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Comparative rearing parameters for bisexual and genetic sexing strains of *Zeugodacus cucurbitae* and *Bactrocera dorsalis* (Diptera: Tephritidae) on an artificial diet

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ABSTRACT

The Sterile Insect Technique (SIT) is an important component of area wide programs to control invading or established populations of pestiferous tephritids. The SIT involves the production, sterilization, and release of large numbers of the target species, with the goal of obtaining sterile male x wild female matings, which yield infertile eggs. A major advance in SIT involved sex-linked, genetic manipulations that allowed the production and release of male-only strains (also termed genetic sexing strains, GSS). The use of GSS avoids matings between sterile males and females, which may divert males from seeking and mating with wild females, and studies show that male-only releases result in greater suppression of wild populations than standard bisexual releases (i.e., those including both males and females). GSS based on sex-linked pupal color exist for *Zeugodacus cucurbitae* (Coquillett) and *Bactrocera dorsalis* (Hendel), two important agricultural pest species, but their rearing characteristics have not been documented in detail. The goal of the present study was to compare the pupal color sexing and bisexual strains for each of these species with respect to important rearing parameters, including egg production and eclosion of larvae from eggs (egg hatch), pupal recovery, and weight, emergence rate, and flight ability. In both species, most of these parameters were significantly greater for the bisexual strain than the GSS, and, for a given number of eggs, the production of flight-capable adults was approximately 2 times greater in the bisexual strains of both species. The potential usefulness of GSS in SIT against *Z. cucurbitae* and *B. dorsalis* is assessed based on these findings.

Introduction

The true fruit flies (Diptera: Tephritidae) include over 4000 species of which approximately 250 species are serious agricultural pests of fleshy fruits and vegetables (White and Elson-Harris, 1992; Dhillon et al., 2005). Females typically oviposit in a variety of host plants, and the damage caused by the developing larvae may render the crops unmarketable. In addition to direct losses, the risk of importing infested commodities often leads fruit fly-free countries to impose stringent quarantine guidelines on exporting countries, thus impeding international commerce (Jang et al., 2014). Despite trade precautions, however, the high dispersal ability of the flies along with ever-increasing levels of global transport of people and goods have increased the invasion threat of pest tephritids (Qin et al., 2015).

Increasingly, an Integrated Pest Management (IPM) approach is used to control invading or established populations of pestiferous tephritids. Broadly, this approach involves the implementation of diverse methods, including synthetic insecticides, protein bait sprays, release of natural enemies, male annihilation technique (MAT), and sterile insect technique (SIT) (Dyck et al., 2006; Vargas et al., 2008, 2015). For relatively small infestations, the SIT is often used to achieve eradication. The SIT involves the production, sterilization (typically, via irradiation), and release of large numbers of the target species, with the goal of obtaining sterile male x wild female matings (Knipling, 1955). Such pairings result in infertile eggs and the subsequent reduction of the intrinsic rate of growth of the pest population. Thus, the effectiveness of SIT depends strongly on the survival, dispersal, and mating ability of the released, sterile males (Calkins, 1984), and, accordingly, sterile males are considered the “primary active agent” in SIT (Franz and McInnis, 1996).

A major advance in SIT occurred with genetic manipulations that allowed the production and release of male-only strains (also termed genetic sexing strains, GSS; Robinson et al., 1986). The so-called “first generation” of GSS (for the Mediterranean fruit fly [medfly] *Ceratitis*

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capitata (Wiedemann); Rössler, 1979) relied on pupal color mutations as a selectable marker, with males having the wild phenotype (brown pupae) and females the mutant phenotype (white pupae). In more recently developed GSS for medfly, pupal color (i.e., sex) has been linked with a temperature sensitive lethal (*tsl*) mutation that allows selective mortality of female embryos by exposing them to a high temperature (Franz and McInnis, 1996). Thus, in a *tsl* GSS the sexes are separated at the egg stage prior to larval development, a marked improvement since rearing resources are devoted exclusively to the production of males.

Independent of the sexing mechanism, the release of males only increases SIT's field effectiveness in two main ways (Hendrichs et al., 1995). The removal of females avoids both "sting" damage by sterile females on host fruits, which may adversely affect their marketability, and matings between sterile males and females, which may divert males from seeking and mating with wild females. Both field (Rendon et al., 2004) and field cage (Robinson et al., 1986; McInnis et al., 1994; Orozco et al., 2013) studies show that male-only releases result in greater suppression of wild populations than standard bisexual releases (i.e., including both males and females).

The subtribe Dacina of the Tephritidae contains many economically important species (Virgilio et al., 2015), and SIT has been used in control and eradication programs against major pests in this taxon, including the melon fly *Zeugodacus cucurbitae* (Coquillett) (following the reclassification recommended by Virgilio et al., 2015) and the oriental fruit fly *Bactrocera dorsalis* (Hendel) (Koyama et al., 1984). In a well-known case, the Japanese government conducted a successful eradication, which included an important role for SIT, against the melon fly (Koyama et al., 2004). Although MAT is most commonly used to suppress *B. dorsalis* populations, SIT has been integrated into management efforts in mango growing areas of Thailand (Sutantawong et al., 2002; Orankanok et al., 2007) and has also been used to eradicate *B. dorsalis* from the Mariana Islands (Koyama et al., 1984; Steiner et al., 1970). In each of these instances, bisexual strains were released. At present, the only genetic sexing mechanism available for *Z. cucurbitae* or *B. dorsalis* is based on pupal color (McCombs and Saul, 1995; McInnis et al., 2004; see also Isasawin et al., 2014 for pupal color sexing in *B. carambolae* Drew & Hancock). Although these strains have existed for > 10 years, there are few data on their characteristics and mass production, and there are no direct comparisons of rearing parameters between pupal color-based GSS and bisexual strains for either *Z. cucurbitae* or *B. dorsalis*. Interestingly, for both species, males from the GSS were highly competitive with wild males in obtaining copulations with wild females in field cage trials, indicating a strong potential for effective SIT using these GSS (Shelly et al., 2000; McInnis et al., 2004; Sookar et al., 2013).

The goal of the present study was to compare rearing characteristics of a pupal color sexing strain and a bisexual strain for both *Z. cucurbitae* and *B. dorsalis*. Detections of these and other related dacine species have increased greatly in the continental US (particularly in California) over the past few decades (Papadopoulos, 2014) and pose a serious threat to agricultural trade. As a result, demonstration of the capability to rear the strains would enhance the development of alternative control strategies that incorporate an SIT component. Here, we present data for all strains on egg production and hatch rate, pupal production, yield, and weight, emergence rate, and flight ability. As described below, in both species, most of these parameters were significantly greater for the bisexual than the GSS strain. The utility of mass-rearing GSS is assessed in light of these findings.

Materials and methods

Insects

All four strains are currently being reared at the USDA-ARS Daniel K. Inouye Pacific Basin Agricultural Research Center, Hilo, HI, and have been maintained for hundreds of generations. For *Z. cucurbitae*, the bisexual strain has been reared since 1976 (approximately 526 generations; R. Vargas, personal communication), while the white pupae strain had been reared since 2001 (approximately 192 generations; McInnis et al., 2004), respectively. For *B. dorsalis*, the bisexual strain was started in 1991 (approximately 312 generations; R. Vargas, personal communication) and the white pupae strain in 1995 (approximately 264 generations; McCombs and Saul, 1995), respectively. Strains are reared following standard protocol (Vargas, 1989), with GSS screened and filtered each generation for recombinant individuals (breakdown of sex linkage of pupal color trait, causing mis-match between pupal color and sex) to maintain stability in the sexing mechanism. Colonies are housed in a building devoted exclusively to rearing and maintained at 22.5 ± 1 °C, 55% ± 3%% rh, and a 14:10 L:D photoperiod.

For each of the rearing parameters measured, data were collected from flies held in 4 cages (replicates) for each of the 4 strains during 3 different sampling periods. Thus, 16 total cages were monitored per sampling period, and 12 cages were monitored per strain over all sampling periods. Sampling periods were initiated at 2-week intervals, with the first group of cages set up in November 2016, and the last group in December 2016. Unless otherwise indicated, the parameters were measured under the same environmental conditions noted above. The procedures used here generally followed the international standards adopted for assessment of strain quality of mass-reared tephritid fruit flies (FAO/IAEA/USDA, 2014).

Egg production

To measure fecundity, 500 newly emerged flies (300 females and 200 males) were placed in a cubical screen cage (25 cm per side) with food (3:1 sugar:yeast hydrolysate mixture) and water provided ad libitum. Eggs were collected during 1 6-h intervals when flies were 14 d old for *Z. cucurbitae* strains or 18 d old for *B. dorsalis* strains. Using a thumb tack, 300 randomly placed puncture holes were made in the surface of a plastic cup (358 mL volume, 7 cm diameter) into which a water-soaked sponge was placed to stimulate oviposition. Cups with lids were then placed inside cages at 0800 h and removed at 1400 h. Eggs were then rinsed from cup with water and allowed to settle in a 10 mL graduated cylinder to determine the total volume of eggs (measured to the nearest 0.05 mL).

Eclosion of larvae from eggs

After making the volumetric measurement, we placed 200 eggs on blotting paper, which in turn was placed in a small cup containing 36 g of larval diet. This small cup was then placed in a larger plastic cup (358 mL volume) with a screen lid and a sand-covered bottom. After 72 h, numbers of hatched and unhatched eggs were recorded using a dissecting microscope.

Pupal production, yield and weight

Again, from the sample of collected eggs, a 0.25 mL aliquot of eggs was obtained using a surgical syringe and placed on 300 g of diet. The diet containing the eggs was placed in a plastic container (13 × 15 × 4 cm), which was then placed inside a larger fiberglass bin, with the bottom covered with heat-sterilized sand and the sides screened to allow ventilation. After allowing 15 d for larval development and subsequent pupation, pupae were sifted from the sand, and the total volume of pupae was measured in a graduated cylinder to the nearest 0.1 mL to estimate pupal production. In addition, the ratio of pupae/eggs (termed pupal yield) was obtained based on volumes of eggs and pupae using the following parameters: *Z. cucurbitae*: 12,000 eggs/mL, 36 pupae/mL; *B. dorsalis*: 22,000 eggs/mL, 50 pupae/mL. In addition, allocations of 100 pupae were weighed to the nearest 0.0001 g using an Adventurer® OHAUS balance (Item No. AR2146, OHAUS Corp., Parsippany, NJ, USA).