve to keep a large amount of organic matter mixed within the water column, thus reducing the availability of DFB to sediment foraging arthropods such as water boatmen and midges. This problem may be avoided by using a larger particle sand granule formulation of DFB that provides a slower release of the pesticide (Cunningham 1986). Using this slow release formulation will allow DFB to reach the sediment layer of the ponds where it can bond with food particles and be ingested by foraging arthropods. Even if DFB is formulated in such a way that it is effectively distributed throughout a pond, the abundance and diversity of prey in these ponds may make this solution unacceptable. Many studies have shown that the distribution of arthropods in both terrestrial and aquatic environments is typically heterogenous (i.e. unpredictable over space and time) (Goss-Custard et al. 1977, Lifjeld 1984, Colwell and Landrum 1993, McCasland 1997). In fact, both Cooper et al. (in press) and Euliss et al. (1991) characterized ponds in this area as containing high standing crops of arthropods with low diversity that were unpredictable over both space and time. Thus, it is possible to apply DFB during a period where most of the arthropod fauna are present in the adult stage and thus be ineffective. In addition, Cooper et al. (in press) found that shorebirds foraging on these ponds are opportunistic feeders, utilizing any prey type that was abundant. Therefore, a single application of DFB may not alter shorebird feeding habits, because shorebirds may simply switch to arthropods unaffected by an application of DFB. One final caution in the use of DFB is related to the possibility of acquired resistance by some of these arthropods. Populations of the housefly (Musca domestica) have been shown to develop resistance to DFB if applications of the pesticide are nearly continuous (Keiding et al. 1992).

Our results indicate that DFB can reduce numbers of corixids. Previous research has shown that numerous species of Diptera, Copepods, Amphipods, and Artemia, the other major invertebrate groups inhabiting evaporation ponds of the San Joaquin Valley, are also affected by DFB. DFB could potentially be effective, at least in the short term, in reducing numbers of invertebrates in evaporation ponds. This action may not curtail breeding shorebirds because the reduced number of invertebrates may still be enough to support breeding shorebird populations.

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