



### Did you know...?

The scientists of the Crop Bioprotection Research Unit are internationally recognized for their expertise. Scientists from around the world come to visit us to receive guidance and training. Here are some current visiting scientists:

- **Brazil...** Gabriel Moura-Mascarin is working with CBP microbiologist Mark Jackson on new methods of culturing insect-killing fungi, and Nilce Kobori is working with CBP plant pathologist Dave Schisler to find new ways that beneficial fungi can be used to control plant diseases.
- **South Korea...** Soo-Jin Kim is working with CBP research leader Alejandro Rooney to come up with new ways to identify and track bacteria in the genus *Bacillus* which are important for many different agricultural applications.
- **Pakistan...** Sahdia Khalil is working with CBP plant pathologist Dave Schisler on new ways of culturing beneficial fungi to control plant diseases.
- **Egypt...** Atef Sayed is working with CBP entomologist Bob Behle on ways to use insect-killing fungi to control fruit flies and leaf miners that plague tomatoes.



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Want to find out more? Contact us today!

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### Our Vision

Research to develop novel biological control methods, processes and products creates new market opportunities for green technologies that reduce the use of potentially harmful chemical pesticides, herbicides and fungicides. CBP strives to be an internationally recognized leader within this field. The impact of our research is recognizable in our highly cited scientific publications and in the technologies developed by us that we transfer to the private sector to help support the success of American enterprise and the security of our nation's food supply.



## Winter 2013

# CBP e-Updates

The electronic research informational for the Crop Bioprotection Research Unit

### Solutions for a New Economy

Research to develop novel biological control agents, products and processes produces new "green" technologies that will help growers increase their yields, and it creates new market opportunities for American companies that want to produce or utilize environmentally friendly products. The Crop Bioprotection Research Unit (CBP) develops novel, performance-competitive biological control technologies that enhance plant health and reduce the use of chemical herbicides, pesticides, and fungicides. CBP conducts research to solve the biggest problems within this field. Specific applications currently being developed by CBP scientists include pheromone-based monitoring systems for the emerald ash borer, novel insect-resistant lines of corn, and living microbial agents to control insect pests, weeds, and fungal diseases of field crops and tree fruits.



Above photo: Highly melanized microsclerotia of the fungus *Mycophotodiscus terrestris* separated from liquid culture fermentation broth.

### Harvesting the Power of Beneficial Fungi

Biopesticides containing beneficial fungi are often grown on grains or other solids, but CBP scientists have found that a liquid diet might be cheaper and better.

The approach, known as "liquid culture fermentation," offers several advantages, including lower material costs and increased yields of certain forms of pest-killing fungi like *Isaria* or *Metarhizium* that can be sprayed directly onto crop plants or applied to soil as a biological alternative to using synthetic pesticides.

For decades, biopesticide makers have cultured fungi like these on moistened grains or other solid substrates to prompt them into churning out billions of specialized cells called "conidia," or spores, which latch onto and then penetrate the cuticles of soft-bodied insect pests, killing them within a few days. Over the past several years, however, CBP microbiologist Mark Jackson and colleagues have sought to improve on the approach using liquid-culture fermentation methods in special tanks called "bioreactors."

"We've made good strides," reports Jackson. "Optimizing fermentation conditions has increased the

yield of spores, and we've identified low-cost nutrients that reduced production costs by 80-90 percent."

One of the greatest reductions has been in costs associated with nitrogen as a primary fungal nutrient. One source, hydrolyzed forms of protein, is typically derived from agricultural commodities like milk casein, which can sell for more than \$6 a pound. Jackson used less-expensive nitrogen sources, including soybean flour or cottonseed meal, which cost 30-50 cents a pound.

Conidia have long been the spores of choice for biopesticide uses, but other fungal cells can be just as effective, including yeastlike structures called "blastospores" and clumps of pigmented fibers known as "microsclerotia." The latter can be easily and cheaply formulated as granules of almost any size for most application needs. Jackson says an advantage of the liquid-culture fermentation technology is that it isn't limited to mass-producing one particular fungal species or even one particular form of fungus. Blastospores can be cultured for use in sprays to control leaf-feeding pests like aphids as easily as microsclerotia for use in granular formulations to control root maggots. "This flexibility opens all kinds of doors," says Jackson.

-This story was first published in Agricultural Research magazine by Jan Suzkiw, ARS Information Staff.

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## Building a better popcorn producer

Insect damage causes hundreds of millions of dollars of losses in the U.S. alone and, in corn is often associated with ear mold toxins. Plant resistance is an economical means to manage insects, but there continues to be a need for determining what genes are involved in producing resistance.

To obtain more information about how the production of insect-defensive chemicals are regulated at the genetic level, CBP entomologist Pat Dowd and molecular biologist Eric Johnson are looking at chromosomal segments of a critical gene involved in the production of an anti-insect protective chemical in corn. The scientists examined genetic material from four different wild relatives of corn that are more insect resistant than today's production lines and identified, for the first time ever, nearly identical segments among all genetic material examined. The scientists believe that these identical segments are likely to influence production of the anti-insect compound. However, confirmatory research in the field is needed. If proven correct, these segments could be used to produce superior lines of insect-resistant corn, ultimately leading to greater corn yield and enhanced quality for growers!



Above photo: CBP entomologist Bob Behle and University of Florida professor Jorge Peña inspect the damage to an avocado grove near Homestead, Florida.

More than 99 percent of the nation's \$322 million avocado crop is grown in south Florida and southern California (less than 1 percent is produced in Hawaii), which makes recent infestations of groves there by invasive wood-boring ambrosia beetles so alarming. A host of counter strategies are in the works, including a biobased foam originally developed by CBP scientists for use against Formosan termites which live underground.

In Miami-Dade County, Florida, avocado growers are contending with *Xyleborus glabratus*, the redbay ambrosia beetle. It tunnels into the sapwood of avocado trees, inoculating them with pathogenic fungi in the process.

Redbay ambrosia beetles specifically attack members of the Laurel tree family (which includes native tree species and avocado).

The culprit is a fungal pathogen that the beetles carry with them into trees and "farm" as food. *Raffaelea lauricola*, the fungus spread by redbay ambrosia beetles, causes laurel wilt disease, which is lethal to avocado and other trees, including native redbay and swampbay species.



Above: a female redbay ambrosia beetle, *Xyleborus glabratus* (about 2 mm long).

"Our research has shown that 3 separate strains of the fungi are fatal to the insects, with fungal-induced beetle mortality greater than 95 percent," Dunlap reports.

-This story was first published in *Agricultural Research* magazine by Jan Suzukiv, ARS Information Staff.

## Avocados aren't just nutritional powerhouses

They're also the chief ingredient in such party favorites as guacamole dip

Spraying avocado groves with insecticides to kill the beetles before they infect trees with the fungi may not be an effective disease-management approach, notes Alejandro Rooney, who leads the CBP Research Unit.

To fight the harmful fungi, Rooney and CBP colleagues Bob Behle and Chris Dunlap are investigating the potential use of beneficial fungi to target the ambrosia beetles. These include entomopathogenic (insect-infecting) species of *Metarhizium*, *Isaria*, and *Beauveria*. Early evidence has been promising, notes Rooney, whose team is collaborating with Jorge Peña and Daniel Carrillo with the University of Florida at Homestead. One such result is from special DNA marker tests devised by CBP chemist Chris Dunlap, which enabled the team to confirm the microbes' ability to infect and kill the beetles.



Above photo insert: tanks of Hydrilla treated with various concentrations of the weed-killing fungus dubbed "MT".

## Fungal Weed-Whackers

Hydrilla (*Hydrilla verticillata*) and Eurasian watermilfoil (*Myriophyllum spicatum*) are invasive aquatic weeds that are a major threat to aquatic ecosystems in the United States and across the world.

Here in the U.S., these species represent two of our most serious aquatic weed problems. Hundreds of millions of dollars have been spent on eradication and control efforts over the past decade. Even more damage in lost valuation has resulted for landowners whose lakes are plagued by these weeds or for home-

owners whose property sits on an infested lake. The ecological impact of the infestations also takes its toll on native plants and fish, which can ruin recreational activities such as boating and fishing.

To solve the problem, CBP microbiologist Mark Jackson and U.S. Army Corp of Engineers scientist Judy Shearer developed the first liquid culture fermentation method for the production of high concentrations of a stable, infective form of the aquatic fungus *Mycocleptodiscus terrestris* (MT). The scientists found that MT can be used

to successfully alleviate Hydrilla infestations in the southeastern U.S. Moreover, smaller amounts of chemical herbicides are needed when used in combination with MT, which is a plus for the environment.

MT technology has been so successful that domestic and foreign patents were issued on it. Recently, a U.S. company has sought to license the technology and hopes to begin production soon. For their efforts, Jackson and Shearer received the Federal Laboratory Consortium Award for Excellence in Technology Transfer in 2010!

## Plant Disease Control

### Fighting Fusarium Head Blight

CBP plant pathologist Dave Schisler has been working on developing beneficial fungi and bacteria to control Fusarium Head Blight (FHB), a nasty wheat disease (see photo insert). One of the microorganisms he developed as a living microbial biocontrol agent is a yeast known as *Cryptococcus flavescens*. Schisler has been able to show that application of the yeast to wheat fields can significantly reduce FHB infection. Before it can be ready for commercial



application though, a way to monitor the yeast is needed so that, in addition to environmental monitoring, scientists can obtain information on the factors that influence its survival and activity.

Recently, Schisler collaborated with scientists at Ohio State University to develop a technique for tracking this yeast by looking for its DNA in the field. They found that the yeast moves rapidly through fields and stays on harvested grain and post-harvest field material. This means prolonged protection of the grain from FHB!

## The Buzz Turning the table on mosquitoes

This past summer, CBP scientists Alejandro Rooney, Bob Behle, and Chris Dunlap began researching new ways to kill Asian Tiger, yellow fever, and floodwater mosquito species. These mosquitoes can spread nasty diseases to both humans and animals. The project aims to see whether insect-killing fungi can be used to kill the eggs of these species before they hatch. Going after the eggs would allow a greater proportion of mosquito populations to be targeted, because after hatching the larvae (and ultimately adults) disperse away from egg-laying sites. To date, no practical chemical treatment is available for this purpose. Some data in the literature and field observations indicate that fungi can penetrate and kill the eggs of certain mosquitoes. Whether or not this can be used as a control is unknown, but if successful, the research will lead to entirely new strategies to control the worst medical and veterinary pest species in the United States.

Below: an Asian Tiger mosquito, *Aedes albopictus*, collected in Peoria, Illinois.

