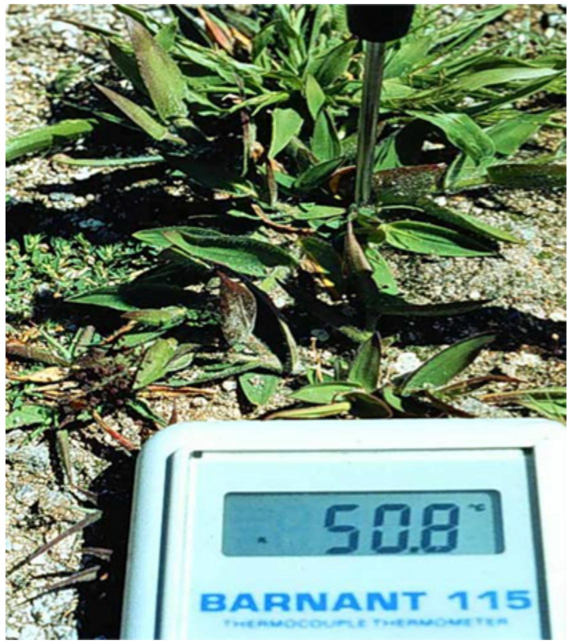
MicroDefender: Curvi (*Curvularia protuberata*)

(Malmberg KEL, Biaggi TM, Rodriguez RJ, Redman RS)



Tropical panic grass ((Dichanthelium lanuginosum) thriving in hot (50^{0}C or 123^{0}F) geothermal soils of Yellowstone National Park

Claim to fame: three-way symbiotic relationship protecting plants against heat stress

Symbiotic relationships that enable partners to thrive in hostile environments. Plants lack locomotion, so if there is something stressful in the environment, they can't just walk away to find a less stressful location. One way plants can adapt to stress is by establishing symbiotic relationships with beneficial microscopic fungi. Plant-fungal relationships are ancient. According to fossil records, this relationship has been around for over 400 million years! It is speculated that the relationship with fungi allowed plant ancestors to move from aquatic environments onto land, where drought stress would be an issue.

When these plants and fungi work together, they have what is called a symbiotic relationship. In this case, it's a mutual benefit because both the plant and the fungus in the partnership can live in more severe climates together than they can apart. As the effects from global climate change increase in severity, the likelihood that plants will encounter stressful situations is imminent, so the significance of this relationship is greater than ever!

Discovering important and useful partnerships. Climate change scientists have a mission to help plants survive extreme temperatures and drought conditions. These scientists decided to see if symbiotic fungi were responsible for native plants surviving in stressful habitats like deserts or geothermal soils. After all, Mother Nature spent millennia developing plants that tolerate stress; no need to reinvent the wheel! The fungi involved in these symbiotic relationships don't look like mushrooms that you see at the grocery store or on a hike. They are teeny tiny, microscopic fungi. These fungi are called *endophytes* because they live inside (endo) the plant (phyte).

Scientists wanted to find some of these endophytes in the wild, so they went to high-stress habitats, including ten geothermal sites in the Lassen Volcanic (LVNP) and Yellowstone National Parks (YNP). Geothermal activity from the depths of the earth was heating up and drying the soil, making these locations extremely stressful for plants. Once there, researchers observed one plant, commonly known as Tropical Panic Grass (*Dichanthelium lanuginosum*) was flourishing, even in the hottest soils. This seemed like a great place to start; perhaps there was an endophyte hidden amongst the leaves and roots?

Curvi: the fungus partner of Tropical Panic Grass. Since endophytes are microscopic and live inside the plant, they are invisible to the naked eye. Researchers had to develop a way to look for them. The best way to do this is to harvest the plants and isolate the fungus from the plant. Scientists can do this by sterilizing the exterior of the plant, cutting the plant open, and putting them onto special fungal growth media plates. Given some time, the nutrients from the media allow the fungus to grow big enough and make special reproductive cells called "spores" that allow scientists to identify the fungal species with a microscope! When they looked at more than 200 tropical panic grass samples from geothermal sites in YNP and LVNP, parks that are more than 1000 miles from each other, they found the same endophyte species (*Curvularia protuberata AKA "Curvi"*) living inside the panic grass.

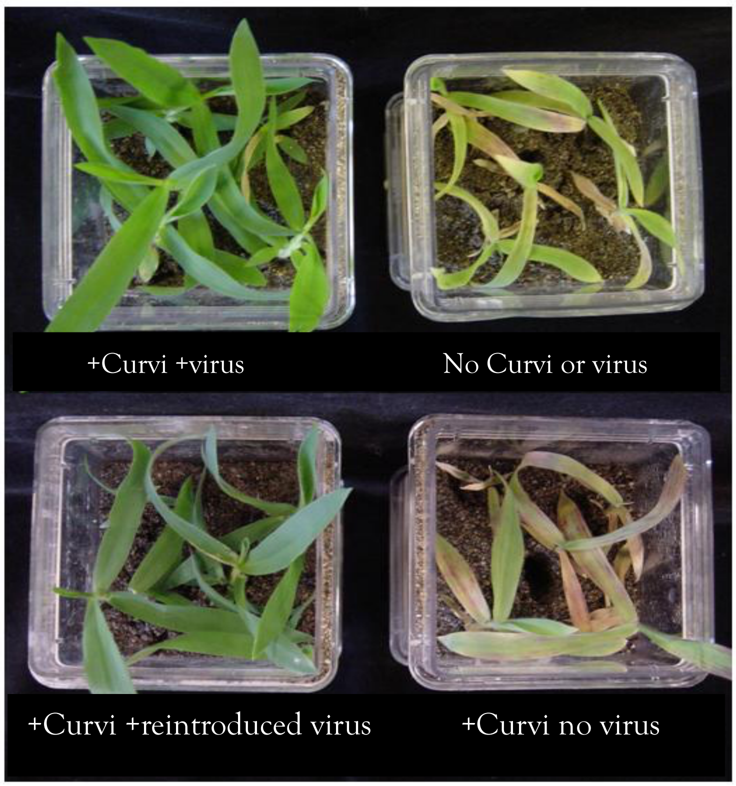
Curvularia protuberata (Curvi) with its brown pigment shown growing through plant roots of Tropical Panic Grass (left panel), the characteristic football shaped brown spores (center panel), and growth on fungal growth media (right panel)

Demonstration that Curvi was responsible for the heat tolerance of Tropical Panic Grass. Researchers isolated some of this special *Curvularia protuberata* that was inside plants from the hottest geothermal site of YNP to conduct additional studies. They first wanted to verify that the presence of Curvi was the reason the plants were doing so well, so they grew Tropical Panic Grass with and without the endophyte.

Field and laboratory experiments showed that when plant root zones were heated up, non-symbiotic plants (plants without the fungi) shriveled and died. In contrast, the plants with Curvi living inside them survived and even thrived! In fact, when grown separately, neither fungus nor plant alone could grow at temperatures above 38^{0}C (100^{0}F). Researchers repeated the heat experiments with different plant species like watermelon and tomato and another stressor - drought stress. The results all indicated that plants symbiotic with this strain of Curvi were able to stay healthy longer than plants without it!

Discovery that the Curvi:plant symbiosis involves a third partner: a virus no less! While conducting these experiments, researchers were surprised to find a virus inside Curvi. This virus, now known as CThTV, had infected the fungus before it moved inside the plant. People often think of viruses as the bad guys of microbiology, but scientists don't like to jump to conclusions, so they repeated the experiment with the virus in mind.

This time, they separated the plant from the fungus and separated the fungus from the virus. They grew the panic grass alone, the panic grass with the fungus infected with the virus, the panic grass with the fungus lacking the virus, and the panic grass with the fungus reinfected with the virus. Only plants with the fungus containing the virus – either the original fungus containing the virus, or treated to remove the virus and then reinfected – were able to survive high temperatures, showing that the virus is a critical part of the three-way relationship. Thus: the fungus alone was not enough to confer thermal tolerance; only the plants that had the fungus *and* the virus were able to thrive in extreme temperatures.



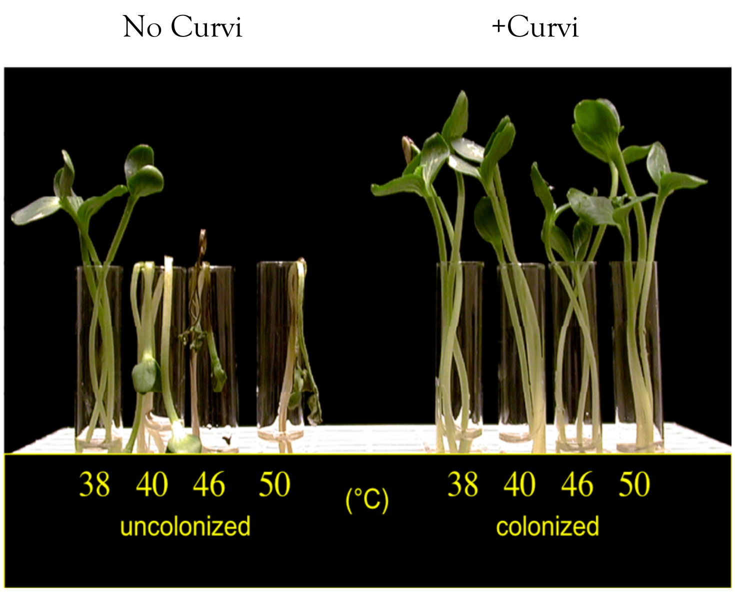
65^{0}C 10 hrs & 37^{0}C 14 hrs for 2 weeks

Good scientists don't make too many assumptions: assumptions can hide routes to new discoveries. It's good that the scientists didn't assume that the virus was dangerous or bad because, in this case, the CThTV virus was an essential part of the puzzle that led to this beneficial relationship. Also, it was exciting to think about three completely different organisms in a three-way symbiosis – a virus, a fungus, and a plant – all working together to achieve thermal tolerance! *It makes you wonder how often there are invisible relationships that we just don't know about yet.*

Curvi, with its virus ally, is a mighty powerful defender of plants under stress!

The importance of Curvi to us: scientists using ancient technology Mother Nature developed to help plants survive extremes

As temperatures and populations increase around the world, it's more important now than ever to help plants in general, and crop plants that feed us in particular, to adapt to our changing climate. Some of the plants that we rely on heavily for food haven't yet been introduced to beneficial endophytes. As it turns out, in nature there are many different species of beneficial endophytes with and without viruses in many different types of plants. Additional studies showed researchers that Curvi containing the CThTV virus could confer high temperature and drought stress tolerance to other plants such as watermelons and tomatoes and more. But, sadly, Curvi was hard to grow in big amounts for large scale use by farmers, so researchers began screening other endophyte species to get the best candidates for commercialization.



Watermelon plants exposed to increasing temperatures: Uncolonized Curvi plants (Left) wilt and die at high temperatures. Plants need Curvi to survive high temperature stress (right).

Untreated tomato plant (left) showing signs of drought stress compared to a Trichoderma product treated plant (right side).*

Scientists took what they learned from the Curvi relationship and then expanded their studies to find other helpful endophytes from plants living in other stressed habitats such as high salt areas. For many years now, scientists have been playing matchmaker and introducing agricultural plants (wheat, soy, corn, rice, alfalfa, tomato, potato and more!) with many different endophytes. After many years of searching, isolating, screening, and testing, scientists took winners of the best candidate endophyte contest. This special group of endophytes, including *Trichoderma* species, have been used successfully in agriculture to help crops deal with stress for many decades and are found all over the world. Like Curvi, they live inside the plant parts that are not eaten and can be easily applied to seeds or plants. With the help of endophytes, crop plants too can feel less stressed, stay cool, hydrated and use less water. A friend of plants is our friend too!

**Trichoderma* endophyte-based product called *BioEnsure* (Seattle, WA. USA)

www.AdaptiveSymbioticTechnologies

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