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A sustainable mass rearing method for western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a common agricultural pest throughout the world (Kirk & Terry 2003). Due to their extremely small size, rapid life cycle (Reitz 2009), and broad host range (Lewis 1997; Nyasani et al. 2013), detecting and preventing the spread of western flower thrips is difficult (Terry et al. 2007). Western flower thrips damage plant matter by feeding, ovipositing, and indirectly vectoring plant pathogenic viruses that can infect a range of horticultural crops (Wijkamp et al. 1995). Today, western flower thrips can be found on nearly all continents, making them one of the most economically significant pests globally (Reitz 2009).

Current control for western flower thrips primarily relies on conventional chemical insecticides despite causing potential negative effects to human health and environmental degradation as well as development of insecticide resistance (Morse & Hoddle 2006; Reitz 2009; Demirozer et al. 2012; Gao et al. 2012). Therefore, it is essential to develop alternative options. Recently, a variety of '-omics' tools, such as genomics, proteomics, and metabolomics, has been used to propel identification of biological targets (Badillo-Vargas et al. 2015; Rotenberg et al. 2020) such as RNA interference (RNAi) as a new management tool for western flower thrips (Leiss et al. 2009; Leiss et al. 2013; Reitz et al. 2019). A mass rearing system is critical for the ability to test various potential biological targets.

Recently, we developed a sustainable mass rearing system that is simple and cost-effective. The new system sustains large thrips populations that we use for various physiological experiments; for example, nano-injection or feeding of potential biological targets to live western flower thrips and observing any physiological or behavioral changes. Included here is a molecular identification for thrips (Ahn et al. 2014) and improved rearing methods from other systems used previously (Murai & Ishii 1982; Tuelon 1992; Steiner & Goodwin 1998; Degraaf & Wood 2009). We have introduced a compact and easy method with optimized rearing techniques that institute a timeline to maintain the quality of host plants in the laboratory and greenhouse, including minimization of mite and mold infestation. These factors are essential to the success of western flower thrips colonies (Loomans & Murai 1997).

Thrips were collected from greenhouses or fields in Corvallis, Oregon, USA. At least 6 different thrips colonies were reared in separated containers until confirmation as *Frankliniella occidentalis*. We used internal transcribed spacer 2 (ITS2) gene as a DNA marker for molecular identification of *F. occidentalis* (Ahn et al. 2014). Adult thrips, 1 to 2 d old, were pooled on ice in a 1.5 mL tube containing a lysis buffer (PureLink RNA Mini Kit, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and manually homogenized using a PYREX® glass pestle tissue grinder (Corning Inc., Corning, New York, USA). Instead of using the genomic DNA kit (normally genomic DNA can be used for ITS), we extracted total RNA from the samples using the total RNA extraction kit according to the manufacturer's instruction with DNase I treatment to remove remaining genomic DNA. Total RNA was quantified by NanoDrop 2000 (Thermo Fisher Scientific, Waltham Massachusetts, USA) and used for complementary DNA (cDNA) synthesis for 3 min using SuperScript III First-Strand Synthesis System and random primers provided in the kit according to the manufacturer's instruction (Invitrogen, Carlsbad, California, USA). The first-strand cDNAs were used as a template to amplify a partial ITS2 gene (475 nucleotides). The primers were designated 5'-TGTGAACTGCAGGACACATGA-3 for forward primers and 5'-GGTAATCTCACCTGAACTGAGGTC-3 for reverse primer. Polymerase chain reaction (PCR) mixture and reaction were performed with DreamTaq polymerase according to the manufacturer's introduction (Thermo Fisher Scientific, Waltham Massachusetts, USA) at 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final 10 min at 72 °C using Veriti 96 Fast Thermal Cycler (Applied Biosystems, Foster City CA). Polymerase chain reaction products were run in 2% agarose gel, purified using a GeneJet Gel Extraction Kit (Thermo Fisher Scientific, Waltham MA), cloned using CloneJET PCR Cloning Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and sequenced by Eurofins Genomics (Louisville, Kentucky, USA). Sequencing results were analyzed using a Geneious 8.1.5 ver. software (San Diego, California, USA) and compared with *F. occidentalis* ITS2 gene (GenBank Accession No. AB063334). We found 2 DNA sequences from the GenBank, and our thrips were identical at 99.79%. Molecular identification should be monitored and re-conducted at least once per yr to guarantee rearing of the correct species.

Shirofumi soybeans (*Glycine max* [L.] Merr.; Fabaceae) were obtained from Fedco Seeds (Monroe, Michigan, USA) and are provided to thrips colonies for nutrition and reproduction, though occasionally red kidney beans (*Phaseolus vulgaris* L.; Fabaceae) are used. Soybean seeds should be planted 10 to 15 d prior to use, no deeper than 5 cm below soil surface, then harvested and stored in 4 °C in a paper-towel lined plastic container (Ziploc®, Racine, Wisconsin, USA). Soybean plants are kept in a climate-controlled greenhouse (22 ± 5 °C, 35 ± 5% relative humidity [RH], and a photoperiod of 16:8 h [L:D]) until ready to harvest. Cotyledons are unifoliate, embryonic leaves from the plant

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that provide thrips with suitable habitat and plentiful nutrients (Murai & Loomans 2001). All cotyledons from soybean sprouts should be harvested between 2 cm to 3 cm in length (Fig. 1A). Discard any cotyledons with apparent damage such as brown bruising and deep cracks or gaps. Harvested cotyledons should be kept in an enclosed container inside a 4 °C cold-room and discarded after 2 to 3 wk because they begin to wilt and are more susceptible to mold growth.

Thrips rearing containers (95 mm outer diam × 40 mm high) with a mesh screen (40 mm diam) for ventilation on the top lid (LabScientific, Danvers, Massachusetts, USA) are used (Fig. 1B). To keep rearing duties for each life stage organized, trays are used to store adult thrips (Tray 1) and immature thrips (Tray 2) separately. Our methods for thrips maintenance are designed to occur 3 times per wk, ideally Monday, Wednesday, and Friday to ensure cleanliness and consistency in food supply during a work wk and over a weekend. Materials needed for thrips maintenance are as follows: 2 clean containers lined with 2 layers of filter paper (90 mm diam) for egg collection, a discard container, soft forceps, a fine tipped (1 mm diam) and medium tipped (13 mm diam) soft paintbrush, a marker for labeling, harvested cotyledons in an enclosed container, and standard Petri dish lids (90 mm) to stack on container lids between rearing periods (Fig. 1B) to prevent cotyledons from drying out. Thrips colonies should be maintained at 25 ± 1 °C, 60% RH, and a 16:8 h (L:D) photoperiod. We recommend using a white piece of paper under the workspace to easily detect thrips inside containers and spot any thrips that may have escaped while working in a colony. During maintenance, harvested cotyledons should be removed from their enclosed container and placed onto a fresh paper towel to absorb excess moisture from cold room storage. All rearing procedures are summarized in Figure 2.

To begin, adult populations (labeled "A" on Tray 1) are maintained. Adult males are roughly one-third smaller than females (Fig. 2A). All cotyledons inside colony container from previous feedings should be pushed to one side of the container and replacement cotyledons should be placed on the opposite side; this ensures easy and accurate transfer of the adults to their new food source. The number of new cotyledons needed will vary between 4 and 6 cotyledons, depending on colony population density with 1 to 2 more cotyledons for each extra d if time between maintenance periods exceeds 2 d. While using forceps to hold each cotyledon, use the medium-sized paintbrush to gently brush each side of an old cotyledon over the new cotyledons, ensuring that all the thrips have been removed from the old food source. Old cotyledons should be saved in a new colony container lined with 2 layers of filter paper, unless the cotyledon is showing signs of mold; if moldy, then place in the discard container instead. Once all old cotyledons are removed from the adult colony, evenly disperse new ones throughout the adult colony container; this reduces stress in the colony due to overcrowding. Repeat this for all adult colonies on Tray 1. Subsequently, all adults will have been given a fresh food source and the container with old cotyledons infested with eggs should be split evenly into 2 containers, both labeled with the date and "E" for egg, and placed on Tray 2.

Combine the 2 oldest (30+ d old) adult colonies with the 2 subsequent aged colonies each time colonies are maintained, because the fecundity of older populations likely has decreased. To do this, use forceps to move donor cotyledons from the oldest colony into the recipient, subsequent aged colony, then use forceps to remove filter paper from donor colony, hold above recipient colony and carefully brush thrips from the filter paper(s) into the recipient colony, carefully avoiding mold, frass, and dead thrips. When maintaining the colony, it is common for adults to jump, flutter, and escape; use the fine-tipped paint brush and gently place the bristles on the thrips, pick it up, and tap paintbrush on colony container.

Tray 2 should house non-egg producing colonies of egg, nymph, and pupa (Fig. 1B). Egg colonies can be identified based only on collection date since the eggs are laid underneath the cotyledon epidermis and are invisible (Fig. 2B). Egg containers will be left to develop for 4 d from collection date but should be monitored for moldy cotyledons. During d 4 to 7, label with "N" for nymphs (Fig. 2C) and feed 8 to 10 cotyledons. A procedure similar to adult maintenance should be followed, but instead cotyledons are discarded because there are no eggs to collect. Nymphs commonly gather around the upper rim or lid of the container, and it is necessary to gently move them back onto cotyledons using the small paintbrush. Remove cotyledons with any signs of mold to prevent spread because substantial mold harms thrips population growth.

When a colony is 8 to 10 d old, label containers with "P" for pupae and feed 2 to 3 cotyledons, at this time because the second instar nymphs mostly have transitioned into pupae and will require less food source. Unless mold is extremely prevalent, it is important not

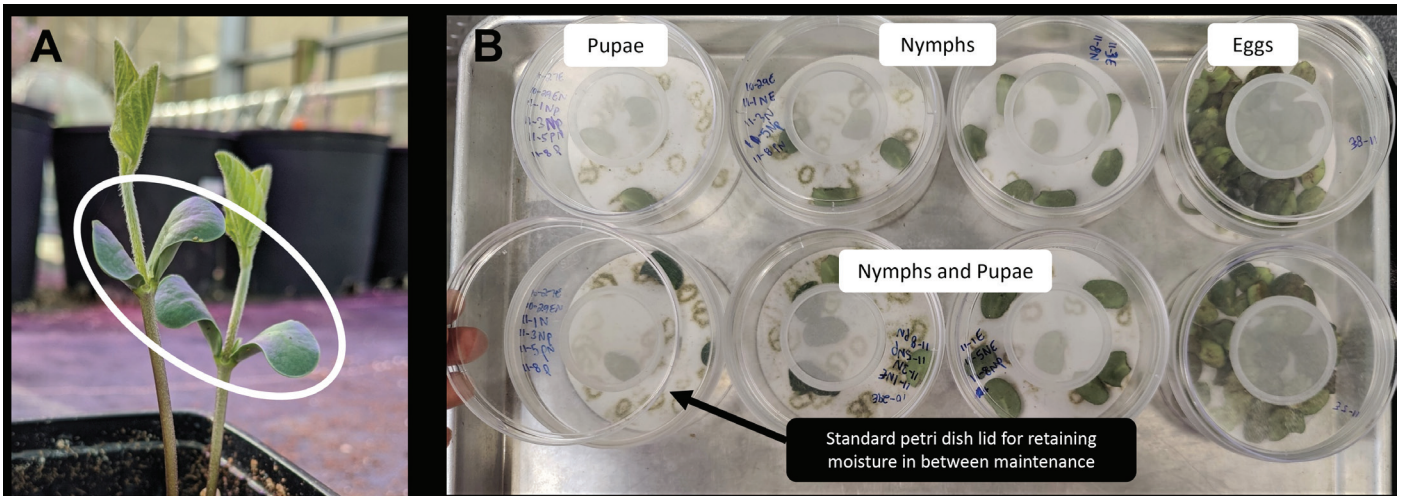

Fig. 1. (A) Shirofumi soybean sprout with cotyledons ready to harvest (circled). (B) Metal tray housing immature stages of *Frankliniella occidentalis* in insect rearing containers with secondary standard Petri dish lids on top of ventilated mesh lids. Records of dates maintained and life stages present in containers are kept on each colony lid.

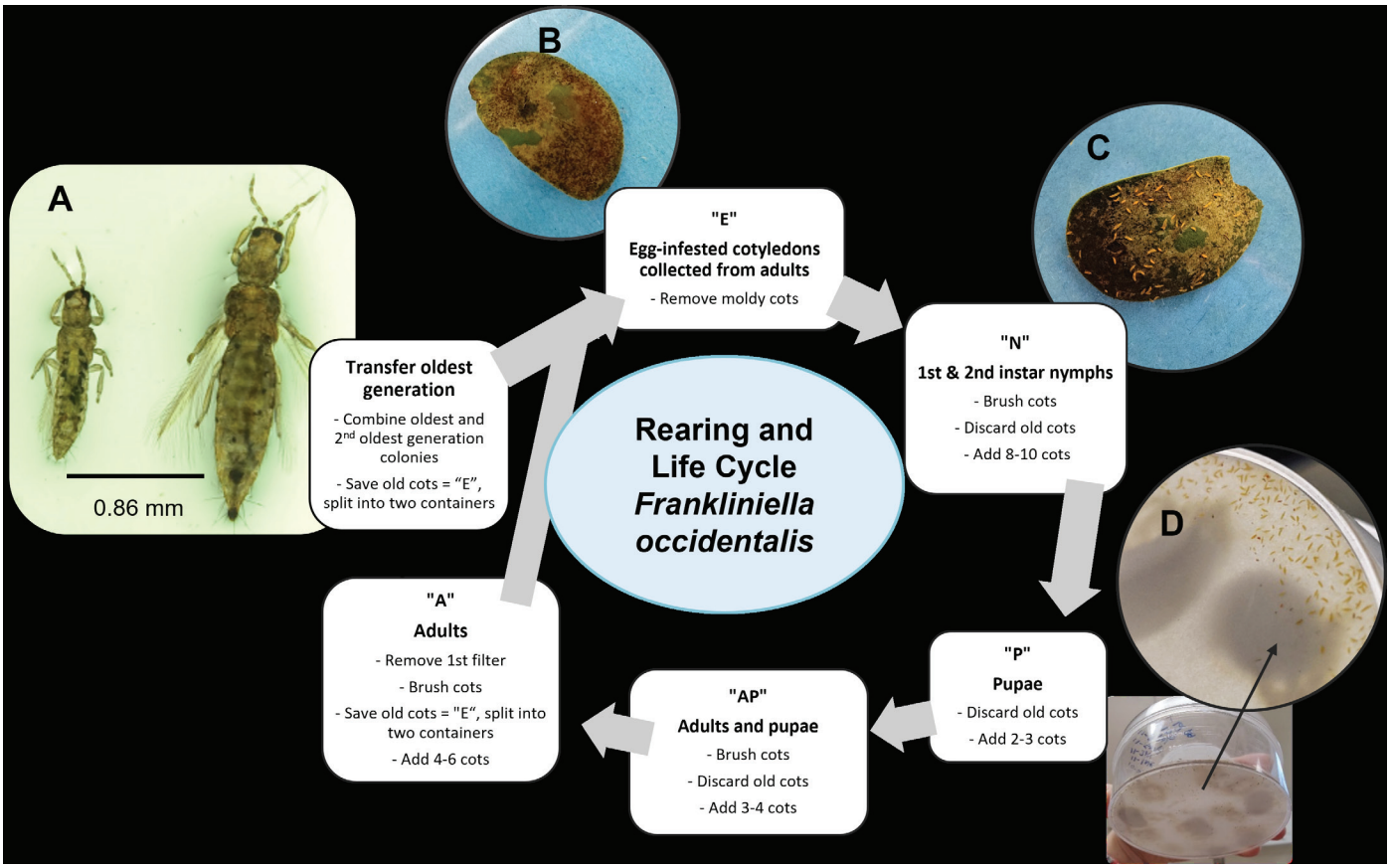

Fig. 2. Diagram of life cycle, rearing procedures, and label coding for *Frankliniella occidentalis*. (A) *Frankliniella occidentalis* male (left) and female (right) adults; (B) egg-infested cotyledon; (C) nymph-infested cotyledon; (D) pupae under filter paper in rearing container; cots = cotyledons.

to disturb the filter paper because the pupae are vulnerable to being crushed. Pupae will appear as small yellow oval specks on the underside of or in between filter paper(s) (Fig. 2D). Once a colony is 11 to 12 d old, label with "AP" for adult and pupae mixed, feed 3 to 4 cotyledons. After 13 d, the colony mostly will be comprised of adults, can be moved to the adult tray labeled with "A," and should be maintained with 4 to 6 cotyledons. At this time, the first layer of filter paper should be removed to prevent mold growth.

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Summary

Here we present a simple, cost-effective, and sustainable mass rearing system for western flower thrips, *Frankliniella occidentalis*, with details of molecular identification. Our rearing methods are improved from other systems used previously because we have organized maintenance responsibilities that occur during a Monday through Friday work wk, and have streamlined environmental and nourishment conditions that have greatly sustained *F. occidentallis* colonies that we use for various physiological experiments.

Key Words: Thripidae; maintenance; life cycle; molecular identification; management

Sumario

Aquí presentamos un sistema de crianza masiva que es sustentable, simple y de costo efectivo para el trips de California, *Frankliniella occidentalis*, con detalles sobre identificación molecular. Nuestros métodos de crianza son una mejoría en comparación a otros usados previamente ya que hemos organizado responsabilidades de mantenimiento estructuradas alrededor de una semana laboral de lunes a viernes, así como la simplificación de las condiciones ambientales y de alimentación que han sustentado enormemente las colonias de *F. occidentallis* que usamos para varios experimentos fisiológicos.

Palabras Claves: Thripidae; mantenimiento; ciclo de vida; identificación molecular; manejo

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