Biocontrol Rearing Facility

1913 SW 34th Street, Gainesville, FL

Department of Agriculture & Consumer Services
Charles H. Bronson
Commissioner

Division of Plant Industry
Richard Gaskalla
Director

Biological Control

The Florida Department of Agriculture & Consumer Services supports the development of various biological control programs in Florida. Biocontrol helps growers reduce reliance on chemical controls and still grow the high-quality produce for which Florida is famous.

Biocontrol is the use of parasites, predators and pathogens for the control or stabilization of pest populations. The Department's Division of Plant Industry conducts several ongoing programs that rear insects which are natural enemies of many pest insects.

Gainesville's DPI houses mass-rearing facilities which produce Caribbean fruit flies and Diaprepes root weevils for research, along with several biocontrol insects, including a new laboratory for production of the Phorid Fly, an Imported Fire Ant parasite. DPI also houses the Florida Accelerator Services & Technology (FAST) facility, an electron-beam linear accelerator, which treats many agricultural commodities. The Sterile Insect Release Facility (SIRF) in Sarasota is a cooperative program between FDACS-DPI and USDA-APHIS which produces millions of sterile Mediterranean fruit flies for weekly release in high-risk areas throughout the state.

Phorid Fly production

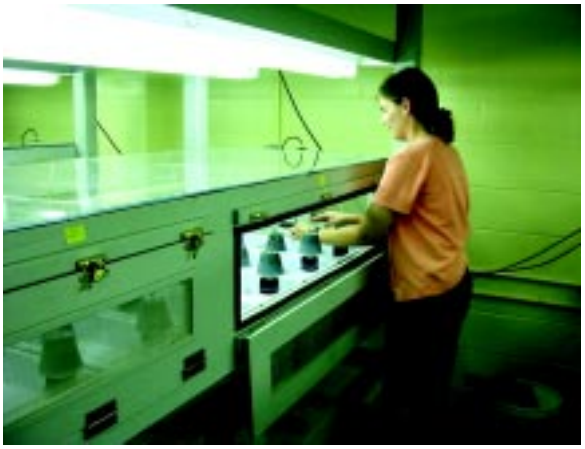
Attack box preparation

Three times a week, 1.2 g of ants and 0.8 g of brood are added to 14 trays in attack boxes designed to encourage ant trailing behavior. Specially designed lifter cups cycle up and down every ten minutes causing the ants to run, allowing the phorid flies to easily parasitize them. Female flies oviposit an egg into the thorax of the ant.

Weighing ants and brood

Sorting ants and brood

Attack trays



Loading an attack box

These attack boxes are held under stringent environmental conditions to mimic the phorid flies' natural habitat. Phorid flies are continually released into the attack boxes from an emergence box located on one end. Adult flies live for several hours to several days.

Parasitized ant holding

Ant holding room

Parasitized ants in holding pans

Parasitized ants are held for 42 days in holding pans with nesting tubes and fed sugar-water. Every other day the dead ants are removed and placed on plaster trays.

Pulling dead ants from holding pan

The phorid fly larvae develops within the ant, eventually migrating to its head and releasing enzymes which cause the decapitation of the ant. Hence the origin of its nickname as **the decapitating fly**. The larvae then consumes all of the ant head's contents and pupates within it.

Phorid Fly shipments

Phorid fly pupae are aspirated off of the plaster holding trays, counted, and placed in plaster shipping cups. About 350 pupae are placed in each cup. These are then sent to field cooperators.

Collecting phorid fly pupae

Shipping container

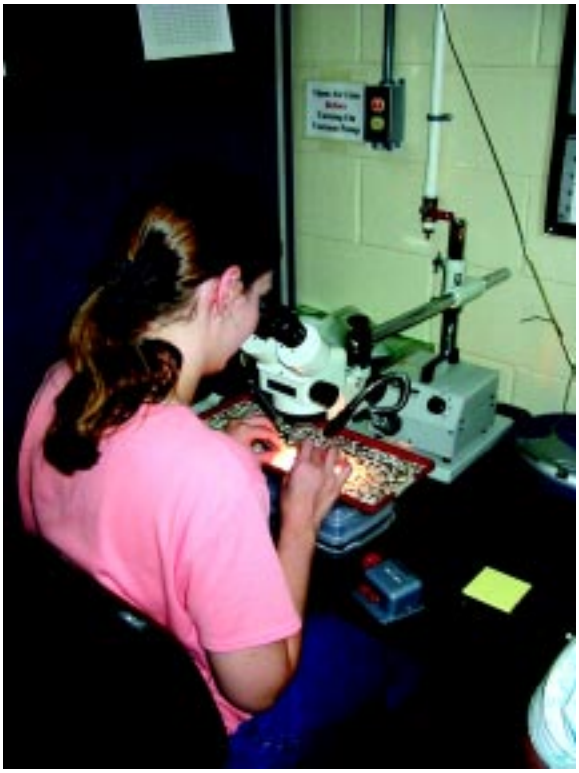
Field cooperators hold the pupae in cages until the adults emerge, then they collect and release the emerged adults daily over a two week period in specific locations. The release sites are monitored for establishment and spread of the phorid flies for several years.

Pupae handling & quality control

Phorid fly pupae holding box

Phorid fly pupae are kept under extremely high humidity levels in special holding chambers for 15 days. They are then placed in an emergence box at the end of the attack boxes. Adult flies emerge ready to mate and oviposit into the provided ants.

Watering phorid fly pupae



Collecting production data

Phorid fly production data is obtained by counting parasitized ant heads on the plaster holding trays under a dissecting microscope. Emergence counts are handled in like fashion after the trays are removed from the emergence box at 25 days. Sex ratios are also performed on shipment flies to make sure adequate percentages are being released.

Phorid Fly Rearing Program

Florida Department of Agriculture & Consumer Services
Division of Plant Industry, Gainesville, Florida
George Schneider, Facility Administrator
and Amy Croft, Phorid Fly Supervisor

The Phorid Fly Rearing Program established at the Florida Department of Agriculture's Division of Plant Industry Facility in Gainesville this past year has progressed well. The goal of the program, a cooperative initiative with the USDA, is field establishment of the phorid fly, *Pseudacteon tricuspis*, as a biological control agent for the Imported Fire Ant (IFA), *Solenopsis invicta*, in the eleven southern states afflicted with this aggressive pest. Over the past year, the Division has modified several rooms at its Biocontrol Rearing Facility to house the rearing rooms and work areas necessary, as well as provided oversight for the rearing program. The USDA-APHIS has provided the funds for these modifications and for technicians to perform the variety of rearing tasks associated with the project. The USDA-ARS has provided the initial phorid fly stock, transferred the rearing techniques, and supplied the fire ants and brood needed for production of the fly. Close cooperation between the three organizations has been essential for the successful start-up of the program.

Currently four technicians and a supervisor perform the numerous duties associated with the rearing process with direction from the Biocontrol Rearing Facility administrator. Additionally the USDA-APHIS has provided a methods development position to investigate rearing enhancement techniques and troubleshoot problems. The Division's maintenance section has performed most of the facility's structural modifications and constructed the elaborate attack boxes necessary to house the adult phorid flies and encourage parasitism of the provided fire ants.

Three times weekly ants from USDA-ARS are exposed to phorid fly attacks for a 48-hour period and then removed. The process begins with the collection of ants from various selected field sites by USDA-ARS personnel. The ants are held in colony boxes at USDA-ARS until they are then shipped along with appropriate amount of brood to the Biocontrol Rearing Facility for processing into the attack boxes. Equal amounts of ants and brood for each tray in the attack boxes are weighed into holding cups and covered for thirty minutes to allow the ants to form a 'bond' with the brood. After thirty minutes one holding cup of ants and brood is placed in every tray of each attack box, where exposure and attack by the phorid flies is immediate.

Every tray in the attack boxes has two cups suspended from strings operating in opposition to one another set on a ten-minute timer. One cup is raised while the other is lowered. Once the ants have been put into the tray they immediately start moving the brood to the lowered cup. When ten minutes have expired, the cups automatically shift in position causing the lower cup to rise and the higher cup to lower to the tray. The function of this process is to cause the ants to 'trail' thereby exposing them to repeated attacks by the phorid flies. While the ants are moving the brood to the new lower cup, the phorid flies parasitize the ants by means of injecting an egg into their thoraxes. As stated earlier, this process continues for a 48-hour period with a nocturnal cycle when the lights

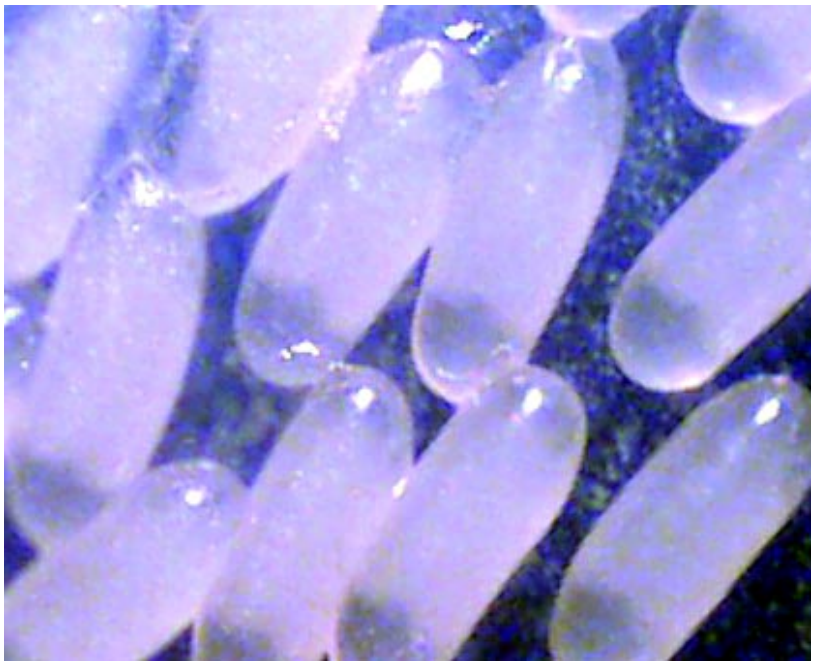
are off and the cups do not move. It is during this time that the ants settle under the lowered cup and the flies roost on various areas in the attack boxes. When the 48-hour exposure is over these ants are removed from the attack boxes before the lights are initiated to prevent the escape of numerous flies. The ants are then separated from their brood and processed into their different colony holding boxes. Fresh ants are then shipped from USDA-ARS to be processed into the attack boxes to start the process over again.

When the ants are separated from their brood and placed in colony holding boxes they are covered and shelved for 42 days. During this 42-day cycle, the boxes are maintained three times weekly, to include removing dead ants, changing the food source (a sugar wick), and watering the moisture block to maintain humidity inside the box. The dead ants for each attack box are collected into one box and then spread out onto plaster trays and moistened to prevent desiccation and then moved to a holding box for two days. After two days, the tray is removed and analyzed under a microscope by a technician to determine the number of parasitized ant heads. Parasitized heads are counted and recorded onto data sheets. The tray is then returned to a holding box for 23 days, proper humidity levels are maintained throughout larval development. Prior to emergence of the flies, the trays are moved from the holding boxes to the attack boxes, where final stages of development occur.

Five attack boxes are on-line and each produces between 400 and 800 hundred phorid flies per day. An additional three attack boxes should be on-line later in the spring. The goal is to get all of the boxes up to peak production, averaging five hundred flies per day, as quickly as possible. This should allow for at least three field releases in the latter part of the spring and have the colony well-established heading into the fall release season.

The USDA-APHIS Gulfport Laboratory will be coordinating the field release efforts with various federal and state cooperators. It is hoped that this particular phorid fly can become successfully established throughout the entire southeastern United States within the next three to five years. It is anticipated that either concurrently or consecutively additional phorid fly species will be mass reared at the Biocontrol Rearing Facility once cleared for field release by the USDA-ARS. These additional species will allow more pressure to be applied to suppress the IFA since each phorid fly species attacks different size IFA workers and at different times of the day. The overall goal is to establish a complex of natural enemies similar to what exists in South America against the IFA throughout the infested regions in this country. This will reduce pesticide usage and give native ant species an opportunity to reestablish themselves in numerous environmental niches.

Diaprepes production



Egg collection

The Diaprepes adults lay eggs between strips of wax paper. These strips are collected daily and placed in a Ziploc bag with a moist wick and incubated for one week.

Neonate transfer

Seven to ten 1-week old neonates are sterilized with a .25% bleach solution and placed in cups of diet with a paint brush, where they remain for 30 days.

Single transfer

As the grubs grow, they are sterilized and placed individually into new cups of diet, where they remain until adulthood.

Larval diet preparation

Several ingredients, including a weevil pre-mix formula, benzoic acid and methyl paraben are added to agar and deionized water and heated. After it is mixed, the diet is poured into 1 oz soufflé cups.

The diet is dried overnight and bagged

To control microorganisms, the diet is irradiated with electron beam technology.

Adult diet preparation

Adult diet preparation includes raising citrus to ensure healthy pesticide-free plants and cutting up organically grown carrots. Adults also eat larval diet, which is added to the adult cages.

The adults are shipped to researchers studying Diaprepes weevil control or are used in Biocontrol Phase II for parasite production.

Caribbean Fruit Fly production

Caribfly egg collection

Adult Caribfly colony cages

Adult Caribbean fruit flies are maintained in cages specifically designed to allow for easy egg collection. Flies are kept in production for one month.

Eggs are collected by rinsing them off rubber-cemented cloth screens. The eggs are then cleaned, measured and incubated for three days prior to infestation on diet material.

Egg collection

Larval diet production

A corn cob-based larval diet is mixed and placed in tray. Each tray is infested with 80,000 eggs placed on Masslinn towel strips. The eggs hatch and the neonates burrow down into the diet material.

Larval diet preparation and infestation

The diet stacks are incubated at 80°F for five days while the larvae mature. The diet stacks are then moved to a room at 70°F so that the larvae do not cook themselves with the metabolic heat they are generating. By day six and seven the mature larvae begin to crawl out of the diet stacks.

Pupae collection & spent diet management

Collection of larvae

Larvae that have crawled out of the diet stacks are collected in gutters filled with vermiculite and placed in pupation trays. These trays are incubated for eight days prior to the pupae being sifted out with a large rotary drum machine.

Sifting machine

The spent diet material is steamed to kill any leftover larvae and then fed to cattle. It is a good way to recycle the mass rearing processes' largest waste product.

Irradiation & quality control

Irradiation cylinder

Pupae are irradiated with 7 krad to sterilize the flies that will soon emerge. They can then be used for SIT releases or other field research initiatives.

Quality control cages

Various quality control tests are performed to ensure high quality flies. These tests include emergence percentages, sex ratios, flight ability, and mating compatibility.

SIT release & trapping

Automated ground release machine

Caribflies are currently being used to evaluate automated ground release machine technology for use in future SIT programs against this and other fruit fly pests.

Caribflies are also being used for research on various soil drench pesticides against Tephritid flies, new bait station technology applications, and mass rearing improvements.

Florida Accelerator Services & Technology (FAST)

This electron beam linear accelerator was built in 1989 funded mainly by the U.S. Department of Energy as a pilot demonstration facility to investigate the feasibility of radiation in agriculture. FDACS accepted the challenge to provide technology necessary to conduct biological control programs, to meet regulatory requirements for other governments, to ensure the safety of our state and national food supply, and to protect Florida's agricultural industry from various pests. A new accelerator was brought online in 1999.

FAST facility

Treatment of agricultural commodities:

- Disinfest insect diets, fruits and vegetables of insect pests, bacteria and fungi
- Extend shelf life by retarding the ripening process
- Treat insects for biological control programs
- Disinfest foods of harmful pathogens, such as salmonella, E. coli and listeria
- Satisfy quarantine requirements for shipping products to certain domestic and foreign markets

Treatment of other commodities:

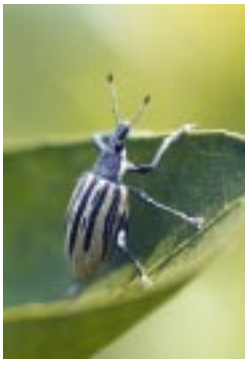
- Sterilization of medical disposables
- Treat semiconductors to modify operational characteristics
- Decontaminate U. S. Postal Service mail

Computerized process control

Facility process area

Products on conveyor passing under electron beam

Biological control phase II



A

B

C

Diaprepes weevil ~
Diaprepes abbreviatus
Parasite: *Quadrastichus haitiensis*

Biological control by egg parasitoids is an important component of the Integrated Pest Management system for *Diaprepes abbreviatus* (A) in Florida. In 1998, *Quadrastichus haitiensis* (B, C) from Puerto Rico was introduced into the quarantine laboratory in Gainesville and mass rearing began in 2000. Over two million specimens of *Q. haitiensis* were produced from the rearing laboratory and released in Diaprepes-infested areas in Florida during 2000-2002. The parasite has been recovered from several locations in Dade, Broward, Glade and St. Lucie counties.

Biocontrol phase II

Asian citrus psyllid ~*Diaphorina citri*
Parasites: *Tamarixia radiata*
and *Diaphorencyrtus aligarhens*

Asian citrus psyllid (A, B), *Diaphorina citri*, is one of the most effective vectors that transmits greening disease, a limiting factor for growing citrus in Asia. The psyllid also damages citrus trees by sucking nutrients from tender leaves, causing trees to weaken, leaves to curl and new shoots to wilt. The Asian citrus psyllid was first discovered in Delray Beach in June 1998. Two specific parasites, *Tamarixia radiata* (C) and *Diaphorencyrtus aligarhens* (D), from Vietnam and Taiwan, were imported and released into Florida following the accidental introduction of Asian citrus psyllid.

A

B

C

D

A

B

Brown citrus aphid ~*Toxoptera citricida*
Parasite: *Lipolexis scutellaris*

Brown citrus aphid (A), *Toxoptera citricida*, is one of the most serious pests of citrus of Southeast Asian origin. It was discovered in Florida in November 1995. Direct damage is caused by removal of nutrients from tender growth, making foliage curled and distorted. However, the primary concern about this pest is due to its efficient transmission of citrus tristeza virus (CTV), one of the most devastating diseases of citrus. *Lipolexis scutellaris* (B) was collected from Guam and sent to Gainesville in August 1999. The permit for field release of this parasite was granted by DOACS-DPI on June 21, 2000. *L. scutellaris* has a short life cycle and a high rate of production.

Special recognition

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