High-Throughput Mosquito and Fly Bioassay System for Natural and Artificial Substrates Treated with Residual Insecticides

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OPERATIONAL NOTE

HIGH-THROUGHPUT MOSQUITO AND FLY BIOASSAY SYSTEM FOR NATURAL AND ARTIFICIAL SUBSTRATES TREATED WITH RESIDUAL INSECTICIDES

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ABSTRACT. A high-throughput bioassay system to evaluate the efficacy of residual pesticides against mosquitoes and muscid flies with minimal insect handling was developed. The system consisted of 4 components made of readily available materials: 1) a CO_2 anaesthetizing chamber, 2) a specialized aspirator, 3) a cylindrical flat-bottomed glass bioassay chamber assembly, and 4) a customized rack.

KEY WORDS Barrier treatment, glass culture tube, aspirator, mosquito control, Deployed War-Fighter Protection Program

A biological assay, or bioassay, is a measurement of the strength of a stimulus based on reactions produced in a living organism (Bliss and Cattell 1943, Hoskins and Craig 1962). For entomological studies of pesticide efficacy, the bioassay is a universal method to evaluate lethal, sublethal, and repellent effects of pesticides against arthropod pests and vectors of disease (Nagasawa 1958). Bioassays involving arthropods to evaluate, in particular, residual insecticides have utilized a variety of treated surfaces, including vegetation (Anderson et al. 1991, Britch et al. 2009), fabric (Rutledge et al. 1989, Britch et al. 2010), and filter paper (Collins and King 1953). The bioassay test chamber has varied in design to include the use of glass tubes (WHO 1996), petri dishes (Allan et al. 2009, Doyle et al. 2009), and plastic cones (WHO 2005). Recent investigations of innovative residual pesticide application techniques on vegetation as well as artificial materials to improve the Department of Defense pest management system (Linthicum et al. 2007, Pages et al. 2010) have created a demand to evaluate large numbers of residual pesticide–treated samples rapidly, using a variety of target insect species. To tackle this demand, we have developed a novel high-throughput bioassay system for the evaluation of mosquito and fly mortality on natural and artificial substrates treated with residual insecticides.

The high-throughput bioassay system consists of 4 core components: 1) a CO_2 anaesthetizing chamber; 2) a Wynn Gun aspirator (Aldridge et al. 2012); 3) cylindrical, flat-bottomed, glass bioassay chambers each with cover and nutrient source; and (4) a customized polyvinyl chloride (PVC) component storage rack for the bioassay chambers. Glass bioassay chambers are used to hold a sample of the exposure surface, i.e., a sample of insecticide-treated material or untreated control material. The Wynn Gun aspirator is designed to transfer CO_2-anesthetized mosquitoes directly into the glass chambers, reducing handling of mosquitoes and greatly reducing setup time.

The bioassay chamber is a cylindrical, flat-bottomed, 130 × 40-mm-diam Pyrex® glass culture tube (Corning, part no. 9850-40; Corning, NY). To prepare a bioassay experiment, glass bioassay chambers are loaded with an exposure surface and labeled accordingly. Exposure surfaces are placed in the bioassay chambers so that test insects may contact as many aspects of the surface as possible. For a given treatment, we typically prepare 5 bioassay replicates. For example, an exposure surface cut from camouflage netting fabric treated with a residual pesticide will be cut into 5 equal 2.5 × 10-cm sections and placed individually in separate bioassay chambers (Britch et al. 2010, 2011). For bioassays to evaluate residual pesticide treatments on vegetation, the exposure surface in a bioassay chamber will consist of a sprig of plant material, i.e., stem with attached leaves, approximately 10 cm in length (Britch et al. 2009). Depending on the design of a particular field experiment with either treated fabric material or treated vegetation as the exposure surface, there may be as few as 5 or as many as 12 separate treatments, each requiring at least 5 bioassay replicates (Britch et al. 2009, 2010, 2011).

Adult test insects are first removed from laboratory colonies in batches of approximately

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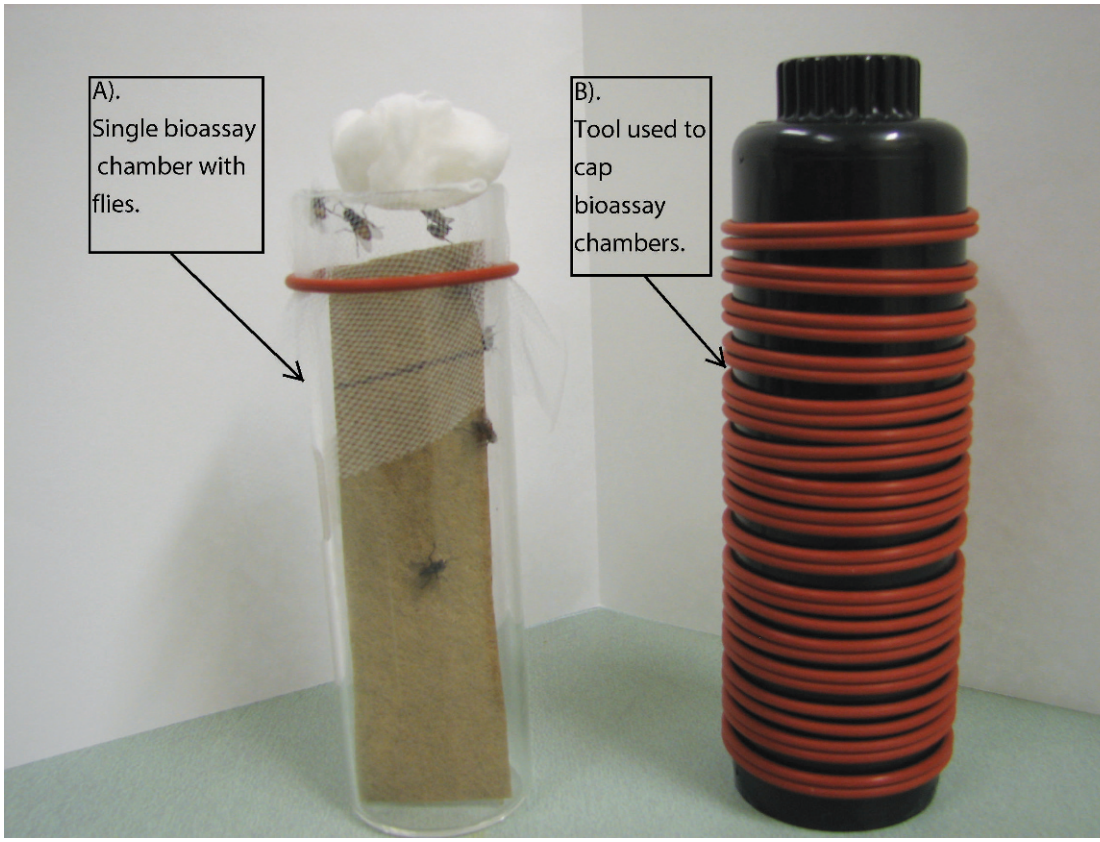

Fig. 1. (A) Completed bioassay chamber is shown at left with insect test subjects and exposure surface visible. (B) O-ring capping device is shown at right. The large open end of the capping device fits snugly over the mouth of the tube, forcing the mesh material down evenly on all sides, and an O-ring is rolled down on to the tube to hold the mesh tightly.

85 mosquitoes or 100 muscid flies using a ''flashlight'' aspirator with mesh-bottomed collection tubes (BioQuip Products, Rancho Dominguez, CA). Collection tubes containing massed insects are then placed in a covered 1-liter cylindrical plastic anesthetization chamber connected by a 6-mm gas delivery tube to the regulator on a bulk CO_2 tank. The chamber is lined with cotton balls to evenly distribute the CO_2 gas and reduce turbulence from the gas delivery tube. Anesthesia is achieved after a 4-min exposure to CO_2 delivered at a rate of 10–12 liters/min. For bioassays with muscid flies, anesthetized *Musca domestica* L. or *Stomoxys calcitrans* (L.), 3–5 days old, are spread carefully over an 11 × 17-in. (27.5 × 42.5-cm) paper sheet and 10 randomly selected flies are transferred by forceps into glass bioassay chambers. For bioassays with mosquitoes, anaesthetized *Culex quinquefasciatus* Say, 3 to 5 days old, are spread carefully over an 11 ×17-in. (27.5 × 42.5-cm) paper sheet and 10 randomly selected females are aspirated with the Wynn Gun directly into glass bioassay chambers. Under development is a larger pickup tube for the Wynn Gun aspirator to permit direct transfer of muscid flies.

The direct transfer method such as described above with the Wynn Gun was originally described by Darwazeh (1969), using a battery-powered aspirator designed by Husbands and Holten (1967) that used an in-line collection tube doubling as a bioassay tube. In Darwazeh's (1969) system, the collection/bioassay tubes were plastic with a mesh bottom that allowed suction to draw mosquitoes directly from a colony into the tube without CO_2 anesthetization, which had previously caused unacceptable levels of mortality in *Culiseta inornata* Williston used in his study. However, in studies conducted by Britch et al. (2009, 2010, 2011), the mortality rate of *Cx. quinquefasciatus* and *Cx. tarsalis* Coq. when using CO_2 anesthetization before transfer with the Wynn gun aspirator was $<0.1\%$. Transfer of test insects from colonies to bioassay chambers is a substantial rate-limiting step in bioassays. Direct transfer from a colony is inefficient because of the need to select only 10 (female) insects from a constantly moving mass of mixed-sex individuals,

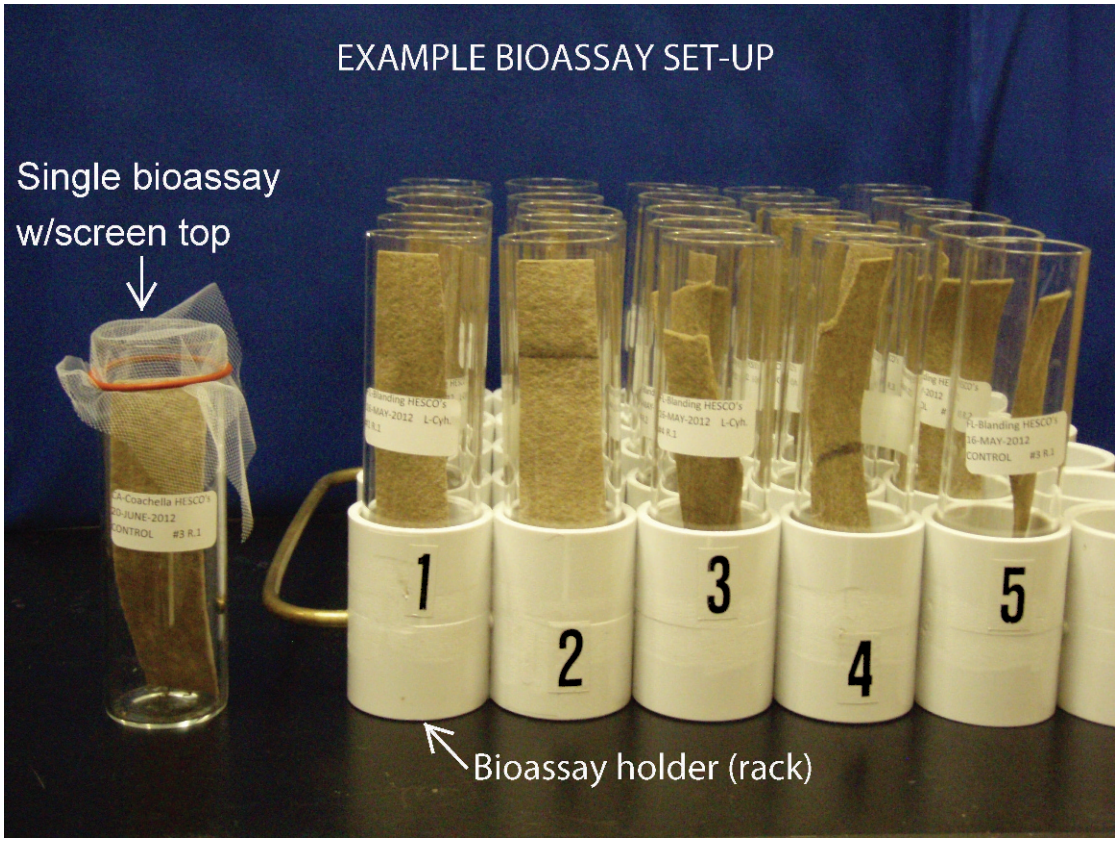

Fig. 2. Bioassay chamber shown at left awaiting cotton ball. At right is a set of 25 bioassay chambers in a 30-slot polyvinyl chloride rack (mesh covers removed for clarity) with brass lifting handle visible on its left side.

and the physical awkwardness of handling the aspirator through the stockinette portal of the colony cage. Speed and precision are greatly increased by bulk removal of insects from the colony, followed by anesthetization and rapid transfer with the Wynn Gun directly into bioassay chambers.

Once a bioassay chamber has been loaded with an exposure surface and test insects, it is covered with a 10 × 10-cm square of 9–10 strands/cm nylon tulle mesh (Wal-Mart, Bentonville, AR), and secured with a silicone S500-70/2-127 O-ring (Spec Seals, Anaheim, CA). A custom PVC O-ring deployment apparatus was developed from a Toro™ (Toro, Bloomington, MN) lawn sprinkler spray body (Fig. 1) to rapidly secure 45 mesh covers in 15 min. The large end of the apparatus fits snugly over the mesh and around the open end of the bioassay chamber to evenly fold the excess mesh downwards so that an O-ring may be rolled onto the chamber. After the mesh cover is secured, a cotton ball soaked to saturation without dripping with 10% sucrose solution is placed on the mesh to provide an ad libitum nutrient source for the duration of the bioassay. The mesh fabric permits ventilation throughout the tube that prevents condensation and limits mold growth, while allowing insects to easily reach the nutrient source. Completed bioassay chambers are shown in Figs. 1 and 2.

Bioassay chambers are inserted into custom-built 30-slot PVC racks (Fig. 2) and positioned inside an incubator maintained at 28 ± 3°C and 30 ± 10% RH with a 14:10 light:dark cycle. The PVC racks are constructed from 1.25-in. (3.1-cm) inner-diam PVC in-line couplers bonded using all-purpose PVC cement (Oatey, Cleveland, OH) in a grid of 5 × 6 units, and fitted with ¼-in. (6.2-mm) brass rod handles that are cemented in place at opposing ends. The PVC in-line couplers have an inner flange that acts as a ledge for the bioassay chambers to rest on and not fall through.

The residual pesticide bioassays are performed on pesticide-susceptible populations of adult female *Cx. quinquefasciatus*, and adult male and female *M. domestica* and *S. calcitrans* maintained at the USDA–Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. Bioassays for each of the 3 test species are conducted consecutively on the same exposure surface sample. Insect samples from each species are separately introduced to exposure surfaces in the glass bioassay chambers

for 48 h in an incubator, and mortality and morbidity are recorded at 24 ± 1 h and 48 ± 1 h. After the 24-h check, cotton balls are remoistened with a 10% sucrose solution using an eyedropper.

Following the 48-h check, the insects are killed with CO_2 or by freezing at 0°C for 30 min to make a final count and confirm that 10 test insects had been present in each bioassay chamber. All exposure surfaces and test insects are removed from each bioassay chamber for the final count to ensure that no insects are missed in the folds and seams of the fabric exposure surface, or the dense leaves and stems in a vegetation exposure surface. The insects, the sucrose-soaked cotton balls, and the tulle mesh are then discarded. The exposure surface is returned to its bioassay chamber to begin a new bioassay with the next of the 3 test insect species. With each new bioassay, new cotton balls and tulle mesh covers are used. Once all bioassays have been completed, the exposure surfaces are returned to storage, the glass bioassay chambers are scrubbed and triple washed with a liquid detergent, e.g., Dawn™ dish soap (Proctor and Gamble, Cincinnati, OH), triple rinsed with water, and dried before repeating the process. The Wynn Gun, silicone O-rings, and 30-slot storage racks are cleaned as needed.

This high-throughput bioassay system has been used successfully in hundreds of experimental bioassays to evaluate treated fabric samples and insecticide-treated vegetation samples for their residual insecticidal effects (Britch et al. 2009, 2010, 2011). The statistical methods used to analyze bioassay results have included the Kruskal–Wallis 1-way analysis of variance on ranks followed by Tukey multiple comparison tests; however, statistical methods may vary depending on experimental design. This purpose-built high-throughput bioassay system is durable, easy to handle, and conducive to rapid repetition. A trained technician can prepare and complete 200–250 bioassays every 2 days using this system.

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