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Title: Establishment of a Chilli Thrips (*Scirtothrips dorsalis* Hood) Rearing System for Virulence Screening of Entomopathogenic Fungi

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes** , all done

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 26
Number of Shots: 56

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Yu-Shin Nai:** Chilli thrips are a well studied pest on multiple crops. Our research is focused on the improvement of the chilli thrips rearing system and extend its application on entomopathogenic fungal screening for future experiments.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What are the most recent developments in your field of research?

1.2. **Hsin-Yu Lin:** Recent studies have focused on mass rearing chilli thrips on whole plants within cages. Some are exploring alternatives like detached leaves, but the clear and detailed methods are still not well-described.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:*

What significant findings have you established in your field?

1.3. **Hsin-Yu Lin:** This study established a new thrips mass-rearing method based on detached-leaf, which can ensure stable chilli thrips colonies. In addition, a stable and reproducible bioassay system was developed for EPF virulence screening.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.8*

What research gap are you addressing with your protocol?

1.4. **Kusum Mushyakhwo:** This protocol addresses the lack of a clear, standardized method for mass rearing thrips using leaf discs, focusing on improving stage management and reducing resource use.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.5. **Kusum Mushyakhwo:** This protocol simplifies developmental stage control, reduces space and labor needs, and provides consistent thrips supply suitable for large-scale and reliable bioassays.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9*

What research questions will your laboratory focus on in the future?

1.6. Yu-Shin Nai: Our laboratory will focus on optimizing mass-rearing methods and applying these protocols to various thrips species, improving environmental and handling conditions to ensure consistent, healthy populations for bioassays. Besides, mass-rearing system can be extended to use on greenhouse trial.

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

NOTE: Authors added the new question during filming

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Collection, Rearing, and Mass Maintenance of Chilli Thrips in Laboratory Conditions

Demonstrator: Hsin-Yu Lin

2.1. To begin, cut leaves and flowers infested with chilli thrips **[1].** Transfer them into a plastic zipper bag, ensuring to seal the bag with some air inside to keep the thrips alive **[2-TXT]**.

2.1.1. WIDE: Talent cutting infested leaves and flowers from the field.

2.1.2. Talent placing the infested plant material into a plastic zipper bag and sealing it with some air inside. **TXT: Transport sample to laboratory within 3 h; If field collection > 3h, place sample into a cooler**

2.2. Next, obtain a glass rearing container measuring 35 millimeters in height and 45 millimeters in inner diameter **[1]**. Place three layers of circular paper towel, each 40 millimeters in diameter, at the bottom of the container **[2]**. Add 8 to 10 drops of filtered water onto the paper towels to maintain humidity **[3]**.

2.2.1. Talent preparing the specified glass rearing container.

2.2.2. Talent placing three layers of 40-millimeter diameter circular paper towels at the bottom of the container.

2.2.3. Talent adding 8 to 10 drops of filtered water onto the paper towels.

2.3. ~~Now, collect a young, red mango leaf from mango fields **[1].**~~Now , wash a young mango leaf thoroughly, examine it under an optical microscope to check for insect eggs or other insect contamination **[2]**.

~~2.3.1. Talent collecting a young, red mango leaf from the field.~~
NOTE: Shot deleted by authors

2.3.2. Talent examining washed leaf under an optical microscope for contamination.

2.4. Using a leather cutter, cut the mango leaf into a circular disc of 40 millimeters in diameter **[1]** and place it on the wet paper towel inside the glass rearing container **[2]**.

2.4.1. SCOPE: Talent cutting the mango leaf into a 40-millimeter diameter disc using a leather cutter.

2.4.2. Talent placing cut discs onto the wet paper towel in the container.

2.5. Observe the chilli thrips under an optical microscope at 10X magnification **[1]**. Using a fine painting brush, transfer 10 adult thrips, comprising five males and five females, into

the prepared glass rearing container **[2]**.
NOTE: Filmed by videographer

2.5.1. SCOPE: View of chilli thrips under an optical microscope at 10x magnification.

2.5.2. SCOPE: Talent using a fine painting brush to transfer 10 adult thrips into the glass rearing container.
Added shot 2.5.2.1 : Talent putting thrips in glass rearing container

2.6. Then seal each glass rearing container with two layers of parafilm **[1]**. Create approximately 40 small holes in the parafilm using a number 00 insect pin to ensure proper ventilation **[2]**.

2.6.1. Talent sealing the glass rearing container with two layers of parafilm.

2.6.2. Talent making approximately 40 small holes in the parafilm using a number 00 insect pin for ventilation.

2.7. Place the sealed glass rearing container into an incubator for 48 hours **[1-TXT]**.

2.7.1. Talent placing the sealed glass rearing container into the incubator set to the specified conditions. **TXT: Incubation: 25 ± 1°C; ~70% RH; Photoperiod (L:D) : 12 h: 12 h**

2.8. Observe the leaf tissues under a stereomicroscope to confirm the presence of eggs, indicating oviposition **[1]**. Then place a fresh mango leaf disc onto the leaf with eggs **[2]** and add 8 to 10 drops of filtered water onto the paper towel to maintain humidity **[3]**.

2.8.1. SCOPE: View of leaf tissues with eggs under a stereomicroscope.
NOTE: Filmed by videographer

2.8.2. Talent placing a fresh mango leaf disc onto the leaf with eggs.

2.8.3. Talent adding 8 to 10 drops of filtered water onto the paper towel.

2.9. For the maintenance of the thrips, directly transfer the leaf containing first instar larvae into a new glass rearing container **[1]**. Observe the chilli thrips daily **[2-TXT]**.

2.9.1. Talent transferring the leaf with first instar larvae into a new glass rearing container.

2.9.2. Shot of the chilli thrips in the container. **TXT: Add 8 - 10 drops filtered water and fresh mango disc every 4 days**

2.10. For the mass rearing of the chilli thrips, first collect female adult thrips from the maintained population **[1]**. Transfer 10 female adult thrips into a prepared rearing container to obtain eggs **[2]**.

2.10.1. Talent collecting female adult thrips from the maintained population.

2.10.2. Talent transferring 10 female adult thrips into the prepared container.

2.11. Then gently transfer approximately 120 first instar larvae into a new glass rearing

container using a fine paint brush **[1-TXT]**.

2.11.1. Talent using a fine paint brush to gently transfer approximately 120 first instar larvae into a new glass rearing container. **TXT: Observe daily; Add water and mango discs every 4 days**

3. Molecular Identification of Chilli Thrips Using PCR and DNA Sequencing

3.1. For the molecular identification, collect one adult chilli thrips into a 1.5-milliliter centrifuge tube **[1]**. Homogenize the chilli thrips using a pellet pestle **[2]**. Then isolate genomic DNA using a commercial kit according to the manufacturer's protocol and amplify using PCR master mix **[3]**.

3.1.1. Talent collecting one adult chilli thrips into a 1.5 milliliter centrifuge tube.

3.1.2. Talent homogenizing the chilli thrips in the centrifuge tube using a pellet pestle.

3.1.3. Shot of isolated genomic DNA in a vial.
AND
TEXT ON PLAIN BACKGROUND:
PCR Master Mix (2x) with a COI primer pair
Forward primer LCO 1490
Reverse primer HCO 2198 (amplifies the partial mitochondrial COI gene)
Video Editor: Please play both shots side by side
Added shot 3.1.4: Place PCR mixture into thermal cycler

3.2. Check the PCR amplicon by electrophoresis in a 1% agarose gel to verify the result of amplification **[1]**. Excise the target band, approximately 658 base pairs in size, from the gel **[2-TXT]**.

3.2.1. Shot of agarose gel after electrophoresis.

3.2.2. Talent excising the target band of approximately 658 base pairs from the agarose gel. **TXT: Purify amplicon using Gel/PCR purification kit then send for sequencing**

3.3. Perform a BLAST *(blast)* search on the NCBI *(N-C-B-I)* database using default parameters to confirm the species identity **[1].**

3.3.1. SCREEN: 3.3.mp4 00:02-00:05, 00:26-00:37,00:50-00:57

4. Preparation of Entomopathogenic Fungal Conidial Suspension for Bioassay Applications

4.1. For the preparation of entomopathogenic fungi, add 2 to 3 milliliters of 0.03% Tween 80 to the surface of a 10 to 14-day-old fungal culture grown on one-quarter strength Sabouraud Dextrose Agar **[1]**. Gently scrape the conidia using a sterile loop **[2]**.

4.1.1. Talent adding 2 to 3 milliliters of 0.03% Tween 80 to the surface of the fungal culture.

4.1.2. Talent gently scraping the conidia from the fungal culture using a sterile loop.

4.2. Transfer the fungal suspension into a clean 15-milliliter centrifuge tube **[1]**. Then homogenize the suspension by vortexing at maximum speed **[2]**. Filter the suspension through filter paper to remove hyphal debris and obtain a pure conidia suspension **[3]**.

4.2.1. Talent transferring the fungal suspension into a clean 15 milliliter centrifuge tube.

4.2.2. Talent vortexing the fungal suspension at maximum speed to homogenize.

4.2.3. Talent filtering the suspension through filter paper to remove hyphal debris.

4.3. Use a hemocytometer to check the number of conidia under a light microscope **[1]**. Dilute the suspension to achieve a concentration of 10^8 conidia per milliliter **[2]**. Then transfer the conidial suspension into a micro-sprayer sterilized with ultraviolet light for subsequent bioassay application **[3]**.

4.3.1. SCOPE: 4.3.1..mp4 00:03-00:20

4.3.2. Talent diluting the conidial suspension to a concentration of 1×10^8 conidia per milliliter.

4.3.3. Talent transferring the conidial suspension into a micro-sprayer sterilized with ultraviolet light.

5. Laboratory Bioassay of Entomopathogenic Fungi Against Chilli Thrips Using Blueberry Leaf Discs

Demonstrator:

5.1. Collect and wash young blueberry leaves from a blueberry farm **[1]**.After air drying them, examine their surface under a stereomicroscope to check for any remaining arthropods or eggs, and remove them if present **[2]**.

5.1.1. Talent washing young blueberry leaves.

5.1.2. SCOPE: View of the blueberry leaf surface under a stereomicroscope while removing remaining arthropods or eggs.
NOTE: Filmed by videographer

5.2. Now, cut a 2.8-centimeter diameter leaf disc using a leather cutter **[1]**. Then disinfect the leaf disc in a 1% sodium hypochlorite solution **[2]** and 3rinse it twice with sterilized water to remove any bleach residue **[3]**.

5.2.1. Talent cutting a 2.8 cm diameter leaf disc using a leather cutter.

5.2.2. Talent disinfecting the leaf disc with 1% sodium hypochlorite solution.

5.2.3. Talent rinsing the leaf disc twice with sterilized water to eliminate bleach residue.

5.3. Next, pour 3 milliliters of 2.5% water agar into small glass containers inside a laminar flow hood **[1-TXT]**. Then gently transfer the leaf disc, adaxial side up, onto the agar and embed it slightly so that only a small portion remains exposed above the surface **[2]**. Once the agar solidifies completely, consider the glass containers ready for bioassay **[3]**.

5.3.1. Talent pouring 3 milliliters of 2.5% water agar into glass containers inside a laminar flow hood. **TXT: Allow agar to cool until it is semi-solid (40 °C)**

5.3.2. Talent placing the disinfected leaf disc onto the agar with the adaxial side up and embedding it slightly.

5.3.3. Shot of the solidified agar surface with the embedded leaf disc ready for use.

5.4. Prior to application, vortex the conidial suspension thoroughly **[1]**. Using a micro-sprayer, evenly apply 0.1 milliliter of the suspension onto the surface of each leaf disc from a distance of approximately 10 centimeters inside the glass container **[2-TXT]**.

5.4.1. Talent vortexing the conidial suspension to ensure homogeneity.

5.4.2. Talent spraying 0.1 milliliter of the conidial suspension onto a leaf disc from 10 centimeters away using a micro-sprayer. **TXT: Air dry the suspension for 30 mins in sterile conditions**

5.5. Now, use a fine paint brush to transfer 10 second instar larvae into each glass container **[1]**. Seal each container with two layers of parafilm **[2]**. Create approximately 30 small holes for ventilation in the parafilm using a number 00 insect pin **[3]**.

5.5.1. SCOPE: Talent transferring 10 second instar larvae into the prepared containers using a fine paint brush.
NOTE: Filmed by videographer

5.5.2. Talent sealing each glass container with two layers of parafilm.

5.5.3. Talent piercing about 30 small holes in the parafilm using a number 00 insect pin.

5.6. Then place the containers in an incubator for 7 days **[1-TXT]**. Observe and record the mortality of chilli thrips under a stereomicroscope at 10X magnification daily for 7 days **[2]**.

5.6.1. Talent placing the sealed containers into an incubator set to the specified environmental conditions. **TXT: Incubation: 25 ± 1°C; ~70% RH; Photoperiod (L:D) : 12 h: 12 h**
Videographer's Note: Use shot 2.7.1

5.6.2. SCOPE: Observation of chilli thrips under a stereomicroscope at 10x magnification to assess mortality.
NOTE: Filmed by videographer

5.7. Keep the dead larvae inside the glass container for mycosis observation to confirm entomopathogenic fungal infection **[1]**.

5.7.1. LAB MEDIA: 5.7.1-(2).jpg, 5.7.1-(3).jpg, 5.7.1-(5).jpg
Video editor: Place all images side by side and highlight from 2 to 5

5.8. For the bioassay of selected entomopathogenic fungi, prepare three concentrations of conidial suspensions **[1-TXT]**. Inoculate the suspensions into different rearing containers **[2].**

5.8.1. Talent preparing three conidial suspensions at concentrations in labelled vials.
TXT: Concentrations: 10^6, 10^7, and 10^8 conidia/mL

5.8.2. Shot of labelled inoculated rearing containers placed in incubator.
Videographer's Note: Use shot 2.7.1

5.9. Transform the mortality data of each fungal strain using an arcsine transformation prior to performing one-way ANOVA to determine significant differences among treatments **[1]**. Then calculate the median lethal time and median lethal concentration values using probit regression analysis **[2]**.

5.9.1. SCREEN: 5.9.1.(1).mp4. 00:09-00:12
5.9.1.(2).mp4 00:07-00:33, 00:50-00:56

5.9.2. SCREEN: 5.9.2..mp4 00:05-00:12, 00:17-00:33

Results

6. Results

6.1. Morphological characteristics of chilli thrips at various life stages were distinctly observed, including the early larval stage, later larval stage**,** and adult stage **[1]**. Polymerase chain reaction amplification targeting the partial COI *(C-O-I)* gene produced a band confirming the identity of field-collected thrips as chilli thrips **[2]**.

6.1.1. LAB MEDIA: Figure 2A-C. *Video editor: Sequentially highlight the images from A to C*

6.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the single bright band next to the yellow asterisk in the mtCOI lane*

6.2. The detached-leaf rearing system successfully supported chilli thrips colony establishment, with a 90% survival rate observed in the control group over the 7-day observation period **[1]**.

6.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the black bars labeled "CK" from days 1 to 7*

6.3. Among tested entomopathogenic fungal isolates, *Cordyceps cateniannulata* NCHU-213 *(N-C-H-U-Two-one-Three)* caused the highest mortality in chilli thrips larvae at 7 days post inoculation **[1]**, followed by Cc-NCHU-298 *(Cord-eye-ceps-cat-eni-annulata-N-C-H-U-Two-Nine-Eight)* **[2]**, while *Beauveria bassiana* showed lower virulence **[3]**.

6.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the dark gray bar labeled "Cc-NCHU-213" at day 7.*

6.3.2. LAB MEDIA: Figure 4*. Video editor: Highlight the light gray bar labeled "Cc-NCHU-298" at day 7.*

6.3.3. LAB MEDIA: Figure 4. *Video editor: Highlight the medium gray bar labeled "Bb-NCHU-157" at day 7.*

6.4. The LT_{50} *(L-T-Fifty)* value of 10^8 conidia per milliliter of Cc-NCHU-213 and Cc-NCHU-298 showed similar values and exhibited shorter days than *Beauveria bassiana* **[1].**

6.4.1. LAB MEDIA: Table 2 *Video editor: Highlight the rows corresponding to "Cc-NCHU-213" and labeled "Cc-NCHU-298"*

6.5. Mycosis was visibly observed on thrips cadavers infected with *Cordyceps* isolates, as seen in the fungal outgrowth covering their bodies **[1]**.

6.5.1. LAB MEDIA: Figure 5. *Video editor: Sequentially show A and B*

6.6. A dose-dependent increase in mortality was evident in thrips treated with Cc-NCHU-213 at 4 and5 days post inoculation **[1]** while the highest mortality of 71.4% observed

at 10^7 conidia per milliliter after 7 days **[2]**, followed by 58.9% at 10^8 conidia per milliliter **[3]**, and 44.4% at 10^6 conidia per milliliter **[4]**.

6.6.1. LAB MEDIA: Figure 6. *Video editor: Please highlight the columns (1 × 10^6 conidia/mL , 1 × 10^7 conidia/mL , 1 × 10^8 conidia/mL) of 4 and 5 DPI*

6.6.2. LAB MEDIA: Figure 6. *Video editor: Highlight the light gray bar labeled "1 × 10^7 conidia/mL" at day 7.*

6.6.3. LAB MEDIA: Figure 6. *Video editor: Highlight the dark gray bar labeled "1 × 10^8 conidia/mL" at day 7.*

6.6.4. LAB MEDIA: Figure 6. *Video editor: Highlight the medium gray bar labeled "1 × 10^6 conidia/mL" at day 7.*

Pronunciation Guide:

1. Scirtothrips

Pronunciation link:
https://www.howtopronounce.com/scirtothrips Collins Dictionary+15How to Pronounce+15How to Pronounce+15

IPA: /sɜr-təʊˈθrɪps/ (American: /sər-təʊˈθrɪps/)
Phonetic spelling: ser-toh-THRIPS

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2. Entomopathogenic

Pronunciation link:
https://www.howtopronounce.com/entomopathogenic Wikipedia+10How to Pronounce+10pronouncekiwi.com+10

IPA: /ˌɛn.tə.moʊˌpæθ.əˈdʒɛn.ɪk/
Phonetic spelling: en-tuh-moh-path-uh-JEN-ik

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3. Cyclic

Pronunciation link:
https://www.merriam-webster.com/dictionary/cyclic glosbe.com+15Merriam-Webster+15Merriam-Webster+15

IPA: /ˈsaɪ.klɪk/ or /ˈsɪk.lɪk/ (US preference /ˈsaɪ.klɪk/)
Phonetic spelling: SY-klik (or SIK-klik)

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4. Thrips

Pronunciation link:
https://www.merriam-webster.com/dictionary/thrips pronouncekiwi.com+5Merriam-Webster+5Wikipedia+5

IPA: /θrɪps/
Phonetic spelling: THRIPS

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5. Hemocytometer

(No audio link found, reliable sources unavailable)
IPA: /ˌhiː.moʊˌsaɪˈtɒm.ə.tər/
Phonetic spelling: hee-mo-sahy-TOM-uh-ter

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6. Parafilm

(No confirmed link found)

IPA: /ˈpær.ə.fɪlm/

Phonetic spelling: PAIR-uh-film