Journal of the American Mosquito Control Association, 25(3):370–373, 2009

SCIENTIFIC NOTE

AN UNUSUAL LARVAL COLLECTION AND SURVIVAL OF *ORTHOPODOMYIA SIGNIFERA* IN THE PRESENCE OF THE PREDATOR *TOXORHYNCHITES RUTILUS SEPTENTRIONALIS*

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ABSTRACT. From a discarded heavy-equipment tire (ca. 57 liter) at an industrial construction site, we collected 655 (86.0%) *Orthopodomyia signifera*, 23 (3.0%) *Toxorhynchites rutilus septentrionalis*, 17 (2.2%) *Aedes japonicus japonicus*, and 67 (8.8%) *Culex pipiens pipiens*. Although larvae of *Aedes albopictus* and *Aedes triseriatus* were not collected from this container, both species were prevalent as host-seeking adults and readily collected as larvae from other containers at this site. Laboratory trials to test the survival of prey (*Ae. albopictus*, *Cx. p. pipiens*, or *Or. signifera*) in the presence of *Tx. rut. septentrionalis* showed that survival of prey larvae differed among species. Multiple comparisons revealed that *Ae. albopictus* had the lowest and *Or. signifera* the highest survival in the presence of *Tx. rut. septentrionalis*. Survival of *Or. signifera* and *Cx. p. pipiens* was not significantly different from one another, but both were different from *Ae. albopictus*. Further testing is warranted to test other factors responsible for differences in the interspecific relationship between *Or. signifera* and other species in tree hole communities.

KEY WORDS *Orthopodomyia signifera*, *Toxorhynchites rutilus septentrionalis*, predation, prey, aposematic coloration

Orthopodomyia signifera (Coq.) and *Toxorhynchites rutilus septentrionalis* (Coq.) are widely distributed in eastern North America and readily use natural containers, such as tree holes, for larval development. Crans (2004) assigned both species to a unique life cycle type in the northeastern USA based on their nondessication-resistant eggs, multiple annual generations, larval diapause, and development of immatures in container habitats. Other species belonging to this life cycle type include *Orthopodomyia alba* Baker and *Anopheles barberi* Coq. This life cycle type is particularly adapted to phytotelmata (plant-held waters), in which the accumulation of organic debris moderates temperatures for diapausing larvae (Kitching 2001, Crans 2004). Waste tires and other artificial containers mimic naturally occurring tree holes, and thus larvae of both species are readily collected from either habitat. New Jersey represents the northernmost extent where the 2 species are sympatric.

Artificial containers, particularly in the peridomestic environment, serve as important larval habitats for many mosquitoes. Biotic and abiotic factors determine the community structure of mosquitoes in container habitats, and predation by *Tx. rut. septentrionalis* may be an important limiting factor exerted on these communities (Clements 1999). *Toxorhynchites* larvae are obligate predators, feeding on container-habitat culicines, including conspecific larvae (Campos and Lounibos 2000). Adult females are autogenous, and both sexes feed only on carbohydrate sources (Steffan and Evenhuis 1981). Because of their affinity for killing prey without consumption, *Toxorhynchites* have been investigated as biological control agents for integrated mosquito management (Focks and Sackett 1985). However, many *Toxorhynchites* are also cannibalistic, have a lower fecundity and longer life cycle than most of their prey, and prefer natural containers to artificial containers (Clements 1999). They are considered impractical as inundative biocontrol agents.

Prey consumption in *Toxorhynchites* is positively correlated with prey density (Hubbard et al. 1988), and their cooccurrence in a habitat may be limited by cannibalistic and predatory behaviors (Campos and Lounibos 2000). Sampling of artificial containers such as waste tires seldom produces more than a few *Toxorhynchites* larvae (A. Farajollahi and B. Kesavaraju, unpublished data). Most often, density of other container habitat mosquitoes is also lower in habitats where predacious *Toxorhynchites* are present. Reduction in prey density is expected in the presence of an efficient predator, and it has been shown that *Toxorhynchites* selectively feed on larger prey, inadvertently increasing the proportion of younger instars within the community (Clements 1999). Additionally, Bradshaw and Holzapfel (1983) have shown further prey selection by *Tx. rut. septentrionalis* at the subcommunity level

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Fig. 1. Discarded heavy-equipment waste tire at industrial site in Burlington County, NJ, where large numbers of *Toxorhynchites rutilus septentrionalis* and *Orthopodomyia signifera* were collected from this single artificial container.

within populations of *Aedes triseriatus* (Say), *An. barberi*, and *Or. signifera*. When *Tx. rut. septentrionalis* were introduced into habitats in the presence of the 3 prey species, total adult emergence declined, but *An. barberi* and *Or. signifera* achieved greater relative emergence success than *Ae. triseriatus* (Bradshaw and Holzapfel 1983). Thus, *An. barberi* and *Or. signifera* are more likely to persist or pupate in the presence of *Tx. rut. septentrionalis*, whereas *Ae. triseriatus* are more likely to decline (Bradshaw and Holzapfel 1983). They have, therefore, classified *Or. signifera* as "predator resistant," although the exact mechanism for this differential predator susceptibility is not clear (Bradshaw and Holzapfel 1983). In New Jersey, in the presence of *Tx. rut. septentrionalis* larvae in waste tires, it is common to observe low densities of other mosquito species and conspecifics. We report on an unusual field association of *Tx. rut. septentrionalis* and *Or. signifera* and provide laboratory evidence on the reduced predator vulnerability of *Or. signifera*.

Entire aquatic contents (ca. 57 liter) of a discarded, heavy-equipment waste tire (Fig. 1) located at an industrial site in central New Jersey (39°58′N, 74°50′W) were collected with a siphon and larval dipper. The site is primarily a recycling plant processing materials such as demolition debris, asphalt, tree stumps, and tires. The northern and eastern edges of the property were bordered by Rancocas Creek, the southern by Marne Highway, and the western edge by a mixed-hardwood forest. Field sampling was conducted on September 12, 2007, and the entire aquatic contents of the tire were filtered with a mesh (0.15 mm) and the filtrate transported to the laboratory in a cooler. All mosquito larvae were counted and identified (Darsie and Ward 2005); no pupae of any species were found. First and 2nd instars were reared in enamel pans with lactalbumin:brewers yeast (50:50) for 5 days before identification.

We tested survival of prey species in the presence of predation by holding ten 4th instars of either *Or. signifera*, *Culex pipiens pipiens* L., or *Aedes albopictus* (Skuse) in 60-ml disposable cups with and without *Tx. rut. septentrionalis*. Predator species were collected from different tires in the same location where the original samples were collected. *Orthopodomyia signifera* that were added to the experiment were from the field sampling described earlier, whereas the *Cx. p. pipiens* were from a laboratory colony. *Aedes albopictus* that were used were from an F1 progeny whose adults were collected as larvae from the field. For predation treatments, a 4th instar *Tx. rut. septentrionalis*, starved for the previous 24 h, was added to each cup. We had 2 treatments (control and predation) crossed with 3 species and each treatment replicated 10 times, providing a total 60 units. Experiments were conducted for 24 h under 26°C in 16L:8D photoperiod, and the surviving prey were counted and analyzed with ANOVA. Survival was converted to proportions and transformed to arcsine square root proportions to normalize the data set. Significant effects were further compared with least square means and adjusted with Tukey's method.

Orthopodomyia signifera (n = 655, 89%) was the dominant prey species collected from the waste tire. Sixty-seven *Cx. p. pipiens* (9%) and 17 *Aedes japonicus japonicus* (Theobald) (2%) were also collected. All *Tx. rut. septentrionalis* larvae were collected as 4th instars (n = 23), and all *Ae. j. japonicus* larvae collected as 3rd instars. *Culex p. pipiens* were primarily collected as newly hatched 1st instars (97%), whereas all instar stages (1st to 4th) of *Or. signifera* were present. No pupae of any culicid species were collected, and no *Ae. albopictus* or *Ae. triseriatus* were detected, although both species were prevalent in other larval collections at this site and were persistently host seeking on the authors during the sampling.

Laboratory experiments showed a significant interaction ($F_{2, 54} = 7.05$, $P = 0.002$) between

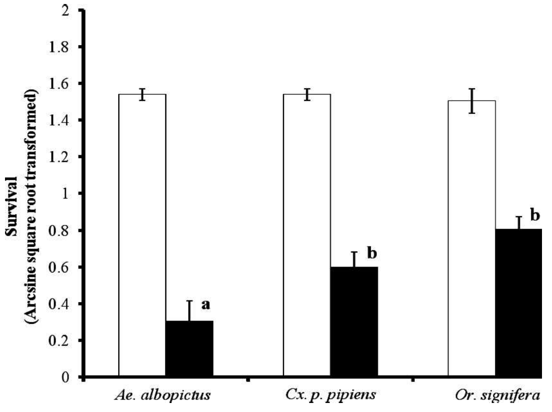

Fig. 2. Mean ± standard error, arcsine squareroot-transformed values of survival for the prey species *Aedes albopictus*, *Culex pipiens pipiens*, and *Orthopodomyia signifera* in the presence of the predator *Toxorhynchites rutilus septentrionalis* under laboratory conditions. Dark bars are predation, and white bars are control. Predation means with similar letters are not significantly different from each other.

treatment (control and predation) and species (*Ae. albopictus*, *Cx. p. pipiens*, or *Or. signifera*), indicating that the survival of the prey larvae was different among species. Multiple comparisons revealed that *Ae. albopictus* had the lowest and *Or. signifera* the highest survival in the presence *Tx. rut. septentrionalis*; however, the survival of *Or. signifera* and *Cx. p. pipiens* was not significantly different from each other but different from *Ae. albopictus* (Fig. 2). Survival in the controls for all the species was not different from each other.

The unusual collection of a large number of co-occurring *Tx. rut. septentrionalis* 4th instars from a single tire in the northeastern USA poses further questions for investigation. Most *Toxorhynchites* species are voracious feeders, particularly in the 4th larval stage, and although they may coexist in nature, they can be found alone or in small numbers because of their cannibalistic behavior (Clements 1999, Campos and Lounibos 2000). As a result, field collectors mostly detect the presence of a single *Tx. rut. septentrionalis* in a container, and, to our knowledge, this relatively large number of co-occurring *Tx. rut. septentrionalis* has not been collected from a single habitat previously. Although we cannot definitively provide evidence to elucidate the specific factors leading to such an occurrence, a plausible expalanation on the coexistence of a large number of 4th instar *Tx. rut. septentrionalis* in a single container could be 1) the large size of the collection tire, which may have supported greater numbers of predators, 2) the presence of large numbers of predator-resistant prey, which may suggest that most predator-prone preys were consumed earlier, and 3) induction of larval diapause in 4th instar *Tx. rut. septentrionalis* that may have reduced prey consumption and cannibalism.

Orthopodomyia signifera and *Cx. p. pipiens* exhibited the greatest survival rates in the presence of *Tx. rut. septentrionalis* followed by *Ae. albopictus*. Bradshaw and Holzapfel (1983) classify *Or. signifera* as predator resistant, mainly because they possess longer, stouter bristles than other container culicines, which may decrease prey capture success of *Toxorhynchites*. *Culex* species also possess similar bristles that may afford protection from predation, but recent studies have shown that *Cx. p. pipiens* also exhibit antipredator behavioral modifications in the presence of predation cues by *Tx. rut. septentrionalis* and hence are less vulnerable to predation (B. Kesavaraju, unpublished data).

Yasuda and Mitsui (1992) stated that the mobility of mosquito larvae affects predator-prey interactions and concluded that *Ae. albopictus* are more vulnerable as a result of their high mobility. In the presence of *Toxorhynchites towadensis* (Matsumura), *Ae. albopictus* approached the predator at a rate of 6 larvae per h, in contrast to only 0.5 larvae per h of *Orthopodomyia anopheloides* (Giles). They concluded that *Ae. albopictus* was more than 7 times more likely to be killed than *Or. anopheloides* because their high larval motility brought them into more frequent contact with *Tx. towadensis* (Yasuda and Mitsui 1992). Kesavaraju and Juliano (2004) showed that *Ae. albopictus* do not show behavioral modifications and hence are more vulnerable to predation by *Tx. rutilus*. These studies support the higher survival of *Or. signifera* and *Cx. p. pipiens* and lower survival of *Ae. albopictus* to *Toxorhynchites* predation in our laboratory experiments. Although not replicated, our field collections support our laboratory results because we collected large numbers of *Or. signifera* in a tire abundant with 4th instar *Tx. rut. septentrionalis*.

Mosquito prey have evolved different responses to escape from predation, and, in small container systems, behavioral modifications are the principal mechanisms of antipredatory response (Juliano 2009). *Orthopodomyia signifera* may also show anti-predator behaviors that make them less vulnerable to predation by *Tx. rut. septentrionalis*. During field and laboratory collections, we have observed *Tx. rut. septentrionalis* capture *Or. signifera* but either release the prey shortly after capture or cease feeding and discard the carcass. This activity is similar to the killing behavior of some pre-pupal *Toxorhynchites* species as described by others (Clements 1999); however, we have observed killing behavior exhibited only toward *Or. signifera*, whereas other species (*Ae. albopictus* and *Cx. p. pipiens*) were fed upon to completion after captured *Or.*

signifera were released by the predator. *Orthopodomyia* species have strongly developed orange, red, or purple epidermal pigments (Zavortink 1968), in contrast with coloration of most other culicine species. We question whether it is possible that this pigmentation may be indicative of aposematic coloration within this genus and if *Orthopodomyia* species may be distasteful by virtue of chemicals they produce themselves or gather from food sources. More investigations are warranted to test this hypothesis of *Or. signifera* antipredator adaptation.

We thank L. McCuiston for valuable laboratory assistance and mosquito-rearing advice, E. Williges for field assistance, H. Rupp for reviewing an earlier draft of this manuscript, A. Afify for laboratory assistance, D. Price for photography assistance, D. Chappine and T. Verna for logistic support, and J. J. Scott for introducing us to the field site. Tarsal Claw Industries is thanked for valued input and resources. This is New Jersey Agricultural Experiment Station publication D-08-08292-11-09, supported by state funds with partial support from the New Jersey State Mosquito Control Commission.

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