GROWING KNOWLEDGE

Series content is coordinated by Dr. Lloyd Nackley, associate professor of nursery production and greenhouse management at Oregon State University in Corvallis, Oregon.

An ongoing series provided by Oregon State University in collaboration with the United States Department of Agriculture and in partnership with the Oregon Association of Nurseries

Thrip threat

New research shows a promising path to managing this agricultural pest

Thrips are a ubiquitous pest in many systems including the strawberry, raspberry and nursery industries of the Pacific Northwest. PHOTO COURTESY OF OREGON STATE UNIVERSITY

BY MAN-YEON CHOI

Thrips represent a global agricultural pest problem. Thrips are tiny, barely visible insects that have cryptic habits and are among the stealthiest insect invaders.

Frequently, thrips are intercepted from insect quarantine in the border areas of United States.

They are increasing now due to more global trade of agricultural products and regional diversity.

Once established, thrips are found on leaves, blossoms, buds, and leaf sheaths of plants almost everywhere such as greenhouses, gardens and fields. Thrips are a ubiquitous pest in many systems including the strawberry, raspberry and nursery industries of the Pacific Northwest.

Small bug; big threat

Western flower thrips (WFT), *Frankliniella occidentalis*, are one of the most economically significant pests, causing severe damage to agricultural and horticultural crops worldwide. In addition to direct damage from feeding on leaves, flowers and fruits, they also transmit economically impactful plant viruses.

Due to their small size and wide host range, detecting and preventing the spread of WFT is extremely difficult. Thrips are consistently listed as a top priority insect pest, producing damage to nursery crops, which are the most valuable agricultural commodity in Oregon at nearly $1.2 billion.

Current control for WFT primarily relies on conventional chemical insecticides. However, WFT commonly develops resistance to insecticides and thus biological control, improved monitoring and more specific insecticides are needed to prevent insecticide resistance.

Our team at the USDA Agricultural Research Service station in Oregon initiated the development of novel biopesticides for thrips using advanced molecular physiological tools, proteomics, genomics, and transcriptomics. A brief overview of the following topics recently published on WFT offers a new approach to developing thrips control. »

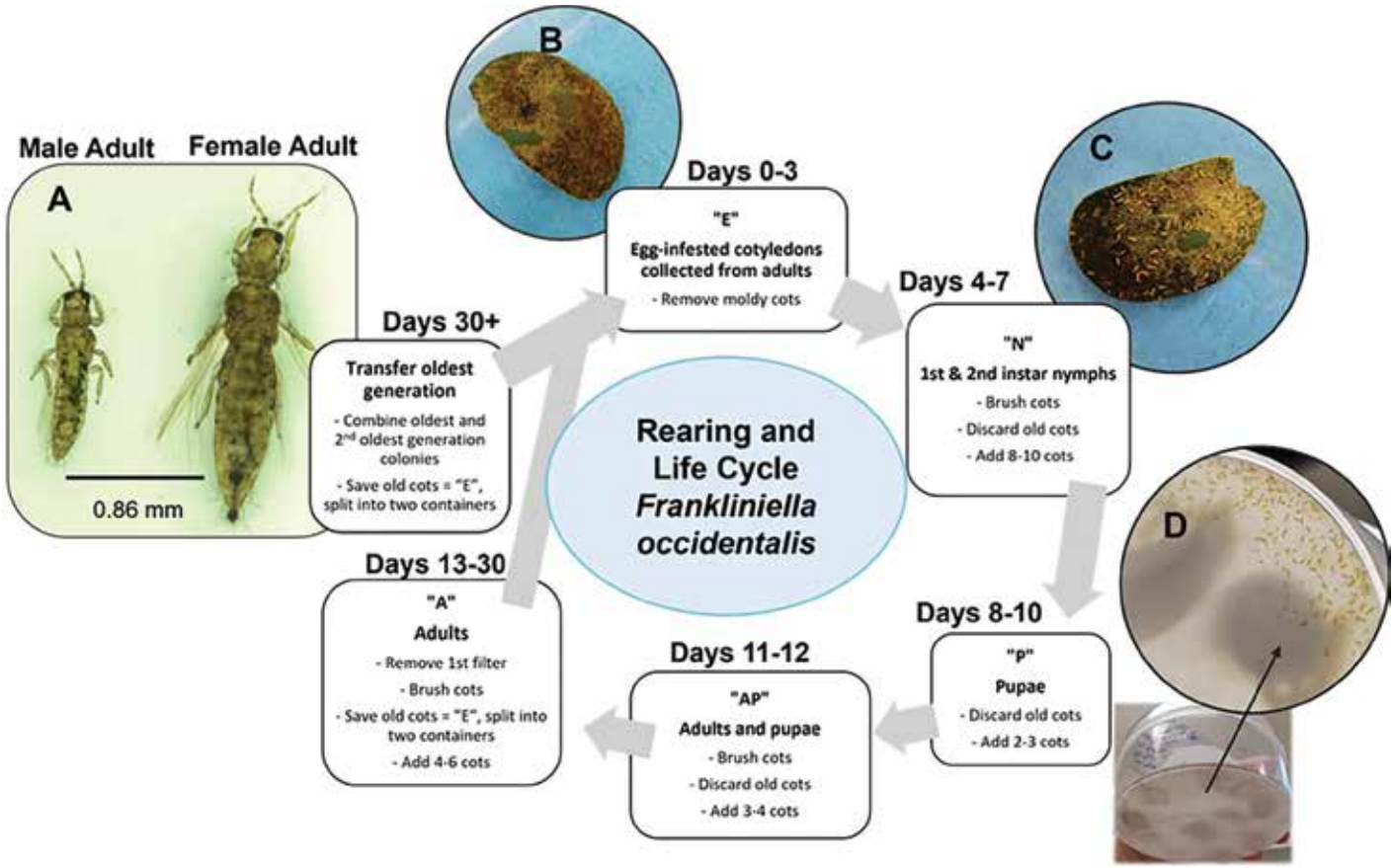

Figure 1: The new system sustains large thrips populations that can be then be used for various physiological experiments. DIAGRAM COURTESY OF OREGON STATE UNIVERSITY

A sustainable mass-rearing method

A new sustainable mass-rearing system is simple and cost-effective in the laboratory (**Fig. 1, above**) and has improved rearing methods as compared to other systems used previously. The new system sustains large thrips populations that can be then be used for various physiological experiments; for example, nano-injection or feeding of potential biological targets to live thrips and observing any physiological or behavioral changes.

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 WFT colonies.

Molecular identification method for thrips

DNA, or deoxyribonucleic acid, is the instruction manual for all living things, including thrips. Genes are specific sections of DNA that determine traits like size, color, or in this case, species identity. To identify thrips at the DNA level, we used a gene called *ITS2* as a marker.

The process started by extracting DNA from individual thrips. Since thrips are tiny, we use a special solution that breaks open their cells, releasing the DNA. Once we had the DNA, we used a technique called polymerase chain reaction (PCR) to make many copies of the *ITS2* gene, a process like photocopying a specific page from a book so it's easier to read. We did this using short DNA sequences called primers, which act like bookmarks to find and copy only the part of the DNA we needed.

After PCR, the DNA was either inserted into a cloning vector for further study or sequenced directly using a method called Sanger sequencing. Finally, we compared our DNA sequences to a known *ITS2* sequence from western flower thrips (WFT) in a public database (GenBank). Our results showed a 99.79% match, confirming the identity of our thrips.

Developed nano-injection method for thrips

Injections are essential in entomological research as they allow for direct delivery of biological compounds into the hemocoel of specimens to find the physiological impact on the insect.

Despite its prevalent use amongst embryonic, larval, and adult stages of insects, most current micro-injections for live insects are restricted to insects over 4 mm in size. Most micro-injection methods (injection volume, µL level) use forcibly immobilized insects, which can affect the fitness and physiology of the insect.

This alteration can lead to inconclusive interpretation of phenotypic responses post-injection. In addition, nano-injection (nL level, 10^{-9} litter) with micro-insects (body size < 3 mm) is challenging, particularly when injecting into the insect without sedation.

We developed a novel nano-volume injection technique for micro-insects (< 3 mm) using WFT, as a model insect. By constructing and using a costum-made

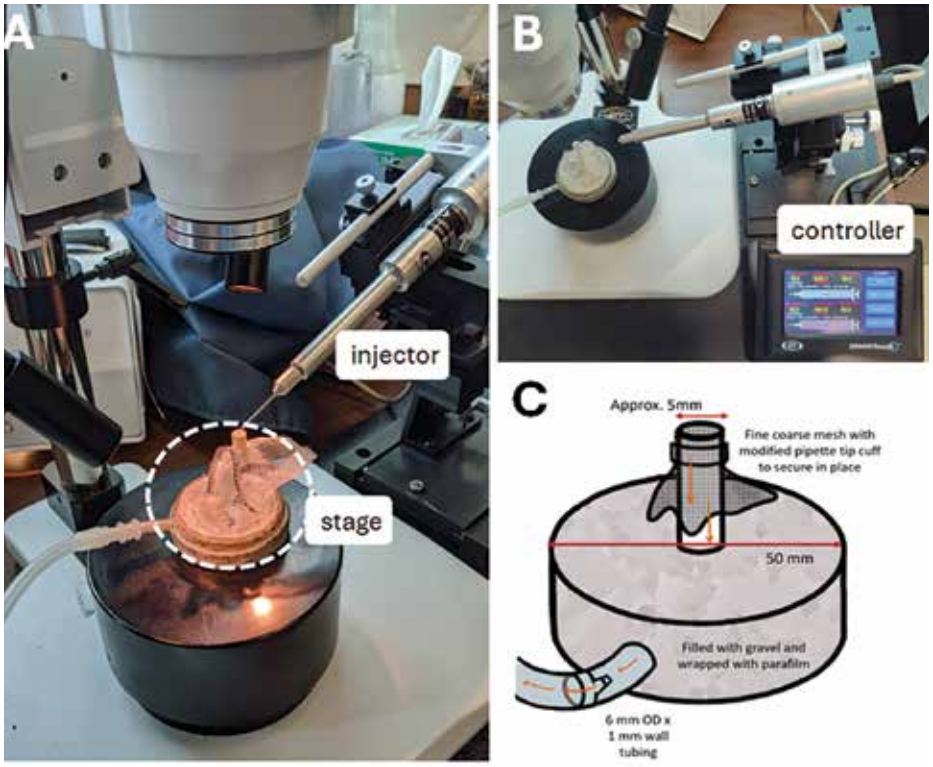

Figure 2: By constructing and using a costum-made vacuum stage connected to a vacuum controlled tube and a nanoinjector under microscope, injections were performed on live thrips, without sedation and physical injury. DIAGRAM COURTESY OF OREGON STATE UNIVERSITY

vacuum stage connected to a vacuum controlled tube and a nanoinjector under microscope (**Fig. 2 left**), injections were performed on live thrips, without sedation and physical injury (**Fig. 3, Page 44**). The nano-injection can be injected up to 10 nL of liquid into the thorax area of the thrips and we confirmed that injected thrips survived without damage compared to uninjected thrips.

A simple survivorship assay in which the thrips were injected with 10 nL water into the thorax or abdomen demonstrated that thoracic injections yield similar survival rates to control thrips that were not injected, while abdominal injections severely limited survivorship. The integrative injection method customizes the vacuum stage, nano-injection tools, injecting volume, and other specifications for live micro-insects (< 3 mm). This technique will facilitate injection of biological »

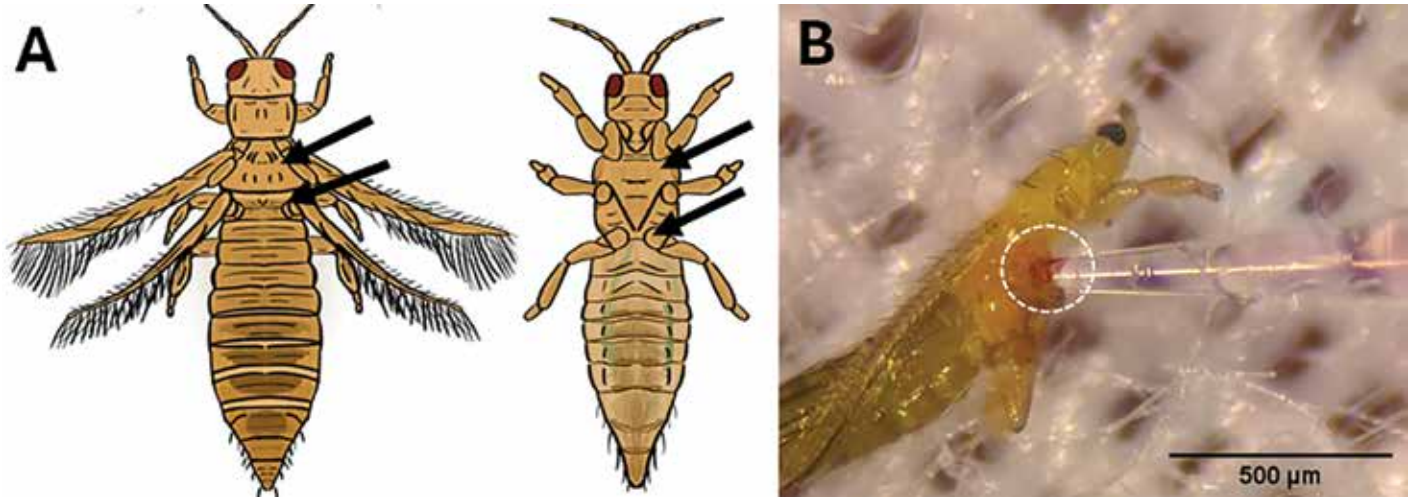

Figure 3: The nano-injection can be injected up to 10 nL of liquid into the thorax area of the thrips and we confirmed that injected thrips survived without damage compared to uninjected thrips. DIAGRAM COURTESY OF OREGON STATE UNIVERSITY

compounds into live micro-insects without any harmful immobilization tools.

Identification and characterization of neuropeptides from WFT

Insect neuropeptides (NPs) are small protein molecules produced in nerve tissues such as the brain. They represent the largest group in the insect hormone system that regulate almost all physiological functions, including feeding, molting, diapause, digestion, diuresis, mating, pheromone production, and many behaviors during developmental and adult stages. These NPs need to be bound to their corresponding receptors to initiate the specific biological processes, which is critical for insect survival.

NPs and their receptors therefore offer biological targets for the development of a new generation of insecticides, particularly biopesticides. We have identified new members of the neuropeptide group, called CAPA and pyrokinin (PK) peptides, that are associated with various physiological functions, including feeding, diuresis, muscle contraction, and pheromone biosynthesis. The fundamental research will assist in the investigation of biological processes at the molecular level, thereby enabling the identification of biological targets that can be employed for the management of thrips.

We studied two specific genes in western flower thrips (*capa* and *pk*) to understand their structure, genetic differences, and how they function in different tissues and life stages. These genes help control important processes in the insect's body.

To see how the proteins made by these genes interact, we tested their binding abilities using receptors from another insect, the brown marmorated stink bug. Think of receptors like locks and proteins like keys—we wanted to see which proteins could "unlock" the receptors.

Since thrips are tiny, collecting enough nerve tissue to study directly is difficult. Instead, we used a special system that allows insect cells to produce these proteins for us, like using a factory to make test samples instead of harvesting them by hand.

Finally, we mapped the nerve cells (neurons) that produce

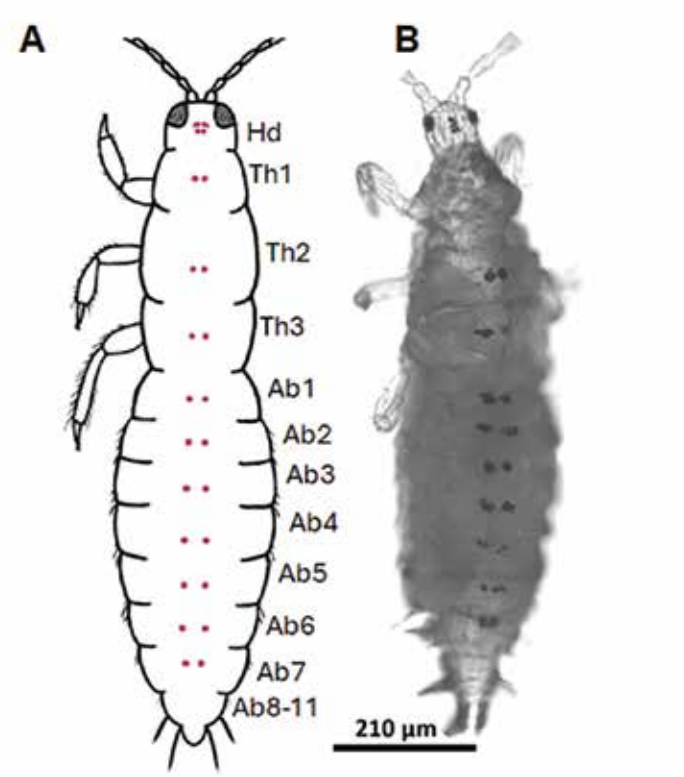

Figure 4: A diagram shows the thorax and abdominal areas of a thrip. DIAGRAM COURTESY OF OREGON STATE UNIVERSITY

these proteins in the thrips' bodies (**Fig. 4, above**). Our results showed that these nerve cells are arranged differently than in other insects, giving us new insights into how thrips control their bodily functions.

Receptor-interference technology

The discovery of new insecticides can improve integrated pest management (IPM), but it is a long iterative process with low chance of success and high risk. An efficient screening process

using a large volume of chemical libraries including natural products is needed.

For decades, insect NPs and their G protein-coupled receptors (GPCRs), have been offered as biological targets for the development of new insecticides, because they are involved in many key biological processes in insects. Disruption of a specific function will lead to novel pest management.

We developed a novel concept and technology that was successfully approved for a GPCR model using insect cell-based expression and phage display peptide libraries. This technology identifies bioactive peptides — small protein molecules — that interfere with a specific physiological function by binding strongly to the target receptor. The technology is called Receptor interference (Receptor-i), and can be applied to any animal pest using a species-specific target GPCR. Receptor-i is distinct from the underlying RNAi technology. ©

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