Burrowing behavior by the fiddler crab *Uca pugilator:* inhibition by the insecticide diflubenzuron

Judith S. Weis[1] & James Perlmutter[2]

[1] Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102, USA
[2] Long Beach High School, Long Beach, New York 11561, USA

ABSTRACT: The burrowing activity of *Uca pugilator* in sand in laboratory trays was not altered when the sand was contaminated with 1.0 mg l⁻¹ diflubenzuron (DFB), indicating lack of avoidance of DFB-contaminated sand. However, crabs that had been exposed to DFB concentrations as low as 0.5 µg l⁻¹ for 1 wk or more exhibited a reduction in burrowing activity, as measured by the number of burrows dug at 15 and 60 min after release and the number of crabs within burrows at those times. The reduction in burrowing activity was not concentration-dependent between 0.5 and 50 µg l⁻¹ DFB, and was unchanged between 1 and 3 wk of exposure to the pesticide. Since fiddler crabs are dependent on burrows for many aspects of their lives, the reduction of this behavior may have serious consequences for the species.

INTRODUCTION

Since wide-spectrum insecticides have been found to have deleterious effects on non-target organisms, there has been a search for less persistent and more specific chemicals. One of the new chemicals that has been developed is the insect growth regulator diflubenzuron (DFB), or Dimilin®, which interferes with chitin synthesis (Post et al. 1974). Because of this mode of action, adverse effects of this pesticide have been observed in the Crustacea, a non-target group of organisms.

DFB has been found to disrupt development and reproduction in Crustacea (Costlow 1979, Christiansen & Costlow 1982). At concentrations as low as 0.5 µg l⁻¹, mysid and decapod larvae had increased malformations and mortality (Nimmo et al. 1980, Tester & Costlow 1981). Mortality was associated with the inability to cast off the molt at ecdysis. Similar effects have been observed on adult crustaceans, but at somewhat higher concentrations (Cunningham 1976, Cardinal et al. 1979, Gulka et al. 1980).

In addition to its effects on the molt cycle and development, DFB has also been observed to cause behavioral changes that cannot be attributed to its mode of action as a chitin synthesis inhibitor. Forward & Costlow (1978) observed changes in swimming speed and phototactic behavior of zoea larvae of *Rhi-*

thropanopeus harrisii at concentrations of 0.3 and 0.1 µg l⁻¹, respectively. Wilson et al. (1984) exposed ovigerous females of *Palaemonetes pugio*, and found effects on the offspring. Zoeae exhibited altered swimming speeds and phototactic behavior at concentrations comparable to those affecting *R. harrisii* larvae. Among freshwater cladocerans, DFB reduced filter feeding rates, body movements, and the normal phototactic response (Miura & Takahashi 1974).

Schaefer & Dupras (1976) found that concentrations of DFB sprayed on field ponds were decreased as a result of adsorption onto sediment and organic matter. While adsorption onto sediment would be likely to reduce the toxicity of DFB to pelagic larvae, it would not necessarily do so for a benthic organism that burrows into the sediment.

The fiddler crab *Uca pugilator* is an intertidal, burrowing species as an adult. Burrows proceed almost vertically downward and may go as far as 1 m. In digging the burrow, the walking legs curve around the excavated mass of substrate and form a basket with the setae. The material is then carried a distance from the burrow and dropped. While males dig only with the legs on the minor side, females dig with either side (Crane 1975).

As a semiterrestrial organism, *Uca pugilator* is found in proximity to supratidal mosquito-breeding areas of

salt marshes that could be sprayed with DFB. As a burrowing species, it comes in close contact with the sediments that could be contaminated with the pesticide. In this study we investigated 2 questions regarding DFB and burrowing of this species: (1) Can fiddler crabs detect and avoid sediment that is contaminated with DFB? (2) Does exposure to DFB alter the burrowing behavior of this species?

MATERIAL AND METHODS

Adult *Uca pugilator* were collected from Scallop Pond, Southampton, New York, USA, in early July 1986. Within 1 d of collection, crabs were tested for their avoidance responses to DFB-contaminated sediments. Tests were all conducted in 12 rectangular plastic trays 43.8 cm × 27.9 cm × 16.5 cm high. Sand was collected from the beach at Scallop pond, where the crabs lived. After 24 h in a muffle furnace followed by sieving, samples of the sand were found to be comprised of 0.48 % total organic carbon. The particle size distribution was 97.4 % sand and 2.6 % silts and clays. Five kg dry weight sand was weighed and added to each of the trays, wetted with seawater, and inclined so that it was 12 cm deep at one end and sloped down toward the other end.

For avoidance experiments, the sand in some of the trays was contaminated with 1 ppm DFB. The DFB was measured from a stock bottle at a concentration of 0.1 mg ml^{-1}, kept at 4 °C. The deep end of each tray was covered with glass to prevent escape of the crabs. A group of 10 crabs, either all males or all females of 15 to 20 mm carapace width, was added to each tray for each test. No ovigerous females were used. The trays were placed under a table in a room adjacent to the laboratory, and thin curtains were hung in front to minimize disturbance to the crabs during the test. Experiments were not timed to correspond to any part of the tidal cycle at Scallop Pond. However, since experimental and control trays were run simultaneously, any effect of the tidal cycle on burrowing activity would affect both experimental and control groups the same way. All experiments were performed during daylight hours and at 23 to 25 °C. Observations were made every 15 min for 1 h. Observations consisted of noting the total number of burrows that had been constructed and the number of crabs that were under the surface at the time of observation. After 1 h, crabs were removed and, in the case of avoidance experiments, crabs in contaminated sediments were rinsed in clean seawater and switched to clean sediments and vice versa for another trial. Data on number of burrows was averaged and analyzed by t-test, while data on number under/over in the 12 trials was analyzed by Chi-square.

In studying the effects of DFB exposure on burrowing behavior, crabs 15 to 20 mm carapace width were exposed in groups of 20 to 30 males or females to 0, 0.5, 5.0, or 50.0 μg 1^{-1} DFB in 25 ‰ seawater, at 23 to 25 °C. Crabs were tested again in groups of 10 as above after 1, 2 or 3 wk. No ovigerous females were used. Again, the tidal cycle at Scallop Pond was not considered, but since experimental and control crabs were tested at the same times, any potential effect of the tidal cycle would be equivalent in all groups. Data on numbers of burrows was analyzed by ANOVA and t-test, and those on crabs under the surface was analyzed by Chi-square and ANOVA.

The diflubenzuron used was the wettable powder WP-25 % (Uniroyal Inc., Naugatuck, Connecticut, USA). This is the principal agricultural formulation for which no solvent is needed. Wilson & Costlow (1986) have demonstrated that it has toxicity comparable to the technical grade formulation to larvae of *Palaemonetes pugio*. It was measured out of a stock bottle prepared at a concentration of 0.1 mg ml^{-1} and kept at 4 °C. Since this is above the solubility of the chemical, the suspension was shaken vigorously before each use. Concentrations were thus 'nominal', in that we did not analyze the water to verify the actual concentrations. Exposure solutions were changed 3 times weekly, and crabs were fed Purina 'Fly Chow' prior to each water change. The experiment was repeated in August 1986 with 1 and 2 wk exposures to DFB.

RESULTS

Avoidance of DFB-contaminated sand

The number of burrows dug and number of crabs under the surface of control vs DFB-contaminated sand (1 ppm) at 15 and 60 min are shown in Table 1. (Although observations were made every 15 min for an hour, the only data presented and analyzed are those at

Table 1. *Uca pugilator.* Avoidance of 1 ppm DFB-contaminated sand. There are no significant differences between control and DFB sand

Sex	Sand	Min	No. of burrows (± SE)	No. of crabs under/over
M	Control	15	2.0 ± 0.8	16/104
M	Dimilin	15	1.3 ± 0.3	18/102
M	Control	60	5.3 ± 1.0	44/76
M	Dimilin	60	3.6 ± 0.8	39/81
F	Control	15	5.0 ± 0.8	60/60
F	Dimilin	15	4.3 ± 0.8	54/66
F	Control	60	7.7 ± 0.6	106/14
F	Dimilin	60	7.0 ± 1.0	91/29

15 and 60 min.) These data represent the means of 12 runs. In comparing the number of burrows in control *vs* DFB sand by t-test, no significant effect of DFB can be seen. Similarly, in comparing the number of crabs under the surface *vs* on top of the sand in the 12 runs by Chi-square, no avoidance of DFB-contaminated sand is seen. A single crab could dig more than one burrow and might emerge from the burrow at the time of observation. Therefore, the number of burrows does not correspond to the number of crabs under the surface at any given time.

Effects of DFB exposure on burrowing behavior

In examining the number of burrows dug by crabs that had been exposed to 0.5, 5.0, or 50.0 μg l^{-1} DFB for 1, 2, or 3 wk, there were no differences observed among the 3 exposure times. Consequently, these were all pooled. ANOVA performed on the number of burrows at the different times for the 2 sexes and 3 exposure concentrations showed that they were fewer than controls, and that the differences were significant for some times, sexes, and concentrations, but not for others. ANOVA showed that in all cases there was no significant difference among the groups exposed to the 3 different concentrations of DFB. Consequently, they were all pooled and compared by t-test with the number of burrows dug by control crabs. These data are shown in Table 2. The t-tests showed that when the

Table 2. *Uca pugilator.* Burrowing by DFB-treated and control crabs. Trial 1: 0.5, 5.0, and 50.0 μg l^{-1} pooled; 1, 2 and 3 wk observations pooled

Sex	Treatment	Min	No. of burrows ($\pm$ SE)	No. of crabs under/over
M	Control	15	2.17 ± 0.33	64/296
M	Dimilin	15	1.00 ± 0.32*	23/337**
M	Control	60	5.10 ± 0.54	145/215
M	Dimilin	60	3.44 ± 0.60*	94/266**
F	Control	15	4.47 ± 0.39	175/185
F	Dimilin	15	3.0 ± 0.34*	145/215**
F	Control	60	7.36 ± 0.45	316/44
F	Dimilin	60	5.39 ± 0.54*	259/101**

* Different from control, p < 0.05 by t-test
** Different from control, p < 0.05 by Chi-square

data are pooled, significant differences are seen in the number of burrows dug by males and females for both time intervals.

In studying the number of crabs under *vs* on top of the surface, again no differences were seen among the 3 times of exposure, so these were also pooled. ANOVA on the DFB groups in these experiments showed that in

all cases there was no significant difference among the 3 different concentrations. Therefore, they were pooled and compared with control crabs. These data are also in Table 2. Significant differences are seen between control and experimental crabs of both sexes and at both time intervals, in that more control crabs were under the surface of the sand. It is apparent that the DFB-treated crabs of both sexes dug fewer burrows than controls and fewer of them were found below the surface. The data also demonstrate a sex difference in that females dug more burrows than males and had a greater tendency to be within burrows.

The experiment was repeated in August with 1 and 2 wk exposures to the same 3 concentrations of DFB. Again, because of no dose or time-dependent differences, the data were pooled and compared with controls by t-test. These data are shown in Table 3. The

Table 3. *Uca pugilator.* Burrowing by DFB-treated and control crabs. Trial 2: 0.5, 5.0, and 50 μg l^{-1} pooled; 1 and 2 wk observations pooled

Sex	Treatment	Min	No. of burrows ($\pm$ SE)	No. of crabs under/over
M	Control	15	3.08 ± 0.28	15/45
M	Dimilin	15	1.25 ± 0.32*	15/105**
M	Control	60	7.75 ± 0.73	43/17
M	Dimilin	60	5.83 ± 0.70	51/69**
F	Control	15	2.04 ± 0.60	21/89
F	Dimilin	15	1.08 ± 0.20	15/105
F	Control	60	6.16 ± 0.61	50/60
F	Dimilin	60	3.41 ± 0.47*	39/81**

* Different from control, p < 0.05 by t-test
** Different from control, p < 0.05 by Chi-square

same trends are seen as in the first experiment, but not all differences are statistically significant. Significant differences were seen in the number of burrows dug by males at 15 min and by females in 60 min, and in the number of male crabs below the surface at 15 and 60 min, and the number of females below the surface at 60 min. Nevertheless, DFB treatment again reduced the number of burrows dug by the crabs and the number of crabs occupying burrows at the times of observation. A difference between this trial and the first is that the striking sex difference in burrowing behavior is no longer apparent. However, the effects of DFB exposure were again seen in both sexes.

DISCUSSION

These investigations reveal that fiddler crabs do not detect and avoid sediment that is contaminated with 1 ppm DFB. Avoidance responses have been observed in

a variety of species to a variety of toxicants, and may serve in some cases to protect the organisms from deleterious effects of the exposure. However, this is not the case for *Uca pugilator* and DFB at the concentration used. It is of course possible that they may be able to sense and avoid higher concentrations of the pesticide.

Exposure for 1 wk to 0.5, 5.0, or 50 µg l^{-1} DFB led to a decrease in the amount of burrowing activity in this species. Fewer burrows were dug and fewer crabs were found under the surface in the experimental containers. This behavioral response was not changed after exposure for 1, 2, or 3 wk and was not concentration-related. For a number of the measurements, the 0.5 µg l^{-1} concentration was found to significantly decrease the burrowing activity. This was the lowest concentration tested. A comparable decrease in activity level (decreased ability to escape from the test containers) was seen by Cunningham & Myers (1987) in juvenile fiddler crabs. These authors suggested that the decreased activity they observed might influence the ability of the crabs to construct burrows.

In the second trial (Aug) males dug more, and females dug less than in the first trial (Jul). Consequently, the sex differences were no longer apparrent. There may be seasonal differences in burrowing rates of the 2 sexes. Ovigerous females might be expected to be reclusive (more likely to stay within burrows), but they were not used in any of the experiments. Few ovigerous females were found in August. It may be that non-ovigerous females also exhibit more reclusive behavior than males during the height of the breeding season.

There may also be semilunar tidal rhythms in burrowing activity. However, Trial 1 included measurements at 1, 2, and 3 wk of exposure, and Trial 2 included measurements at 1 and 2 wk of exposure, and no differences were seen in the measurements in the different weeks. Therefore, a semilunar tidal influence was not apparent in the results.

It is estimated that burrows are excavated on average once a day by *Uca pugilator* (Knopf 1966). Allen & Curran (1974) found that 10 *U. pugilator* completely turned over the soil in a 0.5 m^2 laboratory plot within 1 wk. Powers (1975) observed that the closely related *U. panacea* females dug more burrows than males, sometimes digging as many as 4 or 5 in succession. This sex difference is similar to that observed in our first trial.

Burrows are of great importance to this species. They serve as a refuge from terrestrial predators during low tide, and from aquatic predators during high tide (Crane 1975). They also provide relief from hot and cold temperatures (Wilkins & Fingerman 1965, Powers & Cole 1976). The burrow is also used for mating and egg incubation (Christy 1978), and its privacy and dampness provides an ideal site for molting (Bliss 1968).

Burrows are also important to the marsh ecosystem (Montague 1980). They provide a microhabitat for a community of other organisms (Bright & Hogue 1972), and can aerate marsh soils (Edwards & Frey 1977). The nitrogen excreted by the fiddler crabs within burrows may be available to adjacent plant roots and may enhance growth of *Spartina* (Montague 1980). The organic matter excavated as a result of burrowing behavior can provide another mechanism for organic matter to enter the estuarine system (de la Cruz & Hackney 1977), and can also provide more food for marsh organisms including the fiddler crabs themselves.

Changes in burrowing behavior of crabs have been observed after exposure to other pollutants. Krebs & Burns (1977) found that after an oil spill in Falmouth Harbor, Massachusetts, USA, fiddler crabs *Uca pugnax* dug shallower burrows than normal. The burrows in the oiled areas, instead of being almost vertical, began steeply but became horizontal. These abnormally shallow burrows contributed to winter mortality in these crabs, which were thus not protected from freezing temperatures. Koenig et al. (1976) observed that in DDT-contaminated salt marshes, blue crabs *Callinectes sapidus* did not burrow into the mud as effectively as those in uncontaminated marshes. This was correlated with increased mortality during the winter. The burrowing is believed to buffer the crabs from low temperatures.

Other alterations in burrowing behavior in response to toxicants have been observed in bivalve mollusks (Phelps et al. 1983). The rate of burrowing by the clam *Protothaca staminea* decreased with increasing sediment copper concentration.

Since burrowing behavior is vital to the well-being of the fiddler crabs, and may be important to the overall marsh ecology, the reduction of this behavior in crabs exposed to DFB is a response that may have deleterious effects on the species and on the ecosystem.

LITERATURE CITED

Allen, E., Curran, H. A. (1974). Biogenic sedimentary structures produced by crabs in lagoon margin and salt marsh environments near Beaufort, North Carolina. J. sedim. Petrol. 44: 538–548

Bliss, D. E. (1968). Transition from water to land in decapod crustaceans. Am. Zool. 8: 355–392

Bright, D. B., Hogue, C. L. (1972). A synopsis of the burrowing land crabs of the world and list of their arthropod symbionts and burrow associates. Contr. Sci. 220: 1–58

Cardinal, H., Vernet, G., Sinegre, G. (1979). Quelques effets d'un inhibiteur de croissance: le diflubenzuron sur un crabe *Carcinus meditterraneus* (Czerniavsky). C. R. Soc. Biol. 173: 1105–1108

Christiansen, M. E., Costlow, J. D., Jr. (1982). Ultrastructural study of the exoskeleton of the estuarine crab, *Rhithropanopeus harrisii*: effect of the insect growth regulator Dimilin (diflubenzuron) on the formation of larval cuticle. Mar. Biol. 66: 217–226

Christy, J. (1978). Adaptive significance of reproductive cycles in the fiddler crab *Uca pugilator*: a hypothesis. Science 199: 453–455

Costlow, J. D., Jr. (1979). Effect of Dimilin on development of larvae of the stone crab *Menippe mercenaria* and the blue crab *Callinectes sapidus*. In: Vernberg, W B., Calabrese, A., Thurberg, F. P, Vernberg, F. J. (ed.) Marine pollution: functional responses. Academic Press, New York, p. 355–364

Crane, J. (1975). Fiddler crabs of the world. Princeton Univ. Press, Princeton

Cunningham, P. A. (1976). Effects of Dimilin (TH-6040) on reproduction in the brine shrimp *Artemia salina*. Environ. Entomol. 5: 701–706

Cunningham, P. A., Myers, L. E. (1987). Effects of diflubenzuron (Dimilin) on survival, molting, and behavior of juvenile fiddler crabs, *Uca pugilator*. Archs. environ. Contam. Toxicol. (in press)

De la Cruz, A. A., Hackney, C. T. (1977). Energy value, elemental composition, and productivity of belowground biomass of a Juncus tidal marsh. Ecology 58: 1165–1170

Edwards, J. M., Frey, R. W. (1977). Substrate characteristics within a Holocene salt marsh, Sapelo Island, Georgia. Senckenberg. maritima 9: 215–259

Forward, R. B., Jr., Costlow, J. D., Jr. (1978). Sublethal effects of insect growth regulators upon crab larval behavior. Wat. Air Soil Pollut. 9: 227–238

Gulka, G., Doscher, C. M., Watabe, N. (1980). Toxicity and molt-accelerating effects of diflubenzuron on the barnacle, *Balanus eburneus*. Bull. environ. Contam. Toxicol. 25: 477–481

Knopf, G. N. (1966). Observations on behavioral ecology of the fiddler crab *Uca pugilator* (Bosc). Crustaceana 11: 302–306

Koenig, C., Livingston, R., Cripe, C. (1976). Blue crab mortality: interaction of temperature and DDT residues. Archs. environ. Contam. Toxicol. 4: 119–128

Krebs, C., Burns, K. (1977). Long term effects of an oil spill on populations of the salt marsh crab *Uca pugnax*. Science 197: 484–487

Miura, T., Takahashi, R. M. (1974). Insect developmental inhibitors: effects of candidate mosquito control agents on nontarget aquatic organisms. Environ. Entomol. 3: 631–636

Montague, C. (1980). A natural history of temperate Western Atlantic fiddler crabs with reference to their impact on the salt marsh. Contr. mar. Sci. 23: 25–56

Nimmo, D. R., Hamaker, T. L., Moore, J. C., Wood, R. A. (1980). Acute and chronic effects of Dimilin on survival and reproduction of *Mysidopsis bahia*. In: Eaton, J. B., Parrish, P. R., Hendricks, A. C. (ed.) Aquatic toxicology, STP 707. American Society for Testing and Materials, Philadelphia, 366–376

Phelps, H. L., Hardy, J. T., Pearson, W. H., Apts, C. W. (1983). Clam burrowing behavior: inhibition by copper-enriched sediments. Mar. Pollut. Bull. N. S. 14: 452–455

Post, L. C., DeJong, B. J., Vincent, W. R. (1974). 1-(2,6-disubstituted benzoyl)-3-phenylurea insecticides: inhibitors of chitin synthesis. Pestic. Biochem. Physiol. 4: 473–483

Powers, L. W. (1975). Fiddler crabs in a nontidal environment. Contr. mar. Sci. 19: 67–78

Powers, L. W., Cole, J. F. (1976). Temperature variations in fiddler crab microhabitats. J. exp. mar. Biol. Ecol. 21: 141–157

Schaefer, C. H., Dupras, E. F. (1976). Factors affecting the stability of Dimilin in water and the persistence of Dimilin in field waters. J. agric. Fd Chem. 24: 733–739

Tester, P. A., Costlow, J. D., Jr. (1981). Effect of insect growth regulator Dimilin ± (TH 6040) on fecundity and egg viability of the marine copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. 5: 297–302

Wilkins, J. L., Fingerman, M. (1965). Heat tolerance and temperature relationships of the fiddler crab *Uca pugilator* with reference to body coloration. Biol. Bull. mar. biol. Lab., Woods Hole 128: 133–141

Wilson, J. E., Costlow, J. D. (1986). Comparative toxicity of two Dimilin formulations to the grass shrimp, *Palaemonetes pugio*. Bull. environ. Contam. Toxicol. 36: 858–865

Wilson, J. E., Forward, R. B., Jr., Costlow, J. D., Jr. (1984). Effects of embryonic exposure to sublethal concentrations of Dimilin on the photobehavior of grass shrimp larvae, *Palaemonetes pugio*. In: Vernberg, F. J., Calabrese, A., Thurberg F. P., Vernberg, W. B. (ed.) Marine pollution and physiology: recent advances. Academic Press, New York, p. 377–396