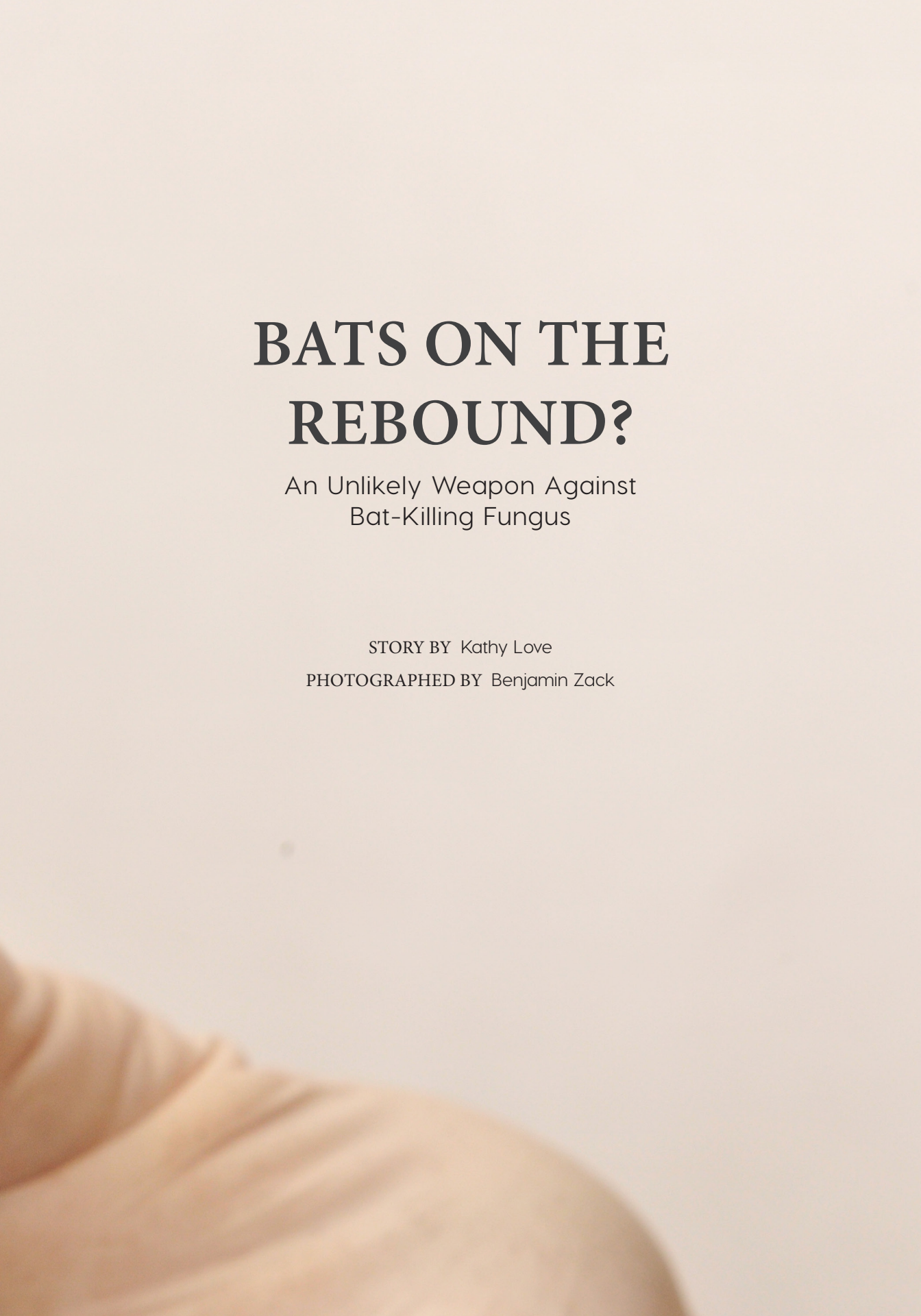
BATS ON THE REBOUND?

An Unlikely Weapon Against Bat-Killing Fungus

STORY BY Kathy Love

PHOTOGRAPHED BY Benjamin Zack



As the sun goes down, Clarissa Starbuck, left, and Sybill Amelon set up a trap site along the Blue Ridge Parkway in North Carolina during the SBDN Bio Blitz. For one weekend a year, biologists from around the south gather for a marathon of intensive bat capturing.

Anna Zack modifies an iButton sensor for use on a bat in the Forest Service "bat lab" at the University of Missouri. The small sensor will be attached to a bat and used to monitor their body temperature while they are studied in an artificial hibernaculum.

Sybill Amelon, a wildlife research biologist with the US Forest Service, handles an Indiana bat in her lab in Columbia, Missouri. Indiana bats are one of several endangered bat species throughout the United States.

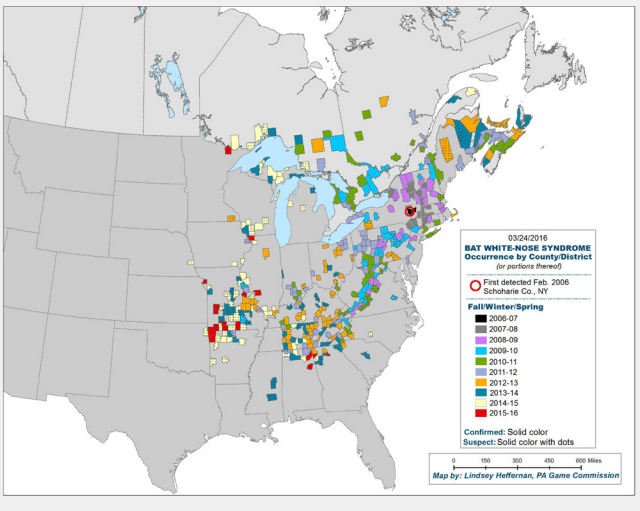
Ten years ago scientists in New York waded knee-deep in dead bats. They noted that the bats – thousands of them – were marked with white noses, leading to a name for the bats' mysterious killer: White-Nose Syndrome (WNS). Since then, 6 million bats have died from the fungus, which starts as tiny white tentacles that infect the bats' tissue. Eighty percent of the bat population in the northeastern U.S. has been wiped out.

The disease is steadily marching west. By 2010, scientists had confirmed the fungus in Indiana, Kentucky and Tennessee. In 2011, the disease appeared in Missouri. By 2013, it reached Arkansas. Suspected cases have emerged among populations in eastern Nebraska and Oklahoma as recently as last year. Now, a multi-state team of scientists is testing a treatment, and it starts from an unlikely place: the grocery store.

Chris Cornelison was a doctoral student at Georgia State University, researching how to discourage mold and delay ripening of fruits and vegetables. "We mixed cells of the naturally occurring bacterium *Rhodococcus rhodochrous* in wax and sprayed it on the interior of boxes of peaches where it prevented the growth of fungus and delayed ripening," Cornelison says. "I was intrigued by the possibility the bacterium could also combat another fungus – the one responsible for WNS."

He appealed for funding from federal resource agencies without success. "Produce – fruits and vegetables – are where the research money is," he says. In 2011, Bat Conservation International stepped forward with $30,000 for Cornelison to work on a cure for WNS. "If BCI had not stepped up in response to our grant request, I'd still be working on peaches," he says.

When the initial funding ran out, Cornelison and a research scientist with the U.S. Forest Service, Sybill Amelon, co-wrote a grant that resulted in $256,000 from the Forest Service to continue the research. Cornelison calls Amelon one of the "leading bat ecologists



Bat White-Nose Syndrome Occurrence by County as of March 24, 2016. White-nose syndrome has since been confirmed in Washington state and documented for the first time in Rhode Island. Map by LINDSEY HEFFERNAN, PA GAME COMMIISSION

"If BCI had not stepped up in response to our grant request, I'd still be working on peaches."

in the country." For the next two years, the two scientists and their colleagues worked on the challenges posed by WNS: how to treat thousands of animals without introducing organisms that would disrupt fragile cave ecosystems.

The non-human hero of their research, the bacterium *Rhodococcus rhodochrous*, evolved traits over eons to prevent the growth of its competitors, fungi that share many characteristics with the cold-loving fungus that causes WNS, *Pseudogymnoascus destructans* (or simply "pd"). While the fungus doesn't kill bats outright, it disrupts their hibernation. Infected bats leave their caves prematurely, wasting precious energy and body fat. The researchers decided a Bacterium v. Fungus match was in order. "The *Rhodococcus* bacterium exists in soils all over the world," Cornelison says. "We were basically just leveraging the ability of this naturally evolved antagonist to combat the fungus."

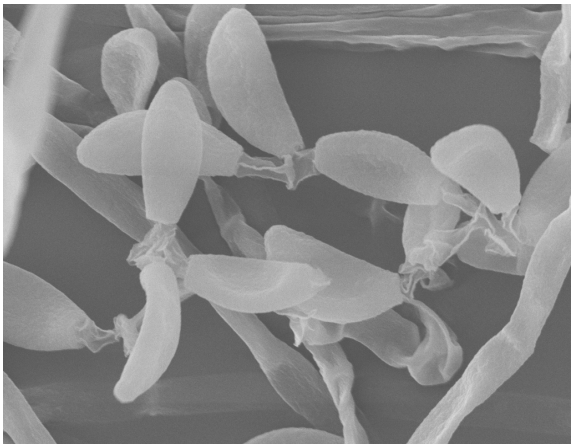
The first experiments used tissue from dead bats infected with WNS. *Rhodococcus* killed the fungus on every tissue sample treated with the bacterium. To experiment with live bats, Amelon had to obtain permits from state and federal regulators. She collected infected bats from the Mark Twain Cave complex near Hannibal and other sites around northeast Missouri, then housed them in an animal lab at the University of Missouri-Columbia.

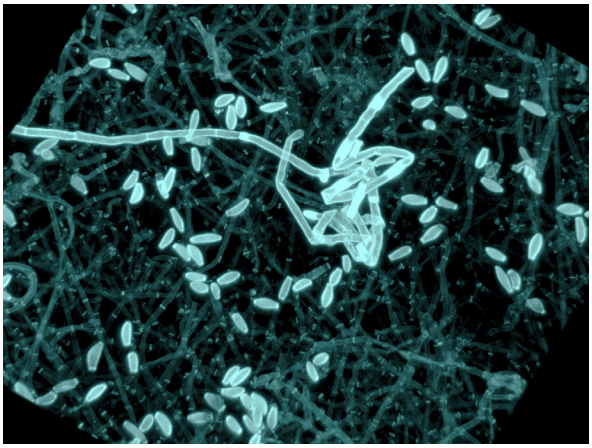
In February 2014, the scientists were ready for controlled experiments using live bats. Those that tested positive for the presence of pd were placed in specially designed coolers for 72 hours, with the fermented *Rhodococcus* bacterium in Petri dishes on the floor. Amelon then transferred the bats to custom refrigerators for several months, where she periodically swabbed them to test for pd. More than half of the infected bats survived and showed no sign of the fungus.

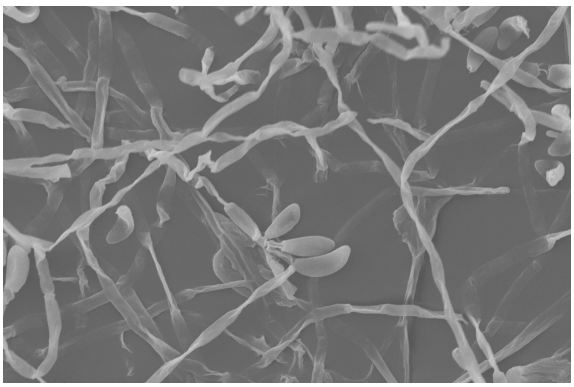
Amelon, watched by her research colleagues and the media, released dozens of healthy but formerly infected bats at the Mark Twain Cave complex May 19, 2015. The bat community was thrilled at the news that a possible treatment for WNS was successful. An article in the *Columbia Missourian* called the experiment "one of the most promising efforts yet." The *Hannibal Courier* described treated bats taking a "victory lap" before disappearing into the forest.

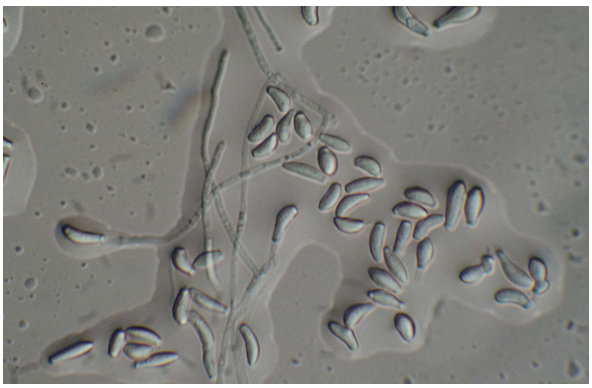
An important next step, said Cornelison, is to find a way to treat large bat populations in their sensitive cave habitats. One scenario might involve using a backpack sprayer that emits chemicals produced by the *Rhodococcus* bacterium. Other researchers are experimenting with swabbing bacteria directly on the wings of bats.

"Our objective is not to get rid of white nose, and we don't anticipate that we'll be able to treat every bat," Amelon says. "Our objective is to increase bat survival to allow them to co-evolve with the disease."









"All images are of P. destructans grown on solid media in the lab. The top left image is of P. destructans conidia acquired using a scanning electron microscope. The top right Is 3D projection of P. desctuctans *mycelia and spores taken using a laser scanning confocal microscope. The bottom left is mycelia and conidia of* P. destructans *taken using a scanning electron microscope. The bottom right image is* P. destructans *conidia taken using differential interference contrast wide field microscopy." JOHN NEVILLE*

Alien Invaders

The fungus that causes White Nose Syndrome is one of many fungi that are seriously threatening species worldwide. A fungus is believed to be responsible for Colony Collapse Disorder that is threatening honeybees. Fungi are thought to cause deformities in several species of reptiles and amphibians, including the endangered Ozark hellbender, found only in Missouri. In the world's oceans, a fungus is attacking and killing coral on a huge scale.

Cornelison says several culprits could be responsible for the damage wrought by fungal attacks. Climate change could improve their conditions for growth. Pesticide use could be responsible for weakening the immune systems of species such as bees. But Cornelison believes globalization bears a huge responsibility for the spread of fungi to locations where they have no natural enemies.

"Materials move around the world now in ways that were impossible or rare before," he said. "(White Nose Syndrome) originated in Europe, for example. Fungi can survive as dormant spores on boots or clothing. Someone enters a cave, picks up the spores on his boots, and travels to North America unaware that he is introducing a non-native microbiological organism that can have enormous consequences on the ecosystem."