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Armies of fighting fungi protect chocolate trees

DURHAM, N.C. -- In discovering that cacao trees are protected by armies of "good" fungi against their "evil" counterparts, biologists have revealed a new and intricate ecological relationship between trees and the ubiquitous fungi that inhabit them.

The researchers believe their discovery represents a significant advance in basic ecology. It also offers the potential for enlisting such fungal armies to protect cacao trees from the pathogens that damage the trees, which are the source of the world's chocolate. Successful cultivation of cacao trees in American rainforests, they said, offers a viable economic alternative to clearing the forests for ranching. Field tests of fungi as biological control agents are already underway, said the researchers.

The biologists published their findings in the Dec. 23, 2003, issue of the Proceedings of the National Academy of Sciences. Lead author on the paper was A. Elizabeth Arnold, a postdoctoral fellow in the Duke University Department of Biology. Other co-authors are Luis Mejía, Damond Kyllo, Enith Rojas, Zuleyka Maynard, Nancy Robbins and Allen Herre of the Smithsonian Tropical Research Institute in Panama. Their research was sponsored by the Research Institute and by grants from the American Cocoa Research Institute, the World Cocoa Foundation, the M&M/Mars Division of Masterfoods, the Andrew Mellon Foundation and the National Science Foundation.

In their study, the scientists concentrated on "endophytes," which are fungi that infect healthy plant tissues without causing disease. Endophytes of woody plants land as airborne spores, burrowing into the plant tissues and living in the spaces between cells -- a process called "horizontal" transmission. According to Arnold, studies on grasses had revealed that fungi passed down from generation to generation -- called vertical transmission -- offered those plants benefits such as resistance to pathogens, drought and herbivores.

"However, the general theory has been that horizontal transmission of a diversity of species into a plant is associated with a pathogenic or parasitic lifestyle," Arnold said. "So, it was expected that these fungi that live inside plant leaves and consume carbon from their host aren't likely to have a beneficial effect on the plants they inhabit."

In their initial field studies, the researchers found that the load of endophyte infection on cacao leaves increases enormously as the leaves age, but that the leaves show no sign of being harmed by these infections. In a survey of endophyte species on cacao trees, they also found evidence that the mix of species varied with spatial location across Panama.

When the researchers studied endophytes infecting three different tree species, they found that the different species were host to overlapping but different arrays of endophyte species. The researchers also found that each tree's preferred endophyte species grew better on nutrient media containing the leaf extracts from those trees, another indication that endophyte species were matched to tree species.

All these findings hinted that endophytes were not just infecting host trees at random, but play a role in the trees' biology, Arnold said.

Such findings prompted the researchers to explore in greater detail the effects of endophytes on disease resistance. In those experiments, they grew cacao seedlings in conditions that prevented infections by endophytes. They next infected some leaves with endophytes alone, some with the major cacao pathogen Phytophthora, and some with both endophytes and Phytophthora.

"We found that Phytophthora successfully infected the leaves that were treated with it, but that in the presence of endophytes, more leaves survived and the ones that survived had less leaf area damaged," said Arnold. Nearly three times more leaves died in the Phytophthora-only infected leaves versus the leaves that were first treated with endophytes, the researchers found. The antipathogen effects were highly localized, and were more important in older leaves that lack the defensive chemicals characteristic of

younger leaves. Such a multifaceted, protective effect of fungal endophytes has never before been demonstrated in angiosperm (broad-leaved) trees, she said. Particularly significant was the pattern of growth of the good and bad fungi in the leaves, Arnold said. "The leaves that had first been infected with endophytes showed just tiny lesions by Phytophthora at the point of entry," said Arnold. "Whereas, in the leaves without endophytes, Phytophthora just ran wild."

According to Arnold, the researchers' findings have led them to launch field trials in Panama, under co-author Herre, in which spores of local endophytes are being grown and sprayed on cacao plants, in an effort to reduce fungal infections. Such production of endophyte spores can be done inexpensively and easily by local farmers or cocoa cooperatives, using only river water and rice, she said.

Arnold emphasized that the initial finding of the role of endophytes in protecting trees constitutes only the barest beginnings of an effort to understand an extremely complex ecological process.

"Our findings indicate that fungal dynamics are really complicated, and that the number of potential interactions -- and their directionality -- is really a big black box," she said. "For example, we see hints that there are direct interactions between endophytes and pathogens, but what those interactions are will require considerable future research," she said.