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Effect of Insect Growth Regulator Dimilin® (TH 6040) on Fecundity and Egg Viability of the Marine Copepod *Acartia tonsa*

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ABSTRACT: Studies of adult LD_{50} concentrations of Dimilin, an insect growth regulator which acts to inhibit chitin synthesis, are misleading when applied to arthropods which experience a terminal molt. The calanoid copepod *Acartia tonsa* Dana has a determinate number of molts after which no growth occurs and the necessity of chitin production is restricted. Survival of adult *A. tonsa* held for 5 d in concentrations as high as 1000 ppb Dimilin is not significantly ($P < 0.05$) different from the controls. Fecundity of *A. tonsa* was not significantly ($P < 0.05$) altered by 1 to 4 d treatment with 1, 10 or 100 ppb Dimilin but the hatch of viable nauplii was reduced from 90+% to less than 50 % in 1 ppb after 12 h of treatment and to less than 5 % in 10 ppb after 24 h of treatment. No viable nauplii were produced by females held in either 1 or 10 ppb Dimilin for more than 36 h. When Dimilin treatment ceased and the females were placed in seawater, the effect of Dimilin was not readily reversed. No viable nauplii were produced by these females for at least the next 30 h after treatment ended.

INTRODUCTION

The insect growth regulator Dimilin (1-[4-chlorophenyl]-3[2,6-difluorobenzoyl]-urea) is an experimental insecticide which acts to inhibit chitin synthesis (Post and Vincent, 1973). More specifically, it is reported to interfere in the formation of the endocuticle (Post et al., 1974). Because of its unprecedented mode of action, it is effective against a wide variety of arthropods. It is highly effective against aquatic Diptera (Mulla and Darwazeh, 1976) and its effectiveness in low concentrations for all developmental stages of mosquitoes (Georghious and Lin, 1975) suggests it as one of the most promising compounds experimentally available for mosquito control (Jakob, 1973).

The chitin-inhibiting effect of Dimilin on non-target arthropods associated with mosquito breeding habitats, especially in the marine environment, is of prime ecological concern because of its possible effect on secondary production. In the case of the effect of Dimilin on non-target arthropods, laboratory bioassays using death of adults as an end-point are not adequate. Calanoid copepods have a determinate number of molts, the last of which is the molt to maturity. After this, no growth occurs and the requirement for chitin synthesis in the adults is restricted to the production of eggs.

It is the purpose of this study to determine the effect of sublethal concentrations of Dimilin for adult *Acartia tonsa* Dana on fecundity and egg viability. Reproductive rates or population birth rates of copepods are directly related to secondary production by Edmundson et al. (1962) and *A. tonsa* is an abundant copepod in Long Island sound (Conover, 1956), the Delaware River (Cronin et al., 1962), in Biscayne Bay, Florida (Woodmansee, 1958) and along the Texas coast (Breuer, 1957). In selecting this persistent and abundant estuarine copepod, we deal with an important component of the food chain. Heinle (1966) suggests that *A. tonsa*, where abundant, is possibly the major planktonic herbivore.

MATERIALS AND METHODS

The calanoid copepods used in these experiments, *Acartia tonsa* were taken from laboratory maintained cultures. Standard culture procedures: Vertical plankton tows were taken within 1 m of the surface off the Duke University Marine Laboratory dock, Beaufort, N.C. (USA), during 3–9 November 1977. *A. tonsa* were

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sorted from the mixed plankton samples after these samples warmed to room temperature overnight. Stocks were maintained in battery jars containing 10 to 16 l of filtered (glass fiber filter) offshore seawater diluted to 10 ‰ S with glass distilled water. Laboratory cultures were kept at room temperature (20 °C ± 2 C°) while constant temperature boxes were used to maintain temperatures during the experiments. All stock cultures were gently aerated and fed every other day *Thalassiosira fluviatilis* and *Pseudoisochrysis paradoxa* in concentrations of approximately 2×10^6 cells ml^{-1}. Phytoplankton were cultured in half-strength medium (Guillard and Ryther, 1962) at 20 °C in 20 ‰ S.

Dimilin concentrations: One ml of the stock solution of 1 ppt Dimilin dissolved in pesticide grade acetone was added to 1 l of 10 ‰ S to make a working stock solution. This working stock solution of 1 ppm Dimilin was prepared daily and mixed thoroughly before appropriate amounts were further diluted with 10 ‰ S to make 0.5, 1.0, 5.0, 10.0, 100.0, or 1000.0 ppb Dimilin concentrations as required. Standard controls were 10 ‰ S and/or 0.5 ppt pesticide grade acetone in 10 ‰ S (acetone-sw control).

Survival: One adult *Acartia tonsa* was placed in each of 90 Boveri bowls with 35 ml of either 1, 10, 100, or 1000 ppb Dimilin, fed and held at 20 °C. Both seawater (sw) and acetone-sw controls were used and the media and food were replaced daily. This experiment was replicated 3 times for the 1, 10, and 100 ppb concentrations. At the end of 5 d the number of live copepods was recorded. Since even the highest concentration of Dimilin (1000 ppb) we tested was found to be sublethal to adult *Acartia tonsa,* we elected to work, for the most part, with 1 and 10 ppb because previous studies indicated these concentrations were effective against crustacean larvae (Christiansen et al., 1978) and larvae of the black salt-marsh mosquito *(Aedes taeniorhynichu)* (Thompson-Hayward Chemical Company, 1974).

Fecundity: One fertilized female *Acartia tonsa* was placed in each of 90 Boveri bowls with either 35 ml of 1, 10, or 100 ppb Dimilin. Thirty more fertilized adult females were exposed to the acetone-sw solution to serve as a control. Observations of the number of eggs laid by each female were made every 24 h and the eggs which collected in the bottom of the Boveri bowls were removed with a capillary-type micropipet. This experiment continued for 4 d. Previously we sought to measure fecundity by removing the females every 12 h to another Boveri bowl containing the appropriate media after which the eggs were counted and incubated in the original bowls. We include the results of that experiment to indicate the effects of excessive handling on *A. tonsa.* Concentrations of 1 to 100 ppb Dimilin had no adverse effect on the fecundity of *A. tonsa* but eggs from these Dimilin treated females were less viable than eggs from untreated females. The following experiments were designed to test the effect of Dimilin on egg viability.

Egg viability: Approximately 50 to 60 adult female *Acartia tonsa* were placed in 1 l beakers containing either 1 or 10 ppb Dimilin or 10 ‰ S or the acetone-sw solution. The specimens were held in the Dimilin solutions for a total of 60 h and every 12 h, the eggs laid over a 4 h period were removed and from 100 to 250 eggs were incubated in 10 ‰ S at 20 °C. The eggs from the sw and acetone-sw controls were treated similarly. Solutions were changed daily and fresh food supplied. The number of eggs hatched after a 48 h incubation period was recorded. This experiment was replicated 3 times. After 60 h of exposure to 1 and 10 ppb Dimilin, the adults were placed in 10 ‰ S to determine if the effect of Dimilin on egg viability was reversible. The experiments then continued for another 30 h. To assess the effect of exposure to Dimilin on the eggs from non-treated females, we determined egg hatching times. Females were allowed to lay eggs in 10 ‰ S for 4 h. From 65 to 335 eggs were incubated in 0.5, 1, 5, and 10 ppb Dimilin and 0.5 ppt acetone-sw solution at 25 °C. This experiment was duplicated and a total of 1615 eggs were observed 3 to 6 times each from the initiation of hatching to the time of 90 % + hatch in the controls. Time to 100 % hatch was predicted by a linear regression equation fitted to the data (Burgis, 1970).

All percentages were arcsine transformed to convert them to angles (arcsine $\sqrt{\%}$; Sokal and Rolf, 1969) before the statistical calculations were made and converted back to percentages for reporting.

RESULTS

Survival

The survival of adult *Acartia tonsa* ranged from 90.6 % (sw control) to 77.5 % (1 ppb Dimilin) during the 5 d experiment. In no case did survival of the Dimilin treated adults differ significantly ($P < 0.05$) from the survival of the control groups (Table 1). We conducted one experiment (37 observations) using 1000 ppb Dimilin and found 91.9 % survival after 5 d.

Fecundity

Fecundity of *Acartia tonsa* females was not significantly ($P < 0.05$) altered by a 1 to 5 d exposure to concentrations of 1 to 100 ppb Dimilin (Table 2). The average number of eggs produced daily by the females held in the acetone control was 28.0 (standard error =

Table 1 *Acartia tonsa*. Percent survival of adults after 5 d in concentrations of 1 to 1000 ppb Dimilin. SW = sea water

Treatment	% survival after 5 d		No. of observations	No. of experiments
	Mean	Std error		
SW control	90.6	2.6	209	6
Acetone control	86.1	4.7	130	3
1 ppb Dimilin	77.5	3.6	93	3
10 ppb Dimilin	81.1	6.6	95	3
100 ppb Dimilin	80.9	4.6	107	3
1000 ppb Dimilin	91.9	–	37	1

± 0.89). These data compare well with the mean daily production of 25.8 (SE = ± 1.84) eggs per female reported for *Acartia tonsa* by Parrish and Wilson (1978). At 1 and 10 ppb Dimilin the fecundity was not significantly ($P < 0.05$) different from the control group (Table 2). We conducted one more experiment to check the effect of an even higher concentration of Dimilin, 100 ppb, on fecundity. The sum of the mean daily egg production did drop to 11.7 eggs per female on the 4th day of treatment at this higher concentration.

In the preliminary experiment when females were transferred to different Boveri bowls every 12 h (rather than the eggs being removed), the average daily fecundity was only 8.1 eggs per female in the control experiment. The specimens in 1 and 10 ppb Dimilin averaged 5.2 and 9.9 eggs d^{-1}, respectively. Perhaps the interpretation of the results of the effect of Dimilin on fecundity of *Acartia tonsa* would not have changed but because of the substantially lower fecundity caused by excessive handling, we redesigned the experiment.

Viability

Eggs produced by female *Acartia tonsa* which were held in 10 ppb Dimilin from 12 to 60 h showed a decrease in % hatch of viable nauplii from 93.4 to 0 % (Fig. 1). Concentrations of 1 ppb Dimilin also reduced

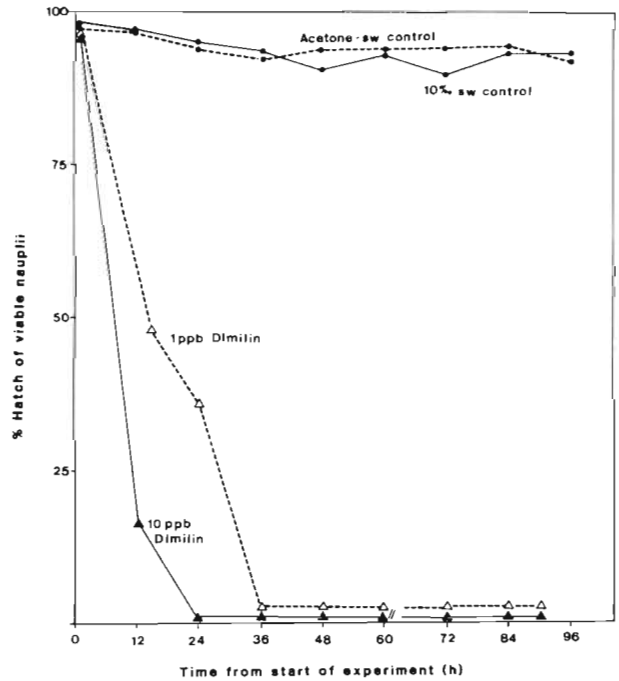

Fig. 1. *Acartia tonsa*. Production of viable nauplii by females exposed to 1 and 10 ppb Dimilin for 12 to 60 h. Dimilin treatment ceased at Hour 61 when the females were placed in seawater. Effect of prior treatment with Dimilin is still evident 30 h after exposure to Dimilin ceased

the egg viability and this effect was most pronounced after 24 h of treatment.

The eggs produced by females exposed to 1 and 10 ppb Dimilin for 12 to 60 h which did not hatch were fully developed (Fig. 2); nauplii were observed to move inside the egg membranes after 24 to 30 h of incubation. In some cases hatching was only partially successful; the chorion ruptured but the nauplius died with the egg membrane still intact (Fig. 3). By the end of the 48 h incubation period no movement of unhatched nauplii was detected and some of the eggs had begun to deteriorate. The normal time to 100 % hatch for *Acartia tonsa* eggs at 25 °C is approximately 25 h.

Nauplii which did hatch during the period were abnormally shaped and failed to molt to the second naupliar stage. The abnormalities most frequently

Table 2. *Acartia tonsa*. Effect of 1 to 100 ppb Dimilin on fecundity during a 1 to 4 d treatment period

Treatment	Mean no. eggs $d^{-1} ♀^{-1}$				Grand mean	Std error
	Day 1	Day 2	Day 3	Day 4		
Acetone control	26.2	29.7	29.4	26.9	28.0	0.9
1 ppb Dimilin	24.4	27.7	27.1	20.3	24.9	1.7
10 ppb Dimilin	31.8	36.2	33.1	29.2	32.6	1.5
100 ppb Dimilin	23.8	–	29.1	11.7	21.5	5.1

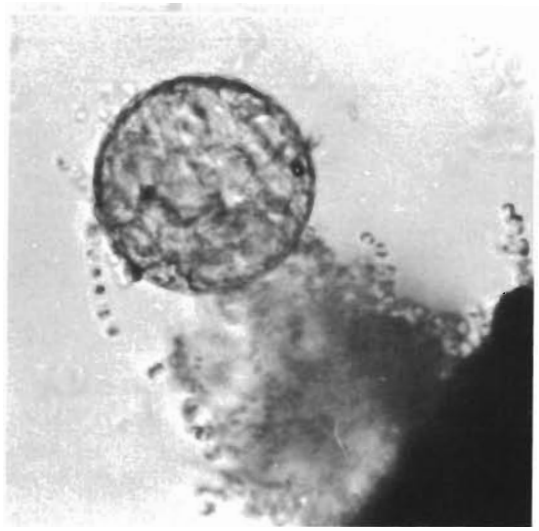

Fig. 2. *Acartia tonsa*. Fully developed embryo from female exposed to 10 ppb Dimilin for 36 h. This embryo was active but failed to hatch after 48 h at 25 °C. Normal time to 100 % hatch at 25 °C is ca 25 h

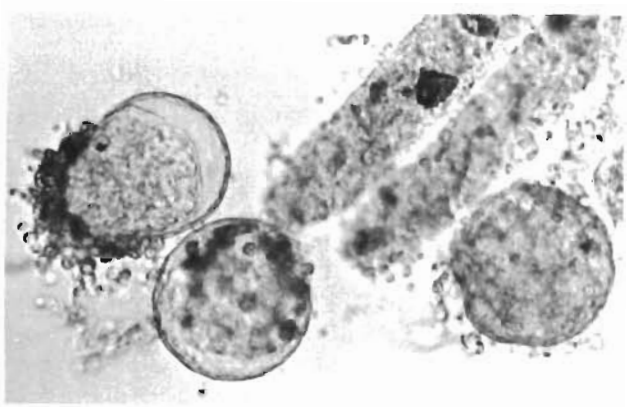

Fig. 3. *Acartia tonsa*. Fully developed embryo, 36 h old, from female exposed to 1 ppb Dimilin for 60 h. The chorion of 1 embryo is ruptured but eclosion is incomplete. Egg diameter ca 75 µm

seen were in body and appendage shape and setae malformation. The nauplii were balloon-shaped as were their appendages (Fig. 4), when compared to a normal nauplius. Frequently the setae on the appendages were twisted and bent.

When treatment was reversed and the copepods were placed in 10 ‰ S after the 60 h of treatment in Dimilin solutions, no viable nauplii hatched from eggs produced during the next 30 h (h 61–90 of the experiment).

Results from experiments testing the effect of Dimilin on eggs from non-treated adults show the treatment of 0 to 4 h old eggs with 0.5, 1, 5, and 10 ppb Dimilin did not produce a significant ($P < 0.05$) difference between the times to 100 % hatch of the control series and treated eggs (Table 3). Here, it should be noted that within each experiment the shortest development times are associated with the highest concentrations of Dimilin. No abnormally developed nauplii were observed from these eggs.

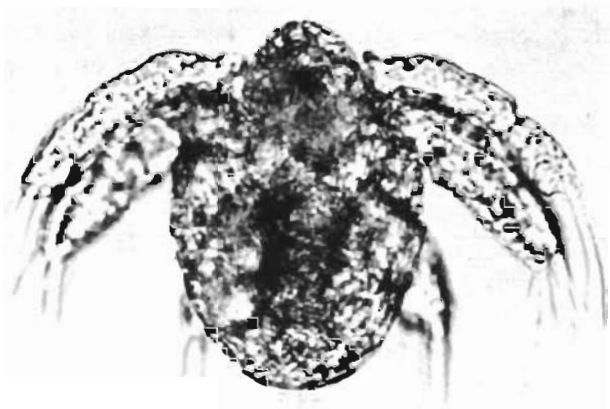

Fig. 4. *Acartia tonsa*. Nauplius I stage with balloon-like body and appendages. These abnormalities are characteristic of those found in nauplii from adults exposed to 1 and 10 ppb Dimilin. Nauplius length ca 80 µm

Table 3. *Acartia tonsa*. Effect of Dimilin on time to 100 % hatch for eggs 0 to 4 h old when treated. Eggs from untreated females. Incubation at 25° C. SW = sea water

Treatment	Time to 100 % hatch (h)	% hatch of total no.	No. total eggs	No. of observations during incubation
SW control	26.3	92	253	4
0.5 ppb Dimilin	26.4	91	126	3
5 ppb Dimilin	25.3	88	114	3
SW control	26.2	97	180	6
1 ppb Dimilin	24.6	92	63	6
10 ppb Dimilin	23.7	95	147	6
SW control	27.4	98	337	6
1 ppb Dimilin	23.4	76	147	5
10 ppb Dimilin	23.3	84	248	5

DISCUSSION

With few exceptions (Cunningham, 1976; Christiansen et al., 1978) the studies on the effects of Dimilin on non-target organisms deal with lethal concentrations to adults. Necessarily, the comparisons of our results on sublethal effects of Dimilin on *Acartia tonsa* draw heavily from the literature on the effect of Dimilin exposure on various insect species.

Our results indicate that concentrations of Dimilin which are sublethal to adult *Acartia tonsa*, while not decreasing their fecundity, can greatly limit the number of viable nauplii produced. McCoy (1978) reports no ovicidal or direct toxic effects on adult citrus rust mites *Phyllocoptuta oleivora* after exposure to Dimilin, and the findings of Mulder and Gijswijt (1973) were similar. Adults of none of the 4 species of insects they tested were susceptible to Dimilin at concentrations up to 100 ppb. Studies of the adult LD_{50} concentrations of Dimilin on any arthropod which experiences a terminal molt have little meaning. Death caused by Dimilin appears to be invariably connected with ecdysis since its effect is to disrupt the deposition of endocuticle (Mulder and Gijswijt, 1973).

The abnormalities of the nauplii produced by Dimilin treated copepods were similar to those reported by Christiansen et al. (1978) for crab larvae exposed to Dimilin. Mulder and Gijswijt (1973) suggest increased internal body pressure and/or deterioration of the mechanical properties of the cuticle was responsible for the balloon-like distortion of insect larvae they observed from Dimilin treated adults.

The hatching of calanoid copepod eggs involves the rupture of the chorion by pressure from within caused by expansion of the inner (egg) membrane (Marshall and Orr, 1954, 1972; Davis, 1959, 1968). Davis describes a sudden change of permeability of the inner membrane just prior to hatching when this inner membrane expands. Within this blister (egg) membrane the unhatched nauplius begins to swim actively. Davis suggests this activity is not necessary for the completion of eclosion and our observations confirm this. The inability of fully developed embryos from Dimilin treated *Acartia tonsa* adults to hatch suggests that changes in the egg could alter this ability to respond to osmotic changes during eclosion. Failure of the egg membrane to change permeability and expand precludes rupture of the chorion. Thirty h old embryos from females exposed to Dimilin for 12 to 48 h were active inside the eggs so it is apparent that eclosion is effected by physiological rather than mechanical means. The failure of fully developed insect larvae to emerge from Dimilin treated eggs has been noted by Mulder and Gijswijt (1973), Moore and Taft (1975) and Wright and Spates (1976). Miura et al. (1976) found that treatment of mosquito (*Culex pipiens quinquefasciatus*) eggs with Dimilin caused weakness of the chorion and interfered with the transverse line of dehiscence in the egg case.

The eggs from non-treated female *Acartia tonsa* which were incubated in 0.5, 1, 5, and 10 ppb Dimilin hatched normally. Since these eggs were already formed and were from 0 to 4 h old by the time their exposure to Dimilin commenced they appear to have been resistant to its effect. Findings of Chaffoy et al. (1978) indicate the importance of the permeability and formation of the embryonic cuticle during the development of *Artemia* sp. embryos. The *Artemia* sp. embryo is quite permeable up to the first gastrula stage; after the second gastrula stage, it is no longer able to take up even inorganic phosphate. In contrast, an embryo (excised from the uterus before gastrula stage and grown thereafter ex utero) which remained completely permeable to inorganic phosphate up to the naupliar stage was malformed and unable to undergo metamorphosis.

Inasmuch as Dimilin is a highly effective mosquito larvicide, its effects on marine crustacea are not surprising. In concentrations sublethal to adults (1 to 10 ppb) Dimilin is toxic to mosquito larvae, megalopae of the commercially important blue crab *Callinectes sapidus* (Costlow, 1979), and to the eggs and nauplii of *Acartia tonsa*. In view of the effect of Dimilin on the larval stages of non-target organisms we suggest the economic and ecological impact of its use be fully considered.

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