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Laboratory evaluation of differential attraction of *Culex pipiens pallens* to fruit-based sugar baits

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ABSTRACT

Mosquito adults usually need to obtain sugar from floral nectaries and damaged fruits/seed pods to replenish their energy reserves. The newly developed attractive toxic sugar baits have been successfully applied in controlling various mosquito species outdoors. However, the attraction of *Culex pipiens pallens* to different fruit-based sugar baits remains unknown. In the present study, we selected nine common fruit species, prepared the fruit-based sugar solutions, and investigated the attractiveness of different sugar baits to newly emerged *Cx. pipiens pallens* in the laboratory. The results showed that when tested against the 5% brown sugar solution, all the sugar baits were significantly attractive to both females and males. When tested together in the mesh-covered cage, there was a significant difference on the attractiveness between different fruit-based sugar baits. The most attractive fruit species included *Broussonetia papyrifera*, *Cucumis melo*, *C. melo var. saccharinus*, *Amygdalus persica* and *Pyrus bretschneideri*, and their seed pods could be potentially used as ingredients in ATSB for controlling mosquitoes outdoors.

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1. Introduction

Members of the *Culex pipiens* complex have been implicated as vectors of a number of arboviruses and pathogens, such as West Nile virus (WNV), St. Louis encephalitis and filarial worms (Farajollahi et al., 2011; Turell, 2012). *Culex pipiens pallens* Coquillett, the most prevalent culicine mosquito species in Northeastern Asia, is the primary vector of wuchereriasis and epidemic encephalitis in China (Li et al., 2002; Fonseca et al., 2009). In addition, *Cx. pipiens pallens* females consume blood from birds, humans and other mammals, which makes them potentially susceptible to infection and viral dissemination of western equine encephalomyelitis virus and WNV (Wang et al., 2010; Jiang et al., 2014). Current mosquito control methods in China depend heavily on synthetic insecticides, whose abusement has resulted in multifarious problems, such as increasing insecticide resistance, environmental pollution and toxic hazards to human beings and other nontarget organisms (Cui et al., 2006; Sun et al., 2006).

Most mosquito species need to obtain sugar to replenish their energy reserves during adulthood (Foster, 1995). Mosquitoes usually acquire sugar from floral and extrafloral nectaries, honeydews and exudates of rotting and damaged fruits/seed pods (Foster, 1995). Based on the sugar feeding behaviour of mosquitoes, researchers successfully developed the attractive toxic sugar bait (ATSB) methods as a new form of vector control (Müller and Schlein, 2006; Schlein and Müller, 2008; Müller et al., 2008, 2010a,b,c; Xue et al., 2011; Beier et al., 2012; Qualls et al., 2012, 2014). Based on the "attract and kill" principle, the ATSB approach targets the sugar-seeking mosquitoes and has been widely applied in controlling various mosquito species by spraying the sugar solutions on vegetation (Müller and Schlein, 2006; Müller et al., 2008, 2010a,c; Xue et al., 2011; Beier et al., 2012) and suspending them on bait stations (Müller et al., 2010b; Qualls et al., 2012). As part of the ATSB optimization process, an increasing number of attractive flowering plants and fruits/seed pods have been identified as potential sugar sources for a variety of mosquito species (Gouagna et al., 2010; Müller et al., 2010d, 2011).

Our previous study revealed that *Cx. pipiens pallens* adults could be differentially attracted by several flowering plant and fruit species (Yu et al., 2016). In the present study, we selected nine common fruit species, developed the fruit-based sugar baits, and investigated their attractiveness to *Cx. pipiens pallens* in the laboratory trials. By comparing the relative attractiveness of these fruit-based sugar baits to mosquitoes, we aimed to screen out the optimal attractive fruit species, which could be used as potential

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Table 1
Taxonomy and common names of the fruits used for preparation of attractive sugar baits.

Family	Species name	Common name
Rosaceae	*Pyrus bretschneideri* Rehder	White pear
Rosaceae	*Amygdalus persica* L.	Peach
Rosaceae	*Prunus salicina* Lindl	Plum
Rosaceae	*Malus pumila* Mill	Apple
Cucurbitaceae	*Cucumis melo* L.	Honey melon
Cucurbitaceae	*Cucumis melo* L. var. saccharinus Naud	Cantaloupe
Moraceae	*Ficus carica* L.	Fig
Moraceae	*Broussonetia papyrifera* (L.) Vent	Paper mulberry
Gramineae	*Saccharum sinense* Roxb	Sugarcane

ingredients in ATSB for controlling mosquitoes in outdoor environment.

2. Materials and methods

2.1. Mosquito rearing

The egg rafts of *Cx. pipiens pallens* were initially collected from water pools in the urban residential areas in Hangzhou (30°16′N; 120°12′E), Zhejiang, China, and commercial rat food (purchased from Zhejiang provincal center of experimental animals, Hangzhou, China) was provided for mosquito larvae. Mosquitoes were reared at 25 ± 1 °C with a photoperiod of 14:10 (L: D) and 75% relative humidity (RH). Adults were maintained in mesh-covered cages (35 × 35 × 35 cm) and offered with a 5% sucrose solution *ad libitum*. Females were provided with a diet of mouse blood for 1 h on three consecutive days, and fully engorged females were allowed to lay eggs on oviposition cups (5 cm in diameter by 7 cm depth). Eggs were transferred into plastic basins (30 cm in diameter by 11 cm depth) filled with dechlorinated tap water. Newly emerged adults intended for use in trials were given access to water only. Experiments were conducted 12–24 h after their emergence.

2.2. Preparation of the fruit-based sugar baits

Based on our previous study on the attraction of *Cx. pipiens pallens* to different flowering plants and fruits (Yu et al., 2016), we selected nine common locally produced fruit species for preparation of the sugar baits (Table 1). The following eight kinds of fruits were purchased from local markets: *Pyrus bretschneideri* Rehder., Rosaceae; *Amygdalus persica* L., Rosaceae; *Prunus salicina* Lindl., Rosaceae; *Malus pumila* Mill., Rosaceae; *Cucumis melo* L., Cucurbitaceae; *Cucumis melo* L. var. saccharinus Naud., Cucurbitaceae; *Saccharum sinense* Roxb., Gramineae; *Ficus carica* L., Moraceae. The seed pods of *Broussonetia papyrifera* (L.) Vent (Moraceae) were collected from the deserted farmland (30°18′N; 120°05′E) nearby the Zijingang campus, Zhejiang University, Hangzhou, China. All the damaged and/or overipped fruits/seed pods were chosen for preparation of the crude juices.They were cut into pieces, added with water at the ratio of 10% (v/w), and crushed by an electric juicer, respectively. The crude juices were filtered by gauze and then stored in the beaker.

The fruit-based sugar baits used in the trials were mainly composed of 75% crude juice of individual fruit species (v/v), 20% water (v/v), 5% red wine (v/v) and 5% brown sugar (w/v). They were mixed well and placed in the beakers sealed with plastic wraps. The sugar baits were left at room temperature for 48 h to ferment, and then 1% sodium benzoate (w/v) was added for preservation of the baits. The sugar baits were stored in 4 °C and were taken out 6 h earlier before their use in the experiments.

2.3. Trap description

The box traps (purchased from Kezhongjie Co., Ltd., Deqing, China) were used for capturing mosquitoes in the present study (Fig. 1A). The trap consists of two dismountable parts: a clear plastic container (10.5 cm in diameter by 10 cm height) with grooves on the inwall (Fig. 1B), and a pedestal (diameter: upper 11.5 cm, lower 9 cm; height: 3.6 cm) with three apertures (9.5 cm in length by 0.7 cm width) along the edge (Fig. 1C). The container could be twisted with the pedestal together through the grooves on the pedestal. Two sticky boards (9.2 cm in height by 8.9 cm width) are inserted into the grooves of the plastic container to capture the attracted mosquitoes. A petri dish (8.6 cm in diameter) filled with 20 mL tested sugar solution is placed in the pedestal. The odorants released by the sugar baits could spread into the surroundings through the apertures spontaneously. When mosquitoes land nearby the traps searching for sugar sources, they could crawl into the traps through the apertures and be drown in the sugar solution or be glued by the sticky boards.

2.4. Attraction of Cx. pipiens pallens to each kind of the sugar baits

The attraction of *Cx. pipiens pallens* adults to each kind of the fruit-based sugar baits was tested in a mesh-coverged cage

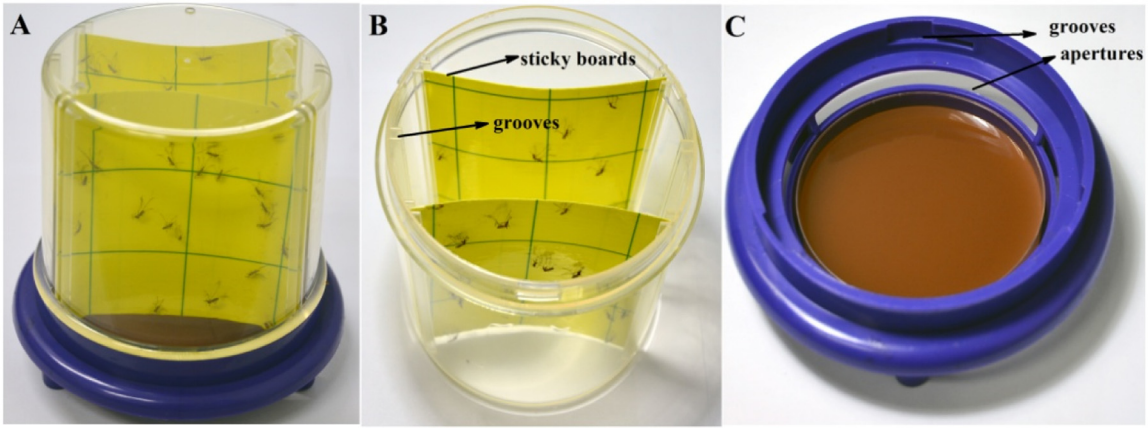

Fig. 1. Photograph of the box trap used in the present study.
(A) The trap consists of a clear plastic container and a pedestal, which could be twisted together through the grooves on the pedestal. (B) Two sticky boards are inserted into the plastic container through the grooves to capture mosquitoes. (C) A petri dish filled with 20 mL fruit-based sugar baits is placed in the pedestal. Three apertures are used for odorants spreading and to allow the mosquitoes to crawl into the trap.

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