ZOOLOGIA 28 (4): 538–540, August, 2011
doi: 10.1590/S1984-46702011000400017

SHORT COMMUNICATION

Aedes aegypti survival in the presence of *Toxorhynchites violaceus* (Diptera: Culicidae) fourth instar larvae

Daniel S. Albeny[1,4]; Gustavo F. Martins[2]; Mateus R. Andrade[1]; Rodrigo F. Krüger[3] & Evaldo F. Vilela[1]

[1] *Departamento de Biologia Animal, Universidade Federal de Viçosa. Avenida Peter Henry Rolfs, 36570-000 Viçosa, MG, Brazil.*
[2] *Departamento de Biologia Geral, Universidade Federal de Viçosa. Avenida Peter Henry Rolfs, 36570-000 Viçosa, MG, Brazil.*
[3] *Departamento de Microbiologia e Parasitologia, Universidade Federal de Pelotas. 96010-900 Pelotas, RS, Brazil.*
[4] *Corresponding author. E-mail: toxorhynchites.sp@gmail.com*

ABSTRACT. The mosquito *Aedes aegypti* (Linnaeus, 1762) is the main vector of dengue and yellow fever viruses. Different methods have been used to control *A. aegypti*, including chemical and biological tools. However, chemical control can lead to a subsequent increase in the mosquitoes' insecticide resistance, and biological control represents an important method as an alternative to insecticide usage. Larvae from the *Toxorhynchites* genus (Diptera: Culicidae) are predators of other mosquitoes and represent a potential natural biocontrol agent of *A. aegypti* larvae. In the present work, *A. aegypti* larval survival was studied in the presence of the neotropical *Toxorhynchites violaceus* (Wiedemann, 1821) fourth instar larvae. *Toxorhynchites violaceus* consumption of *A. aegypti* increased during the 192 hours of the experiment and was more marked in the intervals between 96 and 120 hours and between 168 and 192 hours, when the *A. aegypti* survival reached 0%. During the fourth instar, *T. violaceus* increased its predation on *A. aegypti* larvae, possibly in order to increase its nutrient storage prior to pupation. Otherwise, low prey consumption can lead to a nutritional deficit for the larvae, delaying the adult's sexual development and reducing its egg production. Here we show that *A. aegypti* survival can be reduced by the *T. violaceus* fourth larvae predation under laboratory conditions.

KEY WORDS. Predation; survival.

The mosquito *Aedes aegypti* (Linnaeus, 1762) is an important vector of dengue and yellow fever viruses. *Aedes aegypti* is geographically widespread in Brazil. Larvae can grow in natural and artificial small water-filled containers. To control *A. aegypti* natural populations, chemical products such as larvicides are widely used. However, the evolutionary pressure of indiscriminate insecticide usage may increase the frequency of alleles that provide insecticide resistance in *A. aegypti* (Beserra *et al.* 2007).

To overcome the larvicide resistance, biological control methods have been used, including the use of larvivorous fish and copepods (Cavalcanti *et al.* 2007, Kittayapong *et al.* 2008), the use of the pathogen *Bacillus thuringiensis israelensis* (Bti) (VectoBac WG) (Chen *et al.* 2009) and the use of *Toxorhynchites* spp. larvae (Focks 2007). *Toxorhynchites* spp. larvae live in natural and artificial water containers and are predators of Culicidae, e.g. *Aedes triseriatus* (Say, 1823) and *Aedes albopictus* (Skuse, 1895) (Kesavaraju & Juliano 2004, Kesavaraju *et al.* 2007) and other Diptera, e.g. Psychodidae, Chironomidae and Thaumaleidae (Lounibos & Frank 1987).

Several studies have shown that the *A. aegypti* populations can be reduced by *Toxorhynchites* spp. predation (Tikasingh & Eustace 1992, Focks 2007). Even though *Toxorhynchites* spp. predate during their entire larval lifespan, fourth instar larvae are the most efficient predators (Chowanadisai *et al.* 1984). However, little is known about the interactions of *A. aegypti* with the fourth instar larvae of the Neotropical *Toxorhynchites violaceus* (Wiedemann, 1821). In view of this gap, we have studied the larval survival of *A. aegypti* in the presence of *T. violaceus* fourth instar larvae.

Toxorhynchites violaceus larvae were collected in bromeliads from 'Serra da Piedade' (43°40'N, 19°49'S, altitude 1746 m), Caeté, Minas Gerais, Brazil, by removing the water between bromeliad leaves. Only third instar larvae were transferred to 250 ml plastic pots and taken to the laboratory. They were fed on 20 *A. aegypti* fourth instar larvae per day, until the *T. violaceus* larvae became pupae (experiment was ended at this point). Adult samples were sent to Dr Thomas J. Zavortink (Department of Entomology, University of California, Davis, USA) for

identification. The experiment was conducted at Universidade Federal de Viçosa, Minas Gerais, Brazil.

Aedes aegypti eggs were obtained from Laboratório de Ecologia Química de Vetores (Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil). After eclosion, *A. aegypti* larvae were reared in dechlorinated tap water in 25 × 30 cm plastic trays and were fed on turtle food (ReptoLife®, São Paulo) with a photoperiod of 12/12 hours and kept at 25 ± 3°C.

The experiment was conducted in an incubator in the same light and temperature conditions described above. Thirty-two *T. violaceus* fourth instar larvae divided into eight groups (with four replicates/group) were used in the experiment. Each *T. violaceus* larva was placed individually in 100 ml plastic pots with 50 ml of dechlorinated water. After 48 hours of *T. violaceus* starvation, 40 fourth instar *A. aegypti* larvae were offered to each *T. violaceus* larva (total of 160 per group) at the same time.

The experiment was set with the following observation periods: 24, 48, 72, 96, 120, 144, 168 and 192 hours (named A, B, C, D, E, F, G, and H groups, respectively). After each 24-hours period, predated larvae were counted and one group was discarded. At the end of the experiment, there was only one remaining group (H) that corresponded to 192 hours. Also, after each 24-hours period, the non-predated *A. aegypti* larvae were removed from the plastic pots, counted and replaced by 40 new ones. During this period *A. aegypti* larvae did not receive any food. We analyzed these data using survival analyses with a Weibull distribution, since prey mortality increased with time (Crawley 1993), using the number of unpredated larvae as the response variable. The analyses were done using free software developed by the R Development Core Team (2006) with $p < 0.05$.

The survival curve of *A. aegypti* larvae was plotted as a function of *T. violaceus* predation ($Survival_{A.\ aegypti} = e^{-160.7741\text{^}-2.898551x\text{^}2.898551}$, $p < 0.05$) and it showed that the predation increased with time ($\infty = 2.90$, Fig. 1). *Aedes aegypti* fourth instar larvae survival was almost 98% in the first 24 hours and decreased subsequently, reaching 0% by 192 hours. In addition, *A. aegypti* fourth instar larvae number reduction was more marked between 96 and 120 hours (from 72 to 47%), and between 168 and 192 hours (from 38 to 0%, Fig. 1).

Our result showed that predation of *A. aegypti* by *T. violaceus* increases as the fourth instar larvae age. This was also reported for other *Toxorhynchites* spp. and it is known that an increase in predation assures that sufficient amounts of nutrients are stored prior to pupation (Campos & Lounibos 2000). Insufficient prey consumption can lead to a nutritional deficit in the larvae, delaying sexual development in the adult and reducing egg production (Amalraj *et al.* 2005).

The potential usage of neotropical *Toxorhynchites* spp. in vector control has been poorly studied until now. In the present work it was shown that the predator *T. violaceus* is capable of killing several *A. aegypti* larvae within the same water container in 24 hours. Even though this was tested in laboratory conditions, this study constitutes the first attempt to investigate *A. aegypti* larvae survival in the presence of the *T. violaceus* fourth instar larvae. The use of this predator in the control of the invasive vector *A. aegypti* larvae needs to be further tested in the field.

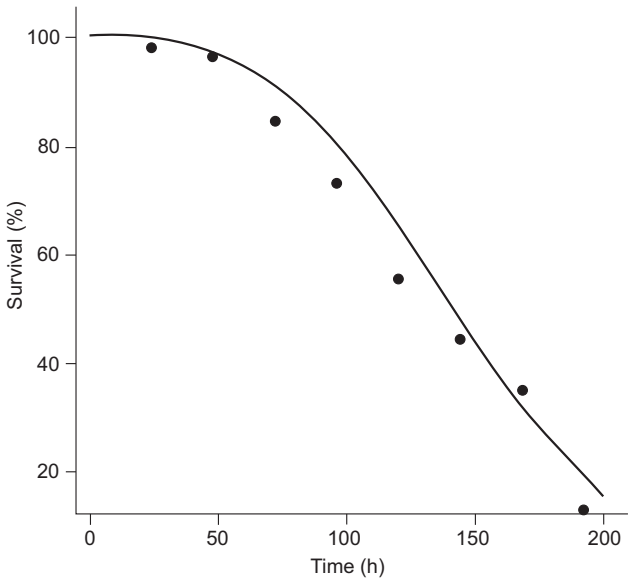

Figure 1. *Aedes aegypti* larvae survival (%) decreases with time (h) in the presence of *T. violaceus* fourth larvae ($Survival_{A.\ aegypti} = e^{-160.7741\text{^}-2.898551x\text{^}2.898551}$, $p < 0.05$). Notice that the prey percentage is almost zero around 200 hours.

ACKNOWLEDGMENTS

We are grateful to Graduate Programs in Animal Biology and in Entomology and to Brazilian government agencies, CNPQ, Fapemig, and CAPES, for supporting this work.

LITERATURE CITED

Amalraj, D.; N. Sivagnaname & P. Das. 2005. Effect of food on immature development, consumption rate, and relative growth rate of *Toxorhynchites splendens* (Diptera: Culicidae), a predator of container breeding mosquitoes. **Memórias do Instuto Oswaldo Cruz 100** (8): 893-902.

Besserra, E.B.; C.R.M. Fernandes; M.F.C. Queiroga & F.P. Castro Jr. 2007. Resistência de populações de *Aedes aegypti* (L.) (Diptera: Culicidae) ao organofosforado temefós na Paraíba. **Neotropical Entomology 36** (2): 303-307.

Campos, R.E. & L.P. Lounibos. 2000. Natural prey and digestion times of *Toxorhynchites rutilus* (Diptera Culicidae) in Southern Florida. **Annals of the Entomological Society of America 93** (6): 1280-1287.

Cavalcanti, L.P.G.; R.J.S. Pontes; A.C.F. Regazzi, F.J.P. Júnior; R.L. Frutuosos; E.P. Sousa; F.F.D. Filho & J.W.O. Lima. 2007. Efficacy of fish as predators of *Aedes aegypti* larvae, under laboratory conditions. **Revista de Saúde Pública 41** (4): 638-644.

Chen, C.D.; H.L. Lee; W.A. Nazni; B. Seleena; K.W. Lau; A.R. Daliza; S.E. Syafinas & A.M. Sofian. 2009. Field effectiveness of *Bacillus thuringiensis israelensis* (Bti) against *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) in ornamental ceramic containers with common aquatic plants. **Tropical Biomedicine 26** (1): 100-105.

Chowanadisai, L.; N. Benjaphong & B. Phanthumachimda. 1984. Laboratory observations on *Toxorhynchites splendens* Wiedemann in Thailand. **The Southeast Asian Journal of Tropical Medicine and Public Helth 15** (3): 337-341.

Crawley, M.J. 1993. **GLIM for Ecologists.** Oxford, Blackwell Scientific Publications, 379p.

Focks, D.A. 2007. *Toxorhynchites* as biocontrol agents. **Journal of American Mosquito Control Association**. **23** (2): 118-127.

Kesavaraju, B. & S.A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. **Annals of the Entomological Society of America 97** (1): 194-201.

Kesavaraju, B.; K. Damal & S.A. Juliano. 2007. Threat-sensitive behavioral responses to concentrations of water-borne cues from predation. **Ethology 113** (2): 199-206.

Kittayapong, P.; S. Yoksan; U. Chansang; C. Chansang & A. Bhumiratana. 2008. Suppression of dengue transmission by application of integrated vector control strategies at sero-positive GIS-based foci. **American Journal of Tropical Medicine and Hygiene 78** (1): 70-76.

Lounibos, L. & J. Frank. 1987. Survival, development and predatory effects of mosquito larvae in venezuelan phytotelmata. **Journal of Tropical Ecology 3** (3): 221-242.

R Development Core Team. 2006. **R: A Language and Environment for Statistical Computing.** R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available online at: http://www.R-project.org [Accessed: 28/XI/2010].

Tikasingh, E.S. & A. Eustace. 1992. Suppression of *Aedes aegypti* by predatory *Toxorhynchites moctezuma* in an island habitat. **Medical and Veterinary Entomology 6** (3): 272-280.

Submitted: 29.XI.2010; Accepted: 13.VI.2011.
Editorial responsibility: Pedro Gnaspini