Partners in Crime

by Jennifer Frazer

Mountain Pine Beetles use fungi to help them kill the west's pine forests in what may be the biggest insect infestation in North American history. But to what extent are the fungi to blame?

Most westerners know all too well that in pine forests from New Mexico to British Columbia, little black beetles are chewing a path of destruction, leaving a reddening, toppling wasteland in their wake. But few realize they're not doing it alone. The beetles are packing fungi to help them finish the job.

Now scientists studying these fungi – which are distant relatives of the Dutch Elm Disease pathogen – are learning that, though essential in some surprising ways, the fungi may not be as actively lethal to trees as once thought. Instead, it's possible the insect – a native called the mountain pine beetle – carries several fungal species as essential supplements that help them make the most of their food under a variety of environmental conditions.

"There's an old kind of myth that bark beetles use fungi to kill trees," said Diana Six, a professor of forest entomology and pathology at the University of Montana. "Some are pathogenic, meaning they can grow into still living tissues of trees, but they really can't kill the tree. We've found these fungi act as a nutritional supplement the beetles carry around."

Beetles, Beetles Everywhere

Millions of acres of lodgepole and related pines have succumbed to mountain pine beetle across the West in the last decade. The epidemic is considered by many to be the largest known insect infestation in North American history. The beetle has spread wildly for many reasons, including drought; historic clear cutting and fire suppression policies that led to large stands of similarly aged, same-species pines; and warmer winters that are killing fewer overwintering beetles. In the last few years, the beetle crossed to the east side of the Rockies in Canada, sparking fears it could spread out of its traditional habitat across the great boreal forest to the eastern half of North America.

Bark beetles have made a business out of cultivating and transporting fungi from tree to tree. They infect the tree in the process of boring in and laying their eggs in galleries under the bark. When this happens, special spore-storing pouches on their mouthparts called mycangia loose the fungi on the tree.

By the time the eggs hatch, the fungi are making themselves at home, growing into the thin sub-bark tissue called phloem, the tree's sugar and nutrient transport system. The fungal filaments contain melanin – the same pigment that

An immature adult mountain pine beetle in a pupal chamber lined with conidia (asexual spores) of pine beetle-associated blue-stain fungi. It must feed on these spores in order to reproduce. Photo courtesy of Kathy Bleiker.

darkens human skin. For some reason, said Six, impregnating the cellulose and lignin matrix of wood with melanin makes it look blue. Eventually, the fungi can grow into the rest of the wood as well. Enterprising woodworkers have tried marketing the result as "denim pine" and have created products from decking to caskets out of the naturally stained wood.

Intriguingly, some of the fungi helping the mountain pine beetles have a distant, notorious cousin: Dutch Elm Disease. That may be misleading, though, Six said. Dutch Elm disease, unlike its relatives, is indiscriminate in its choice of beetles, is extremely virulent, and infects hardwoods. Most bark beetle fungi tend to be less virulent and more dependent on specific conifer-inhabiting beetles.

"It's an oddball for that entire group," Six said.

Pine – and Fungi – for Dinner

Scientists have long sought to find out why mountain pine beetles carry fungi, and why they carry more than one kind. Are they feeding on them? Or are they using them to kill the tree – or both?

Though the liquid in phloem is the most nutritionally-rich part of wood and beetle larvae do drink it, Six said, it is actually poor fare for beetles. They would be unlikely to survive without the help of the fungus, which concentrates nitrogen, a nutrient especially important for growing larvae. The fungi also produce chemicals called sterols. These form the backbone of familiar molecules like cholesterol and testosterone in humans, and insects need them to make a hormone of their own called ecdysone that is essential for moulting and making eggs. The beetles have no source of sterol but their fungi.

"We've shown that if they don't feed on these fungi they actually don't reproduce," Six said.

Young larvae eat the filaments, or hyphae, of the fungus to obtain nitrogen. The adults eat spores that blossom from the walls of their pupal chambers to obtain sterols. In the process, some spores passively get packed into their pocket-like mycangia.

When the beetles arrive at the next tree and bore in, the spores are squeezed out and into the tree's tissues, where they get right to work.

But not just any fungal spore will do. By the time bark beetles finish their work, a dying tree is a botanic garden of filamentous fungi and yeasts. But only the spores of the fungi cooperative with bark beetles make it to the next tree. No one is sure how the beetles do this, though antibiotics or spore shape have been suggested.

"It doesn't appear to be size or shape," Six said. "Some fungi have tiny round spores that should be able to fit, but either they don't come in, or if they do, they don't come out."

Evidence of this nutritional dependence has convinced Six that the fungi are not directly contributing to the death of an individual tree. They indirectly contribute, she said, by allowing beetles to exploit their food.

"Without these fungi, mountain pine beetle would not be the killer it is," she said, "but in the actual killing of a tree, the fungi don't play a role because they're not pathogenic enough."

Sepideh Alamouti, a Ph.D. candidate at the University of British Columbia studying the genomics of mountain pine beetle fungi agrees they are not pathogens per se like Dutch Elm Disease. Though scientists who have artificially inserted these fungi into trees have found they are capable of killing them on their own, in the wild, without beetles, they could not kill pines, she said. And the beetle-fungi combination is not lethal under all circumstances. In California, she said, mountain pine beetles and fungi get into pines and coexist happily for many years.

But the question is not clear cut to everyone. Kathy Bleiker, a research scientist in bark beetle ecology at the Canadian Forest Service and one of Six's former students, studied the nutritional benefits of mountain pine beetle fungi for her doctorate. She finds it difficult to say whether the fungi are actively helping kill the tree or not. It depends, she said, on how you define "dead."

"It's really hard to pinpoint when a tree dies," she said. "If you go out and cut a flower and put it in vase of water, is that flower dead? The buds will still open up after it's cut. Sort of the same thing happens with a tree."

Though a tree may be girdled by beetles or fungi, it will continue to transpire and appear alive for a while, she said. Consequently, since the time of death is up for grabs, it is difficult to determine the exact cause. And providing essential nutrients to fungi and helping kill the tree are not mutually exclusive, she pointed out. Some studies have seemed to indicate the fungi do play a role in the decline of the tree, but it is hard to test directly with proper controls, she said.

"I don't see why the fungi couldn't have a dual role," she said. "That's just one of those questions we'll still be talking about at cocktail parties in 10 years."

The More the Merrier

Bark beetles only associate with the asexual forms of the fungi they work with. The fungi make spores called conidia that protrude on long stalks called conidiophores into the pupal chambers of the beetles. These fungi do have sex, though. They make their sexual spores in sacs called asci inside tiny, black, long-necked flasks called pycnidia that exude spores from their tips. But these spores are produced in an entirely different part of the tree and are never carried by beetles, Six said. So why bother?

In an amazing Swiftian twist, the sexual spores may depend on tiny arachnids called mites. These mites live in old beetle galleries where the sexual spores form, feeding on them and packing them into their own mycangia. And the mites, it turns out, use beetles the way hobos use boxcars.

"They will run down the old [egg] gallery out the larval galleries, jump on the beetles, and off they go," Six said. "How they know it's time, I don't know."

Scientists have also puzzled over the number of different fungi mountain

The black, flask-shaped, sexual spore-making structures (pycnidia) of a species of pine beetle-associated blue-stain fungi. The slimy spore masses at their tips are consumed by mites, not pine beetles. Photo courtesy of Diana Six.

pine beetles associate with. There are at least two – *Grosmannia clavigera* and *Ophiostoma montium*. *Grosmannia* makes much better beetle fodder. One might expect that *Grosmannia* would outcompete *Ophiostoma* and drive it out of the system. So how could the association with *Ophiostoma* persist, as it seems to have, for a very long time?

Six's research indicates that *O. montium* is better adapted to growing in trees during cool weather. Thus the fungi avoid competition by growing at different times. That could be a real benefit for a beetle that lives in climes as varied as hot, arid Arizona to cool, snowy Canada. Even within a single tree, one fungus may do better than another at different times of year. This effect is called environmental buffering, Six said, and allows the beetle to exploit a broader habitat than it could with only one partner.

In fact, the beetle may well be even more fungally promiscuous. Alamouti, who is working with the Tria Project, a Canadian effort to study the genomes of beetle, pine, and fungi, has been studying the genes of the fungi the beetles carry. Typically, bark beetles are associated with two to six different fungi, she said. Her study of fungal genes associated with mountain pine beetle indicates that there are at least four species associating with the beetle in epidemic areas in Canada. One is a cryptic sister species of *Grosmannia* called *Leptographium longiclavatum*, and has also proved pathogenic – and blue staining – in pines.

The fourth species, a white fungus in the genus *Ceratocystiopsis*, is not blue staining or pathogenic. A slow-growing fungus compared to the blue-stain fungi, *Ceratocystiopsis* may provide some unique nutritional benefit, but no one knows. The same combination of fungi has been found in other bark beetle systems, Alamouti said, which provides strong evidence that these fungi are indeed working together with pine beetles.

Bleiker wonders if there may well be other species of fungi, yet undiscovered, that associate with pine beetles in isolated populations, something she is studying herself. That could have important consequences for the future of the outbreak. The climate east of the Rockies is quite different from the West, she said. If the beetle loses its fungal partners due to the change, its expansion would halt. But if the beetle could pick up fungi from local beetles, all bets are off.

All this may inspire the question – would it be possible to control the beetle outbreak by controlling the fungi? The answer seems to be a resounding no. Although fungicides exist that can control the fungi, getting them to the fungi has proved a bit of a problem. In addition to being toxic to a wide variety of beneficial fungi naturally found in forests, there is the delicate issue of getting fungicides into trees. Practically speaking, this involves expensive injections, which isn't feasible across millions of acres of forest. And the fungi, it turns out, are quite hard to kill.

"These fungi are much, much tougher than the beetles," Six said. "We've actually tried this fungicide thing, and you just can't do it."

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