

Science

FINDINGS

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"Science affects the way we think together."

Lewis Thomas

Northwest Forest Plants Defeat Pests and Diseases!



Ellen Goheen

Volunteers from the Oregon Youth Conservation Corps spread western redcedar heartwood chips on a trail in the Rogue River-Siskiyou National Forest. A case study indicates that a chemical compound in the redcedar chips may reduce the invasive plant pathogen that causes sudden oak death and can be spread by hikers who have walked through an infected area.

*"All our wisdom is stored
in the trees."*

—Santosh Kalwar, poet

In the early 1990s, Rick Kelsey, a research forester with the Pacific Northwest (PNW) Research Station, was busy investigating whether extracts from various native or introduced plants could repel forest insects or deer from feeding. When Kelsey met Joe Karchesy, a professor of wood

chemistry at Oregon State University (OSU), they realized they were doing similar work. That was just the beginning.

Karchesy was collecting plants in diverse landscapes—from temperate rainforests to semiarid shrub-steppes—and preparing extracts for testing. Both scientists were keen on finding natural alternatives to modern drugs or pesticides, many of which are made from petrochemicals and often have undesirable side effects.

IN SUMMARY

Societies use biologically active chemicals as medicines and pesticides to protect human and agricultural health. But widespread use of synthetic compounds raises concerns about their safety, and resistance development in targeted pests.

To find safer alternatives, scientists turned to native plants and trees in Pacific Northwest forests using multiyear exploratory surveys. Researchers collected samples from different plant and tree parts for extraction and toxicity testing with a simple brine shrimp bioassay. These bioassays detected strong biological activity in heartwood extracts from four cedar and one juniper species.

In followup experiments, researchers discovered that heartwood extracts from yellow-cedar (*Callitropsis nootkatensis*), incense cedar (*Calocedrus decurrens*), Port Orford cedar (*Chamaecyparis lawsoniana*), and western juniper (*Juniperus occidentalis*) had strong toxicity or repellent activity toward mosquitoes, ticks, or fleas. A patent was issued for compounds with activity toward these pests. It has been licensed to a company interested in developing them into commercial products.

In other experiments, researchers found extracts and compounds from cedar heartwood possess strong antimicrobial activity against *Phytophthora ramorum*, the pathogen causing sudden oak death. To mitigate movement of *P. ramorum* spores by hikers and cyclists, forest health specialists have used western redcedar (*Thuja plicata*) heartwood chips on a popular hiking trail on the Rogue River-Siskiyou National Forest, where retreatment continues to show promising results.

Most native plants and trees defend themselves from diseases and insects by producing their own protective substances. Tree chemists like Kelsey and Karchesy call these “bioactive” chemical compounds, because these substances have effects on living organisms, tissues, or cells. The researchers were eager to discover native plants in Northwest forests that could yield new bioactive compounds that would protect humans, animals, trees, or agricultural products.

Kelsey and Karchesy both viewed the forest as a vast library. Karchesy would say, “Each tree is like a book. If you bother to read that book and do the research, you might find something of value.” Karchesy had been reading his way through the forests and rangelands of western and central Oregon and western Washington. He had also been collecting plant and tree tissues.

“I was curious,” he says. “People around the world use various extracts of forest plants for medicine. We’d sample the heartwood, the sapwood, the inner bark, the outer bark, the needles; it was all very exploratory.” Kelsey’s extraction procedure was similar to Karchesy’s, so they decided to collaborate and combine their collection of plant specimens.

The researchers, along with Karchesy’s students and postdocs, spent many hours preparing extracts and isolating chemical compounds from hundreds of specimens. Karchesy’s daughter Yvette performed the bioassays—short for biological assessment—where brine shrimp were exposed to the

KEY FINDINGS	
• Strong biological activity was detected in extracts from 13 forest plant species and moderate activity from another 27 species from a total of 128 tested.	
• Heartwood extracts from four species of cedar and one juniper species showed strong biological activity, indicating the compounds within the extract might be useful to humans, perhaps as medicine or pesticides. These results led to more thorough follow-up studies.	
• Extracts of cedar or juniper heartwood, and individual compounds in the heartwoods have strong antimicrobial activity against <i>Phytophthora ramorum</i> , the microbe causing sudden oak death.	
• Compounds from the heartwood of yellow-, incense, and Port Orford cedars, and western juniper also demonstrated strong toxicity or repellent activity toward mosquitoes, ticks, or fleas that affect animal and human health. Some have effectiveness comparable to conventional pesticides.	

extracts, like miners using canaries in coal mines to detect lethal gases. “You’re using this live organism as a test,” Karchesy says. “It either kills it or it doesn’t.” If it did, that meant the extract contained bioactive compounds.

Kelsey and Karchesy’s forest surveys and bioassays covered 128 species of plants and trees. Of these, they detected strong bioactive compounds from 13 species and moderate bioactive compounds from 27. The researchers detected strong biological activity in heartwood extracts from four species of cedar and one juniper species. When these initial bioassays were completed, the information was not

immediately published, instead it was used as a guide for further experiments. Discovery of bioactivity toward brine shrimp was just the beginning. Further bioassays of the extracts, and compounds in the extracts, were needed with other organisms. But where to start? It was not an easy, straightforward task.

“But, there was a high probability it would have activity in some other systems, and by that we mean potentially insects, pathogens, or other animals,” Karchesy says. “It was somehow impacting the shrimp’s metabolic processes and subsequently it likely would have some activity against other organisms.”

Purpose of PNW Science Findings

To provide scientific information to people who make and influence decisions about managing land.

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Rhonda Mazza, editor; rmazza@fs.fed.us
Cheryl Jennings, layout; cjennings@fs.fed.us

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Species and their tissues with strong toxicity in the brine shrimp bioassay, listed in descending order of activity

Species	Common name	Active tissue
<i>Angelica arguta</i>	Sharptooth angelica	Roots
<i>Callitropsis nootkatensis</i>	Yellow-cedar	Inner bark, foliage, heartwood
<i>Leucanthemum vulgare</i>	Oxeye daisy	Aerial tissue
<i>Oplopanax horridum</i>	Devil’s club	Root and stem barks
<i>Chamaecyparis lawsoniana</i>	Port Orford cedar	Heartwood

Pitting Extracts Against Pests

Karchesy recalls, “When the survey began there was interest in Lyme disease, a tick-vectored pathogen, and West Nile virus, a mosquito-borne pathogen.” Karchesy also recalls that during his surveys, he met American Indians carving canoes out of western redcedar (*Thuja plicata*) trees and saving the shavings to use as flea control in their dog beds. So, he contacted the Center for Disease Control (CDC) in Fort Collins, Colorado, and told them about his bioassays. They were very

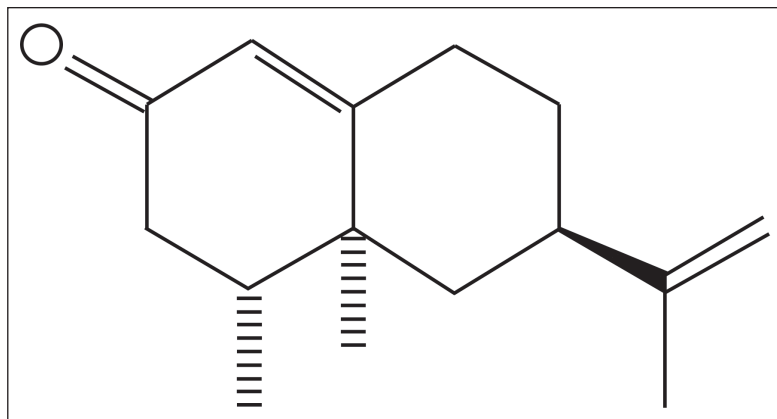
interested in testing the cedar and juniper heartwood extracts on some pests.

Karchesy sent the CDC some extracts and they got back to him, quite excited. They said, “Wow, these extracts are very active. Send more! Isolate the compounds!” he recalls. He ended up collaborating with CDC researchers Nicholas Panella, Marc Dolan, and others on bioassays that pitted extracts from yellow-cedar (*Callitropsis nootkatensis*) with deer



Joe Karchesy

The stump of a yellow-cedar tree showing the yellow heartwood that contains the bioactive compounds. Once beneficial compounds have been identified, they often can be created in a laboratory, eliminating the need to obtain them from trees.



The structure of nootkatone, one of the bioactive sesquiterpene (15 carbons) compounds found in yellow-cedar heartwood, also occurs in grapefruit. Researchers found it repels ticks, and when combined with another heartwood compound, carvacrol, suppressed growth of mosquito and tick larvae.

ticks, known carriers of the bacteria that cause Lyme disease. The researchers found that heartwood components nootkatone and carvacrol repelled ticks at relatively low concentrations and were potentially natural and safer alternatives to synthetic repellents, such as DEET. In a later study, the researchers also found that these two compounds suppressed the growth of mosquito and tick larvae when sprayed on a forest floor.

In yet another study, Karchesy extracted essential oils from the heartwood of incense cedar (*Calocedrus decurrens*), Port Orford cedar (*Chamaecyparis lawsoniana*), and western juniper (*Juniperus occidentalis*). The CDC researchers tested these on ticks, mosquitos, and fleas. These bioassays revealed that incense cedar was the most toxic to all three species, followed by western juniper and Port Orford cedar. They also found that mosquitos were more susceptible to the extracts than ticks or fleas.

At about this same time, Kelsey, Karchesy, and Paul Hennon, a pathologist with the PNW Research Station, conducted a study showing yellow-cedar heartwood chemicals played a major role in preventing snag decay, allowing them to remain standing for 80 years or more at sites experiencing yellow-cedar decline. So, when Kelsey started a new project on sudden oak death, both he and Karchesy knew they had to test some of the most active cedar compounds detected in the brine shrimp bioassays. They collaborated with Daniel Manter, then Karchesy's postdoc at OSU, to pit cedar heartwood extracts against *Phytophthora ramorum*, the microbe that causes sudden oak death.

"And just like that, the research expanded in a new direction," Karchesy says.

Combating a Cloud-Riding Forest Pathogen

Sudden oak death is a tree disease that has affected tanoak trees and several oak tree species on the U.S. West Coast. Since the mid-1990s, the disease has killed about a million trees in California. It affects mainly tanoaks in Oregon, but also causes twig and leaf disease in over 120 species of trees and plants.

"The disease is caused by a water mold," says Ellen Goheen, a forest pathologist with the Forest Service Pacific Northwest Region who leads the management of sudden oak death in Oregon. "*Phytophthora ramorum* is

an aboveground pathogen that moves through the air and thrives in moist environments," she adds. "The spores move in clouds, in water, and in fog. Then they land on leaves. And if there's still a film of water on the leaves, they germinate and penetrate the leaves and the petioles of the smaller stems."

The disease causes cankers or dead tissue to form on leaves, twigs, and stems. It affects more of the tree, until it girdles the trunk and blocks the transport of water and nutrients from the roots up to the leaves.



Oregon Department of Forestry/Flickr

An aerial view of tanoak trees killed by sudden oak death in southern Oregon.

Kelsey, Karchesy, and Manter exposed spores of *P. ramorum* to essential oil extracts from the heartwood of incense, yellow-, and Port Orford cedars; shavings from yellow-cedar heartwood; and four individual compounds from yellow-cedar heartwood. The bioassays showed that these samples stopped cell growth and destroyed the spores.

“They actually caused the membranes of these spores to disintegrate,” Kelsey says. “That was certainly a big surprise to us and we thought it was pretty novel.”

The researchers also noted that yellow-cedar heartwood shavings had potential to prevent the disease’s spread. Shavings used in this experiment had been stored 10 years in a lab, but they performed as well as the individual compounds extracted from heartwood. Fresh shavings could be even more effective as they contain three to seven times the amount of bioactive compounds measured in test samples. In another experiment, the researchers found heartwood extracts from western redcedar also destroyed *P. ramorum* spores. They concluded that spreading shavings in areas near infected trees and where spores might be difficult to control, such as trails and parking lots used by hikers and cyclists, could be useful in minimizing the disease spread.

In August 2009, survey crews found an infected and dead tanoak near a popular hiking trail in the Rogue River-Siskiyou National Forest. The trail was immediately closed to the public. Forest Health Protection applied herbicide, and cut and burned other trees within a 300-foot radius of the dead tree.

“Closing the trail was a real hardship for folks who hiked that trail a lot,” Goheen says. “It’s a popular trail because it goes through an old growth redwood stand and connects to a popular state campground.”

P. ramorum has been known to persist in soils after treatment and to Goheen and her colleagues, closing the trail was not an option. Goheen decided it was a good time to test redcedar heartwood chips on trails within and around the treatment area. “With this invasive pathogen, it’s not business as usual,” she says. “We’re trying hard to not purposefully spread it through human feet.” Youth Corps volunteers spread western redcedar heartwood chips on 200 feet of trails, from the trailhead to center of the treated area. The trail was opened after the chip treatment.



Sharptooth angelica (*Angelica arguta*).

Richard Droker/Flickr



Yellow-cedar (*Callitropsis nootkatensis*).

USDA Forest Service



Devil's club (*Oplopanax horridum*).

Sharon Cooper/Flickr



Oxeye daisy (*Leucanthemum vulgare*).

Manuel/Flickr

These species demonstrated strong toxicity in the brine shrimp bioassay—the first step in a discovery process that may lead to new remedies for treating pests and disease.

Goheen tested the soil a year after and found the *P. ramorum* presence had been reduced. Succeeding soil tests revealed less of the pathogen. “I couldn’t recover the pathogen from the soil a couple of years after the treatment,” Goheen says. “We’ve put down some more chips since then to freshen up the first application.”

As a caveat, Goheen adds that it is hard to know whether it was the chemistry of the chips or that there was enough time for the pathogen to die out. “This was a case study, not a controlled experiment,” she says. “But we’re committed to keeping the chips on the trail, and I would recommend it for other recreation sites.”

Information From the Forest

“All these positive results were very encouraging, and they helped to keep us interested” Karchesy says. Karchesy and his CDC colleagues received a patent to use a group of related compounds, including those in cedar heartwood, as toxins or repellents against arthropods that carry diseases, such as Lyme disease and the Zika virus. The patent has been licensed to a company interested in developing commercial products containing these compounds.

“When it comes to ticks and mosquitos, nootkatone has an activity that’s almost comparable to DEET,” Kelsey says.

All this would not have been possible without the original forest surveys and bioassay results that served as a guide to direct further work. Kelsey, Karchesy, and their colleagues recently published these initial results. The challenge now is for researchers to identify more abundant sources for the bioactive compounds they found in cedar heartwood.

Karchesy says that sometimes he gets questions such as, “Does that mean you want to cut down hundreds of cedar trees just to get heartwood extracts?” His reply? “No, that is unlikely to happen.” He adds, “The trees in the forest have provided the original information, but the same compounds can often be obtained from synthesis or other natural sources. A compound’s activity should be the same whether it is extracted from natural sources or generated by synthesis.”

Kelsey echoes this point.

“You may get the original information from a tree, in other words, the book that you read so to speak,” he says. “But it’s not necessarily where it will be sourced from at the end. For example, nootkatone for some bizarre reason, can be found in grapefruit. When you have something that might have commercial potential, the first thing you do is look for the most abundant and economic source of that material and then attempt production from it.”

“I am happy it all worked,” Karchesy says. “It’s exciting to get positive results and to isolate and identify compounds that I hope will eventually be used, and might even save someone’s life.”

“To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science.”

—Albert Einstein



LAND MANAGEMENT IMPLICATIONS



- Managing forest plant diversity and assisting the survival and sustainability of rare or endangered plants help protect against the loss of important, biologically active natural compounds which have yet to be discovered.
- Trees produce bioactive compounds to protect themselves from enemies. Trees with stronger chemical defenses may be developed through selective breeding or molecular enhancements, but this depends on knowing which species produce active compounds, their modes of action, and the organisms they protect against.
- A plant’s value to society can be difficult to evaluate based simply on visual characteristics, abundance, or absence of a current use. The cliché, “you can’t judge a book by its cover,” applies to bioactive plants as well.

For Further Reading

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Writer’s Profile

Natasha Vizcarra is a science writer based in Boulder, Colorado. She can be reached through her website at www.natashavizcarra.com.



U.S. Department of Agriculture
Pacific Northwest Research Station
1220 SW Third Avenue
P.O. Box 3890
Portland, OR 97208-3890

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Scientist Profiles



RICK KELSEY is a recently retired research forester with the Pacific Northwest Research Station. His research focuses on understanding the complex interactions between trees and the biotic (insects, pathogens) and abiotic (fire, drought, etc.) agents that cause them stress.

His other research is directed toward discovering compounds with biological activity in tree tissues and understanding their ecological importance. Kelsey received his Ph.D. in forestry from the University of Montana.

Kelsey can be reached at:

USDA Forest Service
Pacific Northwest Research Station
Forestry Science Laboratory
3200 SW Jefferson Way
Corvallis, OR 97331

E-mail: rkelsey@fs.fed.us
Phone: (541) 750-7368



JOE KARCHESY is professor emeritus at the College of Forestry in Oregon State University (OSU). His research focuses on natural products chemistry and use of forest plants. Karchesy received his Ph.D. in forest products chemistry from OSU.

Karchesy can be reached at:

College of Forestry
Oregon State University
Corvallis, OR 97331

E-mail: joe.karchesy@oregonstate.edu

Collaborators

N.A. Panella and M.C. Dolan, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Fort Collins, CO

D.K. Manter, USDA Agricultural Research Service, Fort Collins, CO