Scientists in Montpellier are

Exploring the World for Biocontrol Agents

In a beautiful valley near Montpellier in southern France, the European Biological Control Laboratory is home to scientists who fight the battle against Eurasian weeds and insect pests that have invaded the United States.

SCOTT BAUER (K9341-2)

Seasoned travelers, the scientists at the European Biological Control Laboratory (EBCL) will happily oblige the curious with a story or two of misadventure. During a recent trip to Nepal, Tim Widmer learned first-hand just how tricky it can be to shake leeches off your pants and fingers. Rouhollah Sobhian tells of getting blinded in a Tunisian sandstorm, while Alan Kirk recalls being stranded in the Australian Outback. Then, there's Kim Hoelmer, who sampled fried grasshoppers in a Beijing street market, and Franck Hérard, whose team searched for a "lost" member in a thick forest only to find him waiting at their hotel.

Listening to these scientists gives the impression that such misadventures are all part of the job. Aside from world travel, their duties include research on parasites, predators, and pathogens of insects and weeds that have become invasive pests of U.S. agriculture. By one estimate, these pests cost about $46 billion annually in losses and control, not counting ecological damage and harm to wildlife.

From Montpellier's seaside airport, EBCL scientists routinely hop flights to the pests' points of origin in North Africa, the Middle East, the Balkans, and Asia to collect natural enemies. Typically, they'll explore sites where the crops, climate, or habitat matches a particular U.S. region where a pest has become established and a biocontrol agent is needed.

"With all the concern about invasive, nonnative species, we're on the forefront of the only proven, sustainable technology to deal long-term with the problems that have already been introduced," says EBCL director Paul C. (Chuck) Quimby, Jr.

EBCL is about a 20-minute drive from downtown Montpellier on France's Mediterranean coast. USDA's Agricultural Research Service (ARS) established the lab in 1919 and had a new facility built for it in 1999 on a 5-acre plot inside France's prestigious international research campus, called AGROPOLIS. Administered by ARS's Office of International Research Programs, the 1,800-square-meter lab boasts a top-notch quarantine lab with three self-contained greenhouses. EBCL's staff at Montpellier and substations in Rome and Thessaloniki includes 30 scientists, lab technicians, and support personnel from America, France, Britain, Africa, and elsewhere.

SCOTT BAUER (K9325-1)

In southern France, ARS specialist Rouhollah Sobhian (center) and technicians Arnaud Blanchet (left) and Boris Fumanal inspect Russian thistle for potential biocontrol insects.

SCOTT BAUER (K9325-3)

Well camouflaged, a larva of the *Gymnancylie canella* moth burrows into a Russian thistle stem.

From Montpellier's seaside airport, EBCL scientists routinely hop flights to the pests' points of origin in North Africa, the Middle East, Balkans, and Asia where natural enemies can be collected.

EBCL is like an overseas outpost where the natural enemies of pests that scientists have collected can be reared, tested, packaged, and shipped stateside for use in classical biocontrol programs.

Says Quimby, "Our job is first to explore and find natural enemies of designated targets, and then characterize their identity, biology, and host range to make sure they're specific enough for introduction into the United States without causing problems."

To date, this has resulted in nearly 200 different biocontrol agents for use against at least 36 insect and weed species plaguing U.S. agriculture and natural habitats. These agents usually fall into one of four categories:

Parasites—including *Peristenus* wasp species, whose larvae develop inside *Lygus* bug nymph stages.

Predators—such as *Thanasimus formicarius,* a beetle that eats pine shoot beetles, a pest established in 12 states and Canada.

Pathogens—such as *Trichothecium roseum,* a fungus that infects the exotic shrub saltcedar, which in western states increases soil salinity and deprives native plants of water.

Weed Feeders—such as the weevils *Larinus minutus* on knapweeds and *Eustenopus villosus* on yellow starthistle.

One EBCL biocontrol success story is its research on seven parasites and one predator species from western Europe for control of alfalfa weevils. In the 1980s, these biocontrols were released in the United States and resulted in $90 million in yearly savings. In Texas's Rio Grande Valley and southern California, silverleaf whitefly numbers have declined since 1995, thanks to integrated pest management (IPM) tactics that include using insect growth regulators and parasite releases. Kirk and Guy Mercadier contributed further to the $1 billion pest's decline by finding 36 parasite species in explorations to 31 countries, including Spain, the United Arab Emirates, and Pakistan, from 1991 to 1997.

SCOTT BAUER (K9328-1)

Entomologist Franck Hérard inspects gypsy moth larvae raised on an artificial diet. Larvae will be placed on oak trees to trap specific fly parasites, which will be sent to U.S. cooperators.

Currently, EBCL's top weed priorities include yellow starthistle, Russian knapweed, saltcedar, Russian thistle, leafy spurge, hoary cress, perennial pepperweed, spotted knapweed, medusahead ryegrass, and rush skeleton weed.

The insect "hit-list" includes diamondback moth, pink hibiscus mealybug, wheat stem sawfly, gypsy moth, codling moth, apple leafrollers, olive fruit fly, grasshopper, locust, termites, Asian longhorned beetle, and *Lygus* bug.

Codling Moth and Leafroller

Kim Chen, a senior support scientist, is taking aim at codling moths and several leafroller species. These Eurasian insects are apple and pear pests in the northwestern United States. Chen's 1999 and 2000 surveys of three orchards in southern, central, and northern France revealed 65 parasite species, including braconid wasps and tachinid flies, that attack the pests.

One pest fighter is *Colpoclypeus florus,* a eulophid wasp that Chen and technician Jaime Lopez are rearing from collections made in French apple orchards. They will ship *C. florus* this spring to entomologist Tom Unruh, an ARS cooperator in Wapato, Washington, who wants to release a warm-climate strain of the wasp species. He will also receive a braconid wasp that parasitizes leafrollers. Unruh notes that previously released parasites of codling moth in Washington State have already become established.

Lygus Bug, Wheat Stem Sawflies, and Olive Fruit Flies

In addition to the *Lygus* bug, which damages alfalfa, cotton, strawberry, and many other crops, the scientists at EBCL are rearing parasites collected from wheat stem sawflies and olive fruit flies. Kim Hoelmer, entomologist, and Dominique Coutinot, senior support staff member, lead this project.

In Montana and North and South Dakota, sawflies cause roughly $100 million annually in yield losses. Female flies lay their eggs inside the wheat plant's stem, and emerging larvae eat their way down to the base of the stalk and excavate it. Weakened plants may topple over so they can't be harvested.

According to entomologist Tom Shanower, an ARS cooperator in Sidney, Montana, biocontrol is especially appealing against the sawfly because insecticides are too costly to use in wheat, and resistant wheats, called solid stem varieties, currently offer poor yields.

Hymenopterous wasps from Eurasia top the list of biocontrol candidates. Hoelmer's most recent search for them was in Uzbekistan. "This collection is now overwintering," he reports. "The sawflies and any parasitoids they harbor will emerge this spring."

Last fall, he and Coutinot went to Tunisia to find olive fruitfly parasites, of interest to Charles Pickett, a

California Department of Food and Agriculture (CDFA) cooperator. As the fly's population soars, Pickett sees an emerging threat to California's $33.9 million olive industry that will require biocontrol and other IPM tactics to minimize pesticide costs and environmental harm.

Before the fly's arrival, "California olive growers never had to worry about something getting into the fruit," says Pickett, who hopes to import new parasites in addition to one he's already test-released—*Psyttalia concolor.* Hoelmer says, "We found the flies at two different sites and made nice collections, from which we've already gotten two different parasitoids."

SCOTT BAUER (K9324-1)

A state-of-the-art quarantine facility is located next to the European Biological Control Laboratory. Dominique Coutinot, quarantine officer and support scientist, manages the facility under French Ministry of Agriculture rules.

Asian Longhorned Beetles

Franck Hérard, an entomologist, will be exploring Europe to identify natural enemies of cerambycid beetles. He hopes these enemies will be useful biocontrols for battling Asian longhorned beetles. Hérard is working in cooperation with Michael Smith, an entomologist with the ARS Beneficial Insects Introduction Research Laboratory (BIIRL), in Newark, Delaware.

The Asian longhorned beetle made its U.S. debut in New York's Central Park in 1996 and turned up 2 years later in Chicago's Lincoln Park. Between the two cities, 6,400 trees have since been removed, notes Smith. Unchecked, the pest poses a $3.8 billion threat to North America's hardwood and ornamental trees, according to USDA's Animal and Plant Health Inspection Service (APHIS).

While Hérard scours Europe for promising new associations involving cerambycids and parasitoids, Smith's search for natural enemies will focus on the beetle's closest U.S. relatives. Chinese cooperators, meanwhile, will search for enemies that co-evolved with the pest in its native Asia.

Leafy Spurge, Russian Thistle, and Spotted Knapweed

The efforts of Rouhollah Sobhian, now retired from EBCL's biocontrol research team, included biocontrol agents for leafy spurge and Russian thistle (also known as tumbleweed). One agent, a gall midge called *Spurgia capitigena*, will be released by ARS cooperators in Montana against leafy spurge. Another promising biocontrol is *Gymnancylie canella*, a moth that in the caterpillar stage eats tumbleweed shoots and seeds.

Although an icon of the American West, tumbleweed is an invasive species some landowners consider a rangeland pest because of its prodigious seed production, flammability, wind-driven tumbling, and thorny stems. Says Sobhian, whose tumbleweed work is funded by CDFA, "It blows against fences, blocks waterways, and is said to cause accidents on highways."

SCOTT BAUER (K9339-1)

Plant pathologist Tim Widmer sprays a fungus isolated from yellow starthistle on a seedling of the same plant species. The fungus is harmless to humans.

Sobhian says more studies are needed on *G. canella's* overwintering habitat and host specificity before its value as a biocontrol can be fully ascertained.

Sobhian's successor, René Sforza, is renewing a spotted knapweed project in which he'll conduct explorations for biocontrols in Ukraine and Russia—areas formerly restricted during the Cold War. Sforza is also starting a new medusahead ryegrass project.

Saltcedar, Yellow Starthistle, and Other Invasive Weeds

Besides studying giant reed, plant pathologist Tim Widmer and technician Fatiha Guermache are looking for pathogens that cause root and foliar diseases on saltcedar, yellow starthistle, and other invasive weeds. "A plant pathogen," Widmer explains, "is most commonly a fungus, bacterium, virus, nematode, or an abiotic factor, such as ozone or pollution, that continuously disrupts the plant's normal processes."

At CDFA, Widmer's cooperators are interested in pathogens as an alternative to controlling yellow starthistle with herbicides. First detected in the mid-1800s, the thistle now infests 10 million acres in California. Since spreading to other western states, it has become a pest of rangelands, prairies, vineyards, roadsides, and natural habitats. Uncontested, it displaces native grasses and plants that sustain livestock and wildlife. Widmer is currently testing more than 20 fungal strains and 45 bacteria isolated from thistle specimens and soils he collected in France earlier this year.

Pink Hybiscus Mealybug

Entomologist John Goolsby (of the ARS Australian Biological Control Laboratory) and Kirk are exploring Australia, Egypt, Hong Kong, Macau, and other places to collect natural enemies of pink hibiscus mealybug, a soft-bodied pest that attacks 200 different plants.

Because of its broad appetite and insecticide-blocking waxy coat, the bug's 1999 U.S. arrival in California caused much alarm. APHIS cooperators led by Dale Meyerdirk countered with timely parasite releases, including *Anagyrus kamali* wasps from the scientists' Australian collections. This action helped curb mealybug numbers by 98 percent, staving off millions of dollars in losses.

Genes Identify Good Inside the Bad

Molecular biologist Marie-Claude Bon and senior support scientist Nathalie Ramualde are working on genetic characterizations of biocontrol agents and their hosts. One objective is to customize biocontrol strategies based on genetic information about target pests and their natural enemies. They've also begun developing molecular diagnostic tools to detect and monitor parasitism and differentiate among parasites, including *Peristenus* wasps—some species of which have already been introduced into the United States and Canada for *Lygus* bug control.

SCOTT BAUER (K9342-2)

Technician Nicolas Crespy collects diamondback moth larvae from the undersides of wild cabbage leaves.

"We're developing a tool to detect the presence of parasitoid DNA extracted from the host nymphs," says Bon. They use genetic fingerprinting technologies similar to those used in crime labs, such as the polymerase chain reaction.

Formulating Hardy Fungi

Insect pathologist Guy Mercadier and Quimby, along with technician Nicolas Crespy, are testing new ways to formulate fungi into biopesticide products. They have a handy source of microbes to choose from. Since about 1988, Mercadier has amassed 1,000-plus strains of *Beauveria*, *Metarhizium*, *Paecilomyces*, and other fungi isolated from silverleaf whiteflies, diamondback moths, codling moths, African locusts, coffee berry borers, and other insect pests.

The researchers also developed a procedure to make *M. anisopliae* more resistant to drying. In arid regions like Africa's Sub-Sahara, for example, the sun can prevent this fungus from germinating inside its insect hosts. Called the "stabileze" method, their approach calls for shocking the fungus's spores with a dose of sucrose and ethanol, which removes up to 80 percent of cellular water.

"If we devise a good method of formulating a fungal pathogen that's resistant to UV light, high temperature, and degradation in storage," says Quimby, "this technology can probably be used with other fungi against other target insects in the United States."

Passing the Test

Once a parasite, predator, or pathogen has been deemed a worthwhile biocontrol candidate, EBCL scientists carefully package it for quarantined shipment to one of three ARS locations stateside.

The first stop for insects and insect pathogens is either the BIIRL or ARS' Stoneville (Mississippi) Research Quarantine Facility. Weed pathogens go to ARS' Foreign Diseases-Exotic Weed Science Unit, in Fort Detrick, Maryland.

New arrivals must pass a stringent inspection before they're sent to EBCL's cooperators, which include other ARS labs, state agriculture departments, other USDA agencies (including the Forest Service) and non-USDA agencies in the Department of the Interior. The list includes state and land-grant universities, with cooperators in Oregon, California, North Dakota, Montana, Wyoming, Colorado, Illinois, and Kentucky.—By **Jan Suszkiw,** ARS.

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