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TOXORHYNCHITES RUTILUS RUTILUS: EFFECT OF SHIPMENT BY COMMERCIAL AIR CARRIER ON ADULTS

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ABSTRACT. A technique is described for shipping adult *Toxorhynchites rutilus rutilus* by commercial air carrier to allow the production of this mosquito predator at a site distant from the release site. Indoor and field evaluations of the shipping procedure indicated that the technique temporarily increases fecundity but reduces overall adult daily survival and total oviposition.

In evaluations of using *Toxorhynchites rutilus rutilus* (Coquillett) as a biological control agent against *Aedes aegypti* (L.) and other mosquitoes that breed in artificial containers, we shipped laboratory-reared *Tx. r. rutilus* adults from Gainesville, Florida, to New Orleans, Louisiana, by commercial air carrier for release at a test site. Females from several such shipments produced fewer eggs than did unshipped females in earlier experiments (Focks et al. 1978). Because of our interest in the ability to produce adult predators at a central location and ship them quickly to distant release sites and the fact that concurrent quality control tests in the laboratory did not fully assess the extent of the deleterious effects of such air transport, we conducted this field study to evaluate the effects of shipping

roundtrip by commercial air carrier from Gainesville to Atlanta, Georgia, on the longevity and fecundity of *Tx. r. rutilus* females.

METHODS

We reared *Tx. r. rutilus* adults from laboratory colonies by the techniques of Focks and Boston (1979). Normally, *Tx. r. rutilus* adults mate and oviposit ca. 2 and 5 days, respectively, after emergence. Although the mosquitoes used in these experiments were 6–9 days old when released, they had not yet oviposited because we provided no suitable oviposition sites during the holding period. On the day of shipment, we removed the adults from the holding cages by aspirator and transferred them to a refrigerated vault (1–2°C, 45% RH) for immobilization before loading them into shipping containers; the maximum time in the vault was 1.5 hr.

Mosquitoes were shipped in paper cartons (13.3 x 8.3 x 3.2 cm deep), with a set of interlocking paper dividers that formed 20 cubicles (2.5 cm^3) in each carton. One female mosquito was placed in each cubicle. The bottom of each carton was lined with a 0.5-cm layer of dampened sponge, and the lid was lined with a 16-mesh screen to provide a resting site for the insects. For shipping, 64 cartons were placed in a plywood box (61 x 46 x 41 cm) lined with 5 cm of polyurethane insulation.

To reduce temperature fluctuations during transit, 4 flat plastic containers, which held a total of ca. 2 kg of glycol, were also packed in the plywood box. The initial temperature of the glycol was ca. 10°C. Temperatures in the box ranged from 14 to 28°C during shipment. Atmospheric pressure dropped from 760 torr at ground level to 560 torr at 2440 m and above during shipment. Roundtrip flight time between Gainesville and Atlanta was ca. 110 min; total time from packaging to release in the field or transfer to indoor cages was ca. 8 hr.

In indoor tests we measured survival, and fecundity of adult mosquitoes held in clear acrylic cages (0.5 m^3). Mosquitoes were supplied with water wicks, 50% honey-water solution, and a black 0.5-liter oviposition jar half-filled with water.

In outdoor tests we measured survival and fecundity of adult mosquitoes released in a 12.6 ha experimental area on the University of Florida campus. Releases were made in the center of a student housing complex that occupied a 5.3-ha plot that was centrally located in the experimental area. The plot was interspersed with various hardwood and pine trees, shrubs, and open expanses of lawn. It was bordered on 3 sides by a more densely wooded area, and on the fourth side by a lake.

We monitored oviposition in the experimental area with 64 oviposition traps (0.5 liter black jars half filled with water; see Jacob and Bevier 1969 and Tanner 1969) placed at ca. 60-m intervals. Thirty ovitraps were within the housing complex (central area), and the rest were in the outer wooded areas. (Jars were checked daily for *Tx. r. rutilus* eggs, which were removed, counted, and discarded.)

At sundown on July 20, 1978, we released a total of 175 *Tx. r. rutilus* females, which had been packaged and shipped by air to and from Atlanta, at 3 locations within the central area. As a control, on August 10, 1978, we removed a total of 175 females, which were also 6–9 days old, from their emergence cage by aspirator, transported them in small cages ca. 1 km to the experimental area, and released them at sundown in the same 3 locations as on July 20. In both tests, oviposition was monitored daily for 14 days after release. Laboratory tests were conducted with the same cohorts that were released in the field on July 20 and August 10, and a 3rd test was conducted in the laboratory without a field release component.

RESULTS AND DISCUSSION

INDOOR TESTS. Neither shipping nor immobilization produced any mortality

before the female mosquitoes were released in the field or transferred to indoor cages. The results of indoor cage tests on daily survival rate (Sa), fecundity (eggs/female/day; F); and mean oviposition/female (0) appear in Table 1.

Table 1. Effect of immobilization and shipment on the daily survival (Sa) and fecundity (F) of *Toxorhynchites rutilus rutilus* females in the laboratory.

Treatment (No. replications)	Sa[a]	(R^2)	Life expectancy[b] (days)	F±SD eggs/female/day	Mean Oviposition/ female (eggs)
Immobilization (3)	0.954 c	0.90	22	2.8±0.4	28.1
Immobilization and shipment (2)	0.965 c	0.88	29	2.8±0.4	31.0
Control (2)	0.979 d	0.74	48	2.8±0.5	35.0

[a] Sa is the slope of the regression of log numbers alive on days of age. Values followed by different letters are significantly different ($P<0.05$; Duncan's multiple range test).
[b] Life expectancy = (1-Sa)-1.

Analysis of variance conducted for the variables Sa, F, and O on treatment in Table 1 was significant ($P = 0.05$) only for Sa (and hence for life expectancy). The average Sa for the controls (0.979) was significantly different ($P = 0.05$; Ducan's multiple range test) from the average Sa for either the immobilized group (0.954) or the shipped group (0.965). The average Sa values for either treatment group were not significantly different ($P = 0.05$). The Sa value for the controls supports previous estimates of Sa in indoor tests (Focks et al. 1977, 1978). Thus, our data indicate that immobilizing *Tx. r. rutilus* females at 1–2°C for 1.5 hr significantly reduced their daily survival rate under laboratory conditions, and hence their expected lifetimes. However, the additional stress of air shipment after immobilization required for packaging did not seem to further reduce the survival of the mosquitoes in the laboratory.

A plot of F vs. number of days after shipment, for the experiment involving laboratory observations only, appears in Fig. 1. The average fecundity (F) of the control group and of the treatment groups (2.56 and 1.75 eggs/female/day, respectively) was significantly different ($P < 0.01$; t-test) when measured for 28 days. These results and previous observations (unpublished data) indicated that immobilization or shipment, or both, resulted in an increased rate of oviposition for ca. 10 days after treatment. This phenomenon may explain why treated groups with lower daily survival rates did not produce fewer eggs than control groups in the laboratory assays; the total expected oviposition from a group is a function of both fecundity and survival.

FIELD EVALUATIONS. In the following discussion, the data have been adjusted to reflect oviposition by released females only. The data indicate that the shipped females laid fewer eggs in the ovitraps than the control females (315 vs. 497 eggs). Moreover, the shipped females located and oviposited in a lower proportion of the 64 ovitraps within the experimental area (50 vs. 76%) Table 2. From a control perspective, the rate of oviposition near the buildings in the central area is of particular interest; the shipped females located only 51% of all ovitraps within the central area, whereas the control females located 77%. Because *Tx. r. rutilus* is considered to be a sylvan species, we expected the adults to migrate from the central less wooded area where the releases were made to the more densely wooded outer area. Regression of the proportion of eggs recovered in the central area on survival time (days) after release indicated a daily outward movement

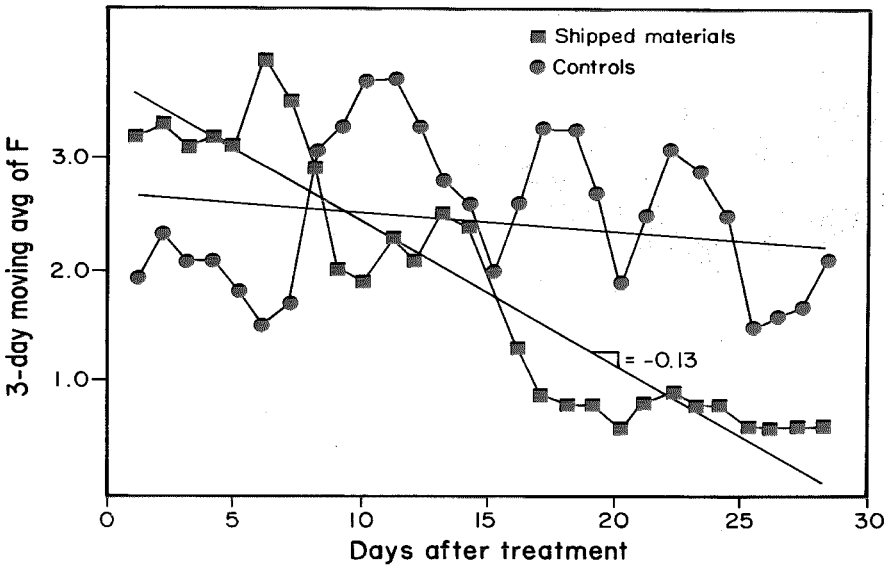

Fig. 1. Fecundity in the laboratory of *Tx. r. rutilus* females shipped roundtrip via commercial air carrier to Atlanta, Georgia, from Gainseville, Florida. (Slope for shipped and control group was −0.13 and 0.01, respectively, with R^2 values of 0.82 and 0.02, points plotted are 3-day moving averages).

Table 2. Comparison of the results of releases of shipped and control *Tx. r. rutilus* in the experimental area.[a]

Parameter	Release	
	Shipped	Control
Sa[b] (R^2)	0.556(0.60)	0.782(0.72)
% Oviposition		
Central area	83	72
Outer area	17	28
% Ovitraps containing eggs		
Total (64 traps)	50	76
Central area (30 traps)	51	77
Outer area (34 traps)	50	73

[a] Adjusted for oviposition by indigenous females.

[b] Slope of regression of log numbers alive on days of age.

of 7.7% (R^2 = .60) and 6.4% (R^2 = .72), respectively, for treatment and control releases. These figures support previous estimates from similar studies in the same area (Focks et al. 1979).

Indoor tests indicated that the probability of surviving from one day to the next (Sa) was a constant, i.e., it did not change with the age of the mosquito (Focks et al. 1978). Assuming that Sa and F were constant in the field (Focks et al. 1979) allowed us to estimate Sa by regressing the reduction in oviposition in the ovitraps on days after release. Regression of log number of eggs/day on number of days yielded estimates of 0.556 (R^2 = .22) and 0.788 (R^2 = .75) for the shipped and control groups, respectively. This reduction agrees with the changes in survival in the indoor tests. The indoor tests indi-

cated that the lowered survival of shipped and immobilized females was somewhat compensated for by a temporary increase in fecundity. The reduced number of eggs deposited by the shipped group as compared with the control in the field evaluation may have been a result of a reduction in daily survival or in the ability to locate oviposition sites. Thus, the deleterious effects of shipping were not fully compensated for by the increased fecundity.

In summary, our techniques are adequate for shipping *Tx. r. rutilus* adults for establishing laboratory colonies, but are inadequate for shipping *Tx. r. rutilus* adults for direct use as biological control agents. These results also suggest that shipping mosquitoes packaged by methods not requiring immobilization by chilling might provide reasonably competitive *Tx. r. rutilus* females for use as biological control agents.

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