EFFECTS OF *TOXORHYNCHITES RUTILUS RUTILUS* (COQUILLETT) LARVAE ON PRODUCTION OF *AEDES AEGYPTI* ADULTS IN LABORATORY TESTS

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ABSTRACT. The effects of various numbers of *Toxorhynchites rutilus* larvae were tested on more or less stable populations of *Aedes aegypti* larvae in artificial containers. Pupation time for the predator was dependent on the number of prey available during the larval stage. Cannibalism did not appear to be a major problem, even when different-sized predators were present in the same container. Weekly introduction of a predator larva to each container gave better overall control of *Ae. aegypti* than did a single initial introduction of one or 2 larvae per container.

INTRODUCTION

Although many *Toxorhynchites* species normally select tree holes as breeding sites, many researchers have also found them in artificial containers where other mosquito species breed (King et al. 1939, Jenkins 1949, Carpenter and LaCasse 1955). *Toxorhynchites* in the larval stage are predators of other mosquito species, and the adult females do not require a blood meal. Thus, interest has recently increased in the possible use of *Toxorhynchites* for the biological control of container-breeding mosquitoes (Brown 1973, Steffan 1975, Gerberg and Visser 1978, Focks et al. 1979). *Aedes aegypti* (Linnaeus), an important disease vector worldwide, has probably generated the most interest for the use of *Toxorhynchites* for this purpose.

Most of the past attempts to control *Ae. aegypti* with *Toxorhynchites* have failed because only small numbers of the adults were released, and subsequent populations were too small to exert control (Payne 1934, Bonnet and Hu 1951, Hu 1955, Peterson 1956). Thus, it became apparent that multiple, inundative releases of *Toxorhynchites* would be required for control of container-breeding mosquitoes (Gerberg and Visser 1978).

The Insects Affecting Man and Animals Research Laboratory, ARS, USDA, Gainesville, Florida, is presently conducting a 3-yr pilot test in New Orleans, Louisiana, to determine the potential of

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Toxorhynchites species to successfully control *Ae. aegypti* in a substandard urban residential area.

Focks et al. (1981) characterized the larval breeding and population density of *Ae. aegypti* in artificial containers found on 6 typical city blocks in the pilot test area. If it is possible to obtain adequate predator oviposition in these containers, information on the frequency of predator introduction required to exert meaningful reductions of the indigenous populations of *Ae. aegypti* will be required. Therefore, we tested the effects of various numbers of *Toxorhynchites rutilus rutilus* (Coquillett) larvae on *Ae. aegypti* breeding in containers receiving regular inputs of *Ae. aegypti* first instars and food.

TESTS AND RESULTS

The rate of consumption of prey is a function of the age and stage of the predator, and the number and stages of prey present (Padgett and Focks 1981, Vongtangswad and Trpis 1980). Existing data on predation rates are inadequate to predict the effects on the adult production and standing crop of prey when various numbers of predators are added as first-stage larvae to this dynamic system. Prediction of the effects of predation are further complicated in *Ae. aegypti* by the random process of egg hatch, a process that is a function of *Ae. aegypti* adult populations, standing crop of viable eggs within the container, and rainfall.

Therefore, we developed a schedule for adding larval food and newly hatched *Ae. aegypti* larvae to laboratory containers which, through density dependent mechanisms over time, established stable age distributions of *Ae. aegypti* immature stages that closely resembled those found in similar containers in the field (Focks et al. 1980). An 8-liter plastic bucket was chosen because it was easy to handle in the laboratory and because it represented a common container in terms of production of *Ae. aegypti* in substandard areas of New Orleans, LA.

DEVELOPMENT OF STABLE DISTRIBUTION OF *Ae. aegypti*. Stable age distributions were first tested in containers without predators to determine the number of first-instar *Ae. aegypti* that should be regularly added to containers of a given size and shape to establish standard populations of *Ae. aegypti* immatures. Two groups of 3 buckets each were included in the test, and 3 liters of well water were added to each bucket. In the first group 50, 100, or 200 newly hatched *Ae. aegypti* larvae were added to the buckets each Monday and Friday (MF). In the second group 50, 100, or 200 larvae were added each Monday, Wednesday, and Friday (MWF). Each time larvae were added to the buckets, a slurry containing 0.1 g of a 3:2 mixture of powdered liver and yeast was added as food. Pupae were removed daily and counted. At the end of 28 days, the number of larvae in the buckets was counted and categorized as early (first and second instars) or late (third and fourth instars). The replicates were started 14 days apart.

RESULTS. Table 1 presents the average number of early and late larvae found in the laboratory containers 28 days after they were set up. Because pupal production did not stabilize until ca. 14 days after the experiment was begun, estimates for the standing crop of pupae were made by doubling the mean daily pupal production observed during days 15–28. The doubling reflected the number of pupae that would have accumulated in the 2-day pupal period had they not been removed on a daily basis. Table 1 also includes the mean standing crop of *Ae. aegypti* immatures found in similarly shaped containers from a substandard residential area of New Orleans, LA, during September and October 1979 (Focks et al. 1981).

It was not surprising to find that pupal production within each group (MF and MWF) was independent of the four-fold differences in the numbers of first-instars added. If the numbers of immatures added significantly affected pupal production, the mean standing crop in the MF 200 and 100 containers would be ex-

Table 1. The standing crop of *Ae. aegypti* immatures in 8-liter plastic buckets provided with various numbers of first instars in the laboratory and in similar containers from a field site in a substandard residential area in New Orleans, LA, as measured after 28 days.

Stage of immatures	Number of immatures						
	Laboratory						
	1st instars added MWF			1st instars added MF			
	200	100	50	200	100	50	Feild[a]
I + II	521	347	81	430	267	12	73
III + IV	270	232	119	269	158	64	55
Pupae	27	31	31	17	23	22	14
Totals	818	610	231	716	448	98	142

[a] Based on Table 5, Focks et al. 1981.

pected to be intermediate to the levels in MWF 200 and 100 and MWF 100 and 50 containers, respectively. The fact that the MWF group received 50% more food than the MF group and produced an almost correspondingly larger number of pupae (43% more) illustrates the role of food in determining pupal production in this density-dependent system.

In terms of the total standing crop of immatures, the field containers from New Orleans were intermediate to the MWF 50 and MF 50 treatments. However, the field containers showed ca. one-half to two-thirds the pupal production found in the MWF 50 and MF 50 containers, respectively.

EFFECTS OF TX. R. RUTILUS ON ADULT EMERGENCE IN *Ae. aegypti*. The predator *Tx. r. rutilus* was tested with maintenance of prey and containers identical to those already described. Predator and prey larvae were introduced into the buckets as follows:

No. *Tx. r. rutilus* (predator) larvae added	No. *Ae. aegypti* (prey) larvae added	
	MF	MWF
0	50	50
1	50	50
2	50	50
1/wk	50	50

After the buckets had stabilized with the *Ae. aegypti*, first-stage larvae of *Tx. r. rutilus* were introduced on day 14 in all buckets and weekly thereafter in the buckets scheduled to be given one predator/wk. Each bucket was screened with nylon mesh, and the daily emergence of adult *Ae. aegypti* was recorded. The number of early and late larvae and pupae of *Ae. aegypti* were counted in each container 14 and 28 days after introduction of predator larvae began. The dates of pupation and emergence of the predators was also recorded. A total of 4 replicates was conducted.

RESULTS. Figure 1 shows the effects of different numbers of predators on the emergence of *Ae. aegypti* adults in containers with different levels of prey. At first, introduction of predators had no appreciable effect on *Ae. aegypti* adult emergence. However, after the predators reached the third instar and, especially, after they reached the fourth instar, emergence of adult *Ae. aegypti* showed a sharp decline in all buckets containing predators. Figure 1 also shows that after pupation of the predators emergence of *Ae. aegypti* adults in the buckets with only one or 2 predators again increased. In those buckets receiving a predator each week, emergence of adult prey was kept at a low level throughout the test.

For days 20–33, the period when con-

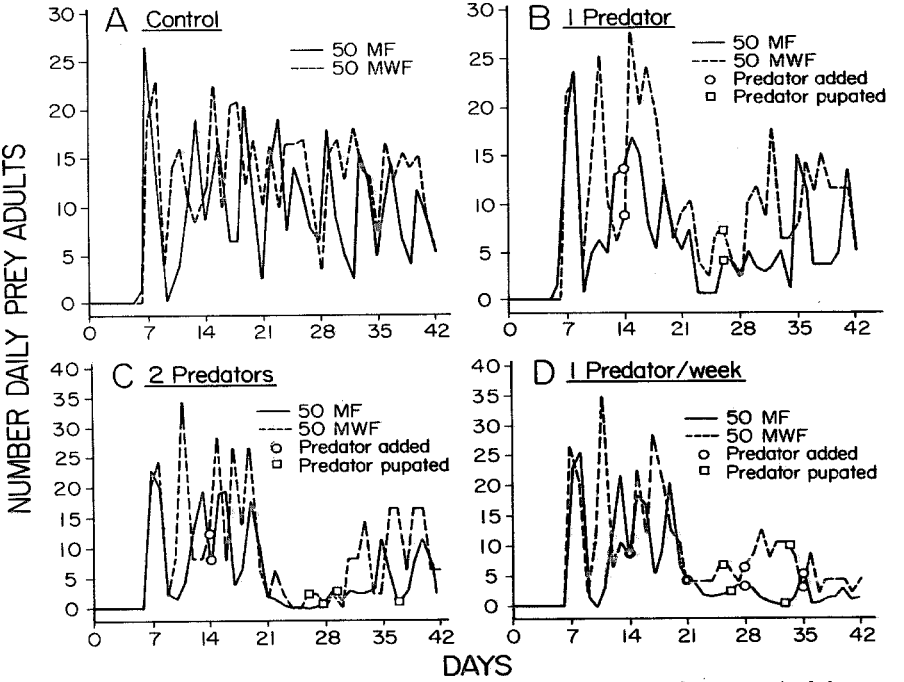

Fig. 1. Effects of various levels of *Tx. r. rutilus* larvae on number of *Ae. aegypti* adults emerging from 8-liter plastic buckets.

tainers with one and 2 *Tx. r. rutilus* showed greatest control of *Ae. aegypti*, buckets with the higher level of prey introduction (MWF) had a higher rate of adult emergence than those with the lower level for equal (MF) numbers of predator larvae (Table 2). Two predators exerted more control during days 20–33 than did either one predator or the weekly introduction of a predator, especially at the higher level of prey introduction. However, the weekly introduction of predator larvae gave more control than one or 2 predators for days 20–42, because buckets had at least one and usually 2 predators at all times. For days 34–42, when emergence of *Ae. aegypti* increased in buckets with one or 2 predators due to pupation of the predators (10 and 30% reduction, respectively) the 1/week introduction of *Tx. r. rutilus* achieved 80 and 66% control of *Ae. aegypti* at the low (MF) and high (MWF) levels of prey introduction, respectively.

Figure 1 also shows that pupation time for the predators differed by density levels of prey. When one predator larva was introduced it pupated after an average of 12 days at the lower (MF) prey level and after 11.7 days at the higher (MWF) level. This insignificant difference indicated that there was sufficient prey available at both levels to allow normal development of one predator.

Table 2. Effects of various levels of *Tx. r. rutilus* larvae on adult production from *Ae. aegypti* larvae in 8-liter plastic buckets.

No. and fequency of prey	No. of predators	Percentage reduction of adult prey for days[a]		
		20–33	34–42	20–42
50 MF	1	62	10	42
50 MF	2	81	30	62
50 MF	1/wk	76	80	78
50 MWF	1	46	13	34
50 MWF	2	75	22	55
50 MWF	1/wk	54	66	58

[a] Avg. daily prey adult emergence in untreated controls was:

	20–33 days	34–42 days	20–42 days
MF	10.1	8.5	9.5
MWF	14.1	11.8	13.1

When 2 predator larvae were introduced initially at the lower level (MF), the mean pupation times were 15.3 days and 23 days. At the higher prey level (MWF) pupation time was 12 and 13.7 days. The longer time required for pupation of the 2 predators at the lower level was undoubtedly due to the reduced food supply, because pupation times were similar at the higher levels of prey.

When a predator was introduced weekly, the first pupa appeared in similar time to those with one predator (12.3 and 11.5 days for the low and high levels of *Ae. aegypti*, respectively). Thus, the second larva added on day 21 did not appreciably reduce the food supply of the first larva before pupation. However, the second pupae required 19 and 19.7 days at the low and high levels of prey, respectively, again reflecting lower food levels during their larval stage. The test was terminated before the third pupae developed in any of the buckets, but their development time would have been in excess of 14 days.

Only one case of cannibalism in *Tx. r. rutilus* was observed in both groups (MF and MWF). However, predator counts at the end of the test did not correspond with input and indicated either unobserved cannibalism or natural mortality. However, even with cannibalism or loss to attrition, introduction of predators at the one/week rate could still significantly control adult emergence. Thus, cannibalism among different-sized predators—i.e., those resulting from weekly releases of predators or asynchronous oviposition from one release—may not be a significant or major problem. Cannibalism was not a problem in the work by Focks et al. (1980).

In general, the introduction of one predator/week most effectively controlled *Ae. aegypti* during the entire test period. However, 2 predators introduced at the same time were very effective for rapid, short-term control at high and low prey levels. With *Ae. aegypti* in nature, however, short-term control is very inefficient, because the eggs from a single oviposition hatch over an extended period of time with each subsequent flooding of the larval habitat. These results demonstrate further that control of container-breeding mosquitoes with *Toxorhynchites* requires repeated, inundative releases to assure that a high percentage of containers with *Ae. aegypti* have a continuing population of predators.

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