PRELIMINARY FIELD OBSERVATIONS ON THE KILLING BEHAVIOR OF *TOXORHYNCHITES AMBOINENSIS* LARVAE

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Various species of *Toxorhynchites* have been widely studied as larvae in the laboratory, and their behavior as predators and as cannibals is well known (Steffan and Evenhuis 1981). In nature, adults of *Toxorhynchites* spp. typically oviposit in small containers in the wild (tree holes, plant axils, rock holes, etc.) that often contain prey mosquito larvae. Because some species of *Toxorhynchites* will oviposit in man-made containers, there is much interest in these species as potential biocontrol agents for pest and disease vector mosquitoes, notably *Aedes aegypti* (Linn.) (Brown 1973, Focks et al. 1979).

An interesting feature of *Toxorhynchites* spp. larval behavior is the so called "killing behavior" of fourth-instar larvae, first described in detail by Corbet and Griffiths (1963). This behavior is characterized by the killing, but not consumption, of prey larvae and sometimes conspecifics. The onset of killing in the fourth-instar depends on the larva reaching a certain weight threshold which also permits pupation (Lounibos 1979), and the killing behavior continues until a day or two before pupation, when the larva has already ceased feeding (Corbet 1985). Such interesting behavior has prompted the "vulnerable pupa" hypothesis, which proposes that the larvae are protecting their own interests before pupation by slaughtering as many of their competitors as possible (Corbet and Griffiths 1963).

More recently, Russo (1983) identified distinct behaviors accompanying killing in five species of *Toxorhynchites* that contrast sharply with normal feeding behavior and suggested that killing evolved as a separate behavior. He implied that killing of prey larvae may deprive earlier-instar competitors of food, slowing their development and probably increasing cannibalism. These earlier-instars are unlikely to be siblings of the "killing" larva due to the ovipositional habits of females. Killing, therefore, seems to discriminate against unrelated individuals and promotes the welfare of cohorts that are siblings. Trpis (1972) found that larvae of *Tx. brevipalpis* (Theobald) destroyed up to an average of 358 *Ae. aegypti* larvae (205 consumed, 153 killed) throughout larval development. Proponents of *Toxorhynchites* spp. as biocontrol agents have accordingly identified killing behavior as an added bonus.

Killing behavior has been reported in at least seven species of *Toxorhynchites* (Steffan and Evenhuis 1981) and widely described in laboratory studies, but as Corbet (1985) and Russo (1983) aptly point out, the behavior has not yet been recorded in nature. The implications of this to mosquito control programs involved in inundative release programs with adult *Toxorhynchites* spp. are obvious. There is no doubt that *Toxorhynchites* spp. are preying upon the *Ae. aegypti* larvae in the various tire yards and substandard neighborhoods where releases have taken place, but perhaps killing behavior is strictly a laboratory artifact and release programs will never be as successful as the laboratory results indicate. The following field observations suggest that killing behavior does indeed occur in one species of *Toxorhynchites*.

Toxorhynchites amboinensis (Doleschall) has been used in release programs and was the species selected for this study. Russo (1983) reported that *Tx. amboinensis* had a high intensity of killing in the laboratory beginning in the fourth instar. Bailey et al. (1983) and Durso et al. (1982) indicated that tires provide ideal habitat for rearing *Toxorhynchites* spp. as well as *Ae. aegypti* larvae.

Eighteen used automobile tires known to contain *Ae. aegypti* larvae and eggs were salvaged from a dump. The tires were cut leaving a hinged portion on the lower half of one side, which could be lifted for ease of inspection. Tires were staked upright in groups of three in a partially shaded outdoor location, and filled with water enriched with a leaf-grass infusion (ca. 4–5 liters/tire). After several weeks, *Ae. aegypti* larvae were well established in all tires. Minimum-maximum temperatures during the 12-day observation period ranged from 12–28°C.

Each tire then received one cohort (age 8 days, 4th-instar) of laboratory reared *Tx. amboinensis* larvae. Corbet (1985) suggested that one of the reasons killing has not been observed in nature is that the corpses of killed larvae decompose too rapidly to be found, so tires were monitored once or twice daily by lifting the hinged portion and examining the water carefully with a flashlight. Dead *Ae. aegypti* larvae were removed and examined under a microscope to confirm cause of death. Larvae showing signs of physical dam-

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age were judged by the standard of Russo (1983): greater than 20% of the body intact were considered "killed." Observations continued until all *Tx. amboinensis* had pupated, and all pupae were then blotted dry and weighed to the nearest 0.1 mg.

Laboratory studies documenting killing in *Toxorhynchites* spp. have provided a prey/predator ratio of 30–50/liter (Trpis 1972, Russo 1983) in containers with 100–200 ml of water. *Aedes aegypti* larval counts (2nd–4th instar and pupae) at the inception of this experiment ranged from 50 to 300 per tire, which represent typical population levels for Florida. Tires with counts below 50 *Ae. aegypti* were supplemented with laboratory-raised larvae prior to the observation period.

All of the 18 *Tx. amboinensis* larvae placed in the tires at the start of the experiment survived to emergence. Killing of *Ae. aegypti* larvae was confirmed in 12 of 18 tires. The "nonkilled" *Ae. aegypti* larval mortality was negligible in all tires. *Toxorhynchites amboinensis* larvae were observed to kill as early as age 9 days, and killing continued until pupation (days 15–19). The total number of *Ae. aegypti* killed per tire ranged from a minimum of 6 to a maximum of 126 over the 12-day period ($\bar{X} \pm$ SE per tire 34.0 ± 42.2). With the exception of 2 pupae, only third- and fourth-instar larvae were found killed. Killed larvae were recovered both floating on the surface of the water and among the detritus at the bottom of the tires. Killing intensity peaked on days 14 and 15 when 132 and 181 killed larvae were recovered from all the tires. *Toxorhynchites amboinensis* pupation occurred over days 15–19; 15 of 18 larvae pupated on days 17–19. Pupal weights ranged from 34.9 to 52.1 mg (43.0 ± 4.7 mg).

These preliminary data indicate that the killing behavior of fourth-instar *Tx. amboinensis* does occur under field conditions and is not a laboratory artifact. Development of the *Tx. amboinensis* larvae in the tires follows roughly the patterns outlined by Russo (1983) for this species in the laboratory, even though the temperatures encountered during these field observations were lower. Killing intensity peaked at the same age as Russo's 1983 study. Pupal weights also indicate that development in the field followed the weight pattern described by Russo (1983) in laboratory specimens before pupation, assuming that the weights of fourth-instar larvae and pupae are not significantly different, as shown by Trpis (1972) for *Tx. brevipalpis*.

There was a direct correlation between *Ae. aegypti* larval density and killing intensity (r = 0.55, $P < 0.018$, n = 18), and this interaction requires further investigation.

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