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EntGuide

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Rearing *Toxorhynchites* For Field Releases

C. J. Jones, Ph.D.
Veterinary Entomologist, Illinois State University, Urbana, IL
and
E. T. Schreiber, Ph.D.
Medical Entomologist and Research Leader, Mosquito Biological and Alternative Control Section,
John A. Mulrennan, Sr. Arthropod Research Laboratory, Florida A & M University

INTRODUCTION

Mosquitoes in the genus T*oxorhynchites* are predaceous as larvae on the immature stages of other mosquito species. Adult *Toxorhynchites* feed on nectar and other naturally occurring carbohydrate sources but never take blood meals (Figure 1). These biological facts have led many entomologists to evaluate this mosquito's potential to reduce pest and disease causing mosquitoes. In nature, *Toxorhynchites* larvae are commonly found in tree and rot holes, rodent–eaten coconut husks, and bamboo stems that hold water. These sites are also often excellent larval developmental sources for pest mosquito species including those capable of causing yellow fever, dengue, and other diseases.

Human habitation brings with it many discarded items such as tires, buckets, and cans that can be used by pest mosquitoes as larval developmental sites. Fortunately, *Toxorhynchites* adults will lay their eggs in most types of water-filled containers. However, under natural circumstances, this predator mosquito doesn't lay enough eggs to keep pest and vector populations in check. Studies that have used predator mosquitoes to reduce pest mosquitoes have relied on rearing and releasing additional *Toxorhynchites* adults to boost naturally occurring populations and get ahead of pest mosquito production.

Various species of *Toxorhynchites* have been released and evaluated in control studies over the past fifteen years in the Caribbean and Pacific rim with some success. Unfortunately, limited studies in the United States have not

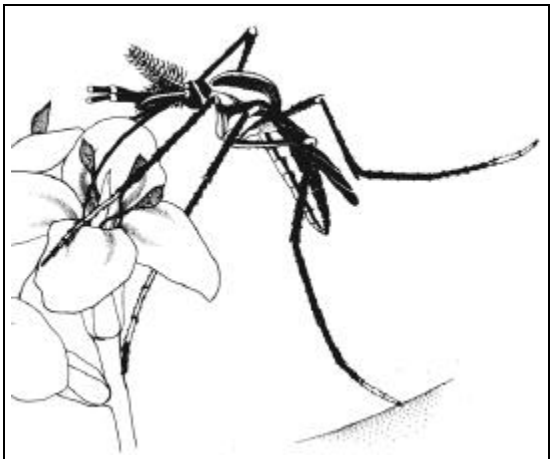

Figure 1. Adult *Toxorhynchites* feeding on flower.
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shown continuous larval control with this predator. However, with successes in the Caribbean and south sea islands, we believe that *Toxorhynchites* release programs still have potential to provide some degree of pest mosquito control (especially in the southeastern U.S.) while at the same time, provide tremendous public relations opportunities for mosquito control districts. One of the advantages of a *Toxorhynchites* release program is that the adult mosquito can disperse and lay eggs in areas most likely to escape treatment with insecticides. This method of biological control can result in favorable press coverage and often gives a mosquito control district the

opportunity to stress ecologically sound methods while controlling mosquitoes.

We have put together this bulletin for those mosquito control districts who wish to initiate their own program of rearing and *Toxorhynchites* release. One species of predator mosquito that we have evaluated (and currently colonized at John A. Mulrennan, Sr. Arthropod Research Laboratory [JAMSARL]) is *Toxorhynchites splendens.* This colony originated from Myanmar, Burma and came to JAMSARL in 1987 by way of University of Notre Dame and New Orleans Mosquito Control District. Another predator species that can be used for releases in Florida (but no longer colonized at JAMSARL) is *Toxorhynchites rutilus rutilus*. This mosquito species had been colonized using field collected larvae obtained from Jacksonville FL. The same rearing procedures outlined below for *Tx. splendens* can also be applied to *Tx. rutilus rutilus*.

PART I. PREY COLONY

If you are going to raise *Toxorhynchites* you must raise the prey that these larvae need for food.. We use *Culex quinquefasciatus* larvae. *Culex* eggs must be kept in water and are difficult to cool down and store for long periods of time. As a result, a constant supply of eggs must be available in order to have sufficient numbers of prey larvae available for predator larvae. Therefore, a main (or parent) colony of *Cx. quinquefasciatus* must be maintained. We will refer to this colony as: "*Culex* colony". Larvae obtained from this colony as food for *Toxoryhnchites* larvae will be refered to as "prey colony". Unless specifically stated, all colony rearing is generally conducted between 32 and 34°C (90 to 94°F).

Aedes aegypti or *Aedes albopictus* larvae can be used as prey and can be easily field collected in Florida and colonized. *Aedes* eggs can be kept dry and stored for relatively long periods of time until needed. However, they must be stored in a sealed container with high humidity to avoid dessication.

A. *Culex Colony & Prey Colony Egg Collection*

Egg collection to maintain a *Culex* colony and provide larval prey is accomplished by placing bowls with oak infusion water in a cage of adult *Culex* mosquitoes and allowing females to lay egg rafts on the water's surface. Oak infusion water is created by filling a 25-gallon plastic garbage can one–third to one–half full of water to which a similar amount of oak leaves is added. The can is covered and allowed to remain undisturbed for one week. Adding water as needed to the can to maintain water level will afford a continual source of ovipositional water.

After *Culex* females have laid eggs on water in bowls, take groups of about 60 to 70 egg rafts each and place them into separate plastic bowls with water. Some of these eggs will be used to maintain the *Culex* colony and some (after hatching) will become prey for developing *Toxorhynchites* larvae. Egg rafts may be kept in bowls of water in a refrigerator or incubator at 10°C (50°F) for emergency purposes.

B. *Larval Colony/Prey Rearing*

After *Culex* egg rafts hatch, the subsequent larvae must be fed on a continual basis. Many different diets exist for nonpredatory mosquito larvae and everyone who works with them has a tendency to develop their own. We have had excellent success rearing *Culex* larvae with a diet consisting of three parts liver powder with two parts yeast. Dry ingredients are mixed in advance, generally in half gallon containers, and stored in a dry place until needed. We add 120 ml of dry mixture to two quarts of water. Contents are then shaken vigorously in a covered container and about 50 ml placed in each tray of 10,000 larvae. Overfeeding is generally not a problem, when rearing *Cx. quinquefasciatus* larvae. However, *Ae. albopictus*/*aegypti* larvae are susceptible to pollution problems if overfed. Larvae are overfed if the bottom of the pan they are reared in becomes cloudy.

Bookkeeping procedures ***are essential*** because you will be rearing three different colonies, i.e., 1.) a main *Culex* colony, 2.) prey from this source and 3.) a *Toxoryhnchites* colony. If colony caretakers are not aware of the correct time to begin various larval groups, it will be impossible to have the right number of prey, at the right time, for predator larvae and the predators may turn cannibalistic. Refrigerating larval prey to slow development is an option when they develop faster than predators or not enough prey are available when needed. If you refrigerate prey, make sure to feed them and keep them at about 10°C (50°F). At that temperature, prey will develop but very slowly.

C. *Adult Colony Rearing*

Adults of the *Culex* colony can be maintained in 18–inch cubical screened cages for blood feeding and egg collection. Blood meal sources for adults can utilize young poultry or alternative-feeding procedures may be developed that do not use birds directly as hosts. At JAMSARL we feed mosquitoes using cotton pads soaked with poultry blood obtained from a poultry-rendering plant. Orthophagous (bird feeding) mosquitoes, such as *Cx. quiquefasciatus*, can be adapted to beef blood but such acclimation can take months. Poultry blood collection from rendering plants is best done during cooler portions of the year to decrease clotting and spoilage. Heparin or sodium ethylenediamine tetraacetate (EDTA) are added to the blood as "anti–clotting" agents and are available through scientific or chemical

supply companies, such as: Fisher Scientific, Pittsburgh, PA (800-766-7000).

Generally 200 grams of EDTA plus 20 liters of water is initially mixed at the blood collection site, 400 ml of this solution is added to a container in which 4-liters of blood is to be held. Blood from the jugular veins of freshly slaughtered chickens is collected at the rendering plant via a halved 8-inch PVC drainpipe. As the blood is being collected it is thoroughly mixed with a wire whisk and poured through a steel mesh sieve to filter out feathers and blood clots. Blood is then poured into screw–top plastic containers and immediately placed on ice. Blood should be kept frozen. In this way, blood need only be obtained once or twice a year. Open containers can be refrigerated for up to a week at 33° F.

On the day of blood feeding, a jar of blood is removed from the freezer that morning. Hot tap water is used to thaw the blood slowly while keeping it warm for feeding that afternoon. Adult mosquitoes will not feed readily on cold blood.

As an energy source, 10% sucrose solution soaked cotton (here referred to as sugar cotton) is provided adults ad lib, as well as water-soaked cotton as an additional moisture source. Sugar cottons are removed from cages early in the morning when mosquitoes are to be blood fed later that afternoon for egg laying.

PART II. *Toxorhynchites* REARING

The following is a daily routine checklist to facilitate *Toxorhynchites* rearing. ***Note: do not attempt to vary this routine until several cycles of this mosquito have been reared successfully***. This will allow those in charge of rearing to familiarize themselves with timing various life stages and become familiar with the number of prey larvae required for each stage of *Toxorhynchites* larvae. After this has been accomplished, then the following checklist can be tailored to individual constraints of rearing space, personnel, light, temperature and humidity.

Day 1. Remove sugar cottons from adult *Culex* colony cages where egg rafts will be eventually collected. Blood feed adults. (This assumes you already have a *Cx. quinquefasciatus* colony in production).

Day 2. Remove blood meal sources on this day from adult *Culex* colony cages.

Days 3 and 4. Check cottons in all *Culex* and *Toxorhynchites* adult cages, change if covered with black specks. This is a sign of fungal and/or bacterial growth that can be deterimental to colonies. Check cottons daily from now on, re-wet or change as necessary. **Note**: blood excretion, in case of *Culex*, will also occur on cottons after blood feeding and should be changed when fungal and/or bacterial growth appears.

Day 5. Collect *Toxorhynchites* eggs and *Culex* egg rafts using oak leaf infusion water. *Toxorhynchites* eggs are collected by placing a black cup or blackened mason jar with oak leaf infusion water into a cage of *Toxorhynchites* adults.

***Day 6*. This day is pivotal to successful colonization and rearing.**

A) Place groups of 30 of the largest *Culex* egg rafts (there are usually between 100-300 eggs/raft) into each of 6 bowls in order to maintain a *Culex* colony. Divide remaining groups of egg rafts into 3 bowls containing 55 rafts each to be used as larval prey for *Toxorhynchites* larvae. Leave bowls with eggs at room temperature. Extra bowls of egg rafts should be set up for additional prey in a refrigerator at 10°C and will be used later. Any egg rafts not needed can be discarded.

B) Using an eye dropper, place 2-3 drops of liver/yeast larval food in each bowl of *Culex* egg rafts before covering with a plastic lid. Placing a lid on bowls prevents other mosquitoes, that may be loose in the room, from laying eggs in that container.

C) Recover *Toxoryhnchites* eggs from the oviposition jar by pouring its contents through a fine screen (about 20 mesh) to catch eggs. Dip screen with eggs into a bowl of fresh tap water and remove a portion of eggs about the diameter of a dime into each bowl. Set up as many bowls as desired.

D) All larval trays (one tray per bowl) are set up in anticipation of newly hatched larvae for the following day. We use white trays 19 inches wide by 32 inches long by 4 inches deep. Trays are filled with tap water to a depth of about three inches. Aeration of trays can be done at this time or left until the following day.

E) Remove sugar cottons from adult *Culex* colony cages where egg rafts will be collected and blood feed adults.

Day 7. Generally, *Culex* colony, prey and *Toxorhynchites* eggs hatch on this day.

A) Place one bowl of newly hatched larvae from *Culex* colony egg rafts into each larval tray. Set up as many trays (i.e., one bowl per tray) as needed. to maintain adult production for *Culex* colony. Feed larvae in each tray 50 ml of liver/yeast larval food.

B) Set up trays of *Toxorhynchites* larvae with prey larvae. Add three bowls of prey larvae and one bowl of *Toxorhynchites* larvae per tray. Set up as many trays as needed. Prey need to be fed 25 ml of liver/yeast food per tray.

C) Remove blood from adult *Culex* colony cages.

D) Return sugar and water-soaked cotton to *Culex* adult colony cages.

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Day 8. If *Culex* colony, prey and/or *Toxorhynchites* eggs are delayed in hatching, follow procedures from day 3. Assuming hatch has begun:

A) Feed all larval *Culex* colony trays (50 ml each) and prey trays (25 ml each). Start aeration if not already done.

B) Set up one or two new trays of prey from refrigerated bowls of egg rafts marked "prey". These will be used to eventually feed the current *Toxorhynchites* larvae as they get older. Use methods from Day 7 on how to set up.

Day 9.

A) Feed larvae in *Culex* colony trays, if majority are third instar increase food to 100 ml per tray and feed twice/day.

B) If majority of *Culex* prey in with *Toxorhynchites* are third instar then increase liver/yeast food to 50 ml per tray and feed prey twice per day .

C) As larval prey become depleted, set up another set of prey from refrigerated bowls of egg rafts marked "prey". These will be used to eventually feed the current *Toxorhynchites* larvae as they get older. Use methods from Day 7 on how to set up.

Day 10.

A) Feed larvae in *Culex* colony and prey trays twice a day.

B) Check abundance of prey in relation to *Toxorhynchites* larvae. **Remember:** hungry *Toxorhynchites* larvae will attack their siblings. If there are less than 4 prey larvae per *Toxorhynchites* larva add additional prey of about the same size (instar) currently in tray. Check twice a day, once in morning and last thing in afternoon and add accordingly.

C). Place oak leaf infusion water in previously blood–fed adult *Culex* colony in order to obtain egg rafts.

D) Put oak leaf infusion in jar and place in adult *Toxorhynchites* cage to obtain more eggs.

E) As larval prey become depleted, set up another set of prey from refrigerated bowls of egg rafts marked "prey".

Day 11.

A) Feed *Culex* larval colony and prey colony in trays.

B) Feed *Culex* prey to *Toxorhynchites* larvae.

C) Check if *Toxorhynchites* larvae are fourth instar or if pupation has begun. If pupae are present, separate them from larvae, using a screen (as a "dipper") and place pupae in bowls of 50 per bowl.

D) Place pupal bowls in cage set up for *Toxorhynchites* adult rearing. Place a cone over each bowl of pupae to allow emergence of adults but prevent oviposition by other adults. Remove cone once or twice daily to assist adults in leaving cone or water's surface. One large water cotton and one honey water dish should be supplied in each pupal cage.

E) Leave pupal bowls in cages for five to six days before removing.

F) Separate newly laid *Culex* egg rafts into prey and colony groups. See Day 6 for methods.

G) Collect and separate *Toxorhynchites* eggs into bowls. See Day 6 for methods.

H) Return sugar cotton to *Culex* adult colony cages.

Day 12.

A) Feed *Culex* larval colony and check for pupation. When 70% of colony has pupated, pour larval trays through sieves in order to reduce water volume and concentrate larvae and pupae. Transfer water. larvae and pupae to smaller containers or bowls, and place in adult cages. Allow adults to emerge. Place a water and sugar cotton in each cage.

B) Pupae in prey trays can be picked out and discarded as necessary.

C) Take sugar cotton out of adult *Culex* colony cage. Blood feed.

D) Feed *Culex* prey to *Toxorhynchites* larvae, repeat rearing procedures from Day 7 through Day 11.

E) When *Toxorhynchites* larvae start to pupate, separate pupae as mentioned on Day 11.

F) When *Culex* prey and *Toxorhynchites* eggs hatch, place in one tray, three bowls of larval prey with each bowl of *Toxorhynchites* and continue procedures from Day 6.

Day 13.

A) Check *Culex* colony, *Toxorhynchites* colony and prey for pupation in trays. Process accordingly. **If you do not have pupation of** *Toxoryhchites* **larvae by this time, you may have temperature, food problems, or overcrowding**. **Larvae are taking too long to develop at this point and remedial measures should be taken**

B) Feed larval *Culex* colony and prey colony.

C) Add prey to *Toxorhynchites* larvae as necessary. At this point, *Toxorhynchites* larval feeding rate may have increased, check prey availability as often as three times per day.

D) Remove blood from adult *Culex* colony cages. Return sugar and water-soaked cotton to *Culex* adult colony cages.

Day 14.

A) Check for pupation in *Culex* colony, *Toxorhynchites* and prey colony. Process accordingly.

B) Feed *Culex* colony and prey larvae in trays.

C) Feed *Culex* prey to *Toxorhynchites* larvae, as necessary.

Day 15.

A) Check for pupation in *Culex* colony, *Toxorhynchites* and prey colony. Process accordingly.

B) Feed *Culex* colony and prey larvae in trays.

C) Feed *Culex* prey to *Toxorhynchites* larvae, as necessary.

Day 16.

A) Check for pupation in *Culex* colony, *Toxorhynchites* and prey colony. Process accordingly.

B) Collect rafts from adult *Culex* colony and feed *Culex* prey to *Toxorhynchites* larvae.

Day 17.

A) Check *Toxorhynchites* pupation.

B) Set up prey bowls from remaining *Culex* colony egg rafts.

C) Feed *Culex* colony and prey larvae in trays.

D) Feed *Culex* prey to *Toxorhynchites* larvae, as necessary.

Day 18.

A) Majority of *Toxorhynchites* larvae should have pupated and adults emerged in cages. If a few stray larvae or pupae are remaining, discard. Clean trays and repeat rearing cycle.

B) Remove oviposition bowls from adult cages of most recent *Culex* and *Toxoryhnchites* colonies. Continue maintenance of colonies. New rearing cycle begins again with procedures starting from Day 1.

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General Notes on Adult Colony Maintenance

In general, caged adult *Culex* last no longer than a month without severe decline in egg productivity. At this point, **clean cages with bleach, rather than soap, and rinse thoroughly**. **Note**: Bleach must be used because survival of adults and larvae may decrease no matter how thoroughly cages are rinsed if only using antibacterial soaps.

Note: Do not recycle into *Culex* colony larval or pupal prey which escape from being eaten by *Toxorhynchites* larvae. This may result in developing a strain of prey mosquitoes which are behaviorally "resistant", in the larval stage, to *Toxorhynchites* predation.

Adult *Toxorhynchites* Colony Maintenance

Adult *Toxorhynchites* can be maintained in cages about six feet high and three feet on a side. Three of the sides are constructed from 0.25-inch thick plexiglass. The remaining side consist of a wooden frame covered with ordinary window screen for maximum ventilation. Access to the cage interior is through a six inch diameter hole drilled into the plexiglass and covered with surgical stockinette. Stockinette may either be taped over the hole or (and more preferably) firmly sandwiched between two pieces of plexiglass and attached around the six inch diameter opening from the inside of the cage .

Adults are nutritionally maintained on a honey water (50:50) mixture which they obtain from open plastic petri dishes covered with screen mesh material. Mesh is necessary so that adults do not drown or get caught in the stickiness of the liquid. In addition, water-soaked cotton is placed in cages and provides additional moisture.

PART III. ADULT *Toxorhynchites* RELEASES

Adult *Toxorhynchites* used for release are placed, as pupae, in 19–liter white plastic buckets with netted lids (we use hair nets) and allowed to emerge. Emerged adults, which usually have a sex ratio of about one to one, are given cotton soaked with honey and water *ad libitum* and kept in these buckets for about six days until released (oviposition is rarely observed prior to this time). This also allows adult females plenty of opportunity for sugar feeding which they need in order to deposit eggs.

Release of adults should be done in several locations of about 200 adults per block rather than all at once at a central release point. Frequent releases may be needed of 1000 adults every two weeks at a rate of about 50–100 per block, because recycling (as well as overwintering) of *Toxorhynchites splendens* adults has not been demonstrated in Florida.

Monitoring at weekly intervals for adult dispersal and survival can be accomplished by looking for presence of *Toxorhynchites* larvae in monitoring stations by using water filled black pint mason jars. Caution is warranted in interpretation of results since dark colored containers are preferred ovipositional sites and may bias results. Limit the number of monitoring stations to 1–2 jars per block.

If mosquito adulticide measures, via ULV sprays, are to be done in concert with *Toxorhynchites* releases, we strongly recommend adherence to the following schedule without exception: ULV spray schedules **should precede releases by at least one day with follow up sprays no sooner than five days after release**. Thus, coordination with the night spray supervisor is essential.

As with most biological control programs, be prepared for substantial amounts of effort directed at fine tuning the program for your particular circumstances. *Toxorhynchites* release programs need to be timed ahead of estimated increases in mosquito nuisance/vector populations. In addition, use of predator mosquitoes has the added requirement of nectar availability to complicate timing.

Remember; do not expect perfect control. Biological control procedures seldom result in complete control of the targeted population. Rather, the necessary complexity of *Toxorhynchites* creates interesting programs that can be filled with wonderful public relation opportunities. Such an approach to mosquito control can allow emphasis of the basics of integrated pest management in a useful and interesting fashion.

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