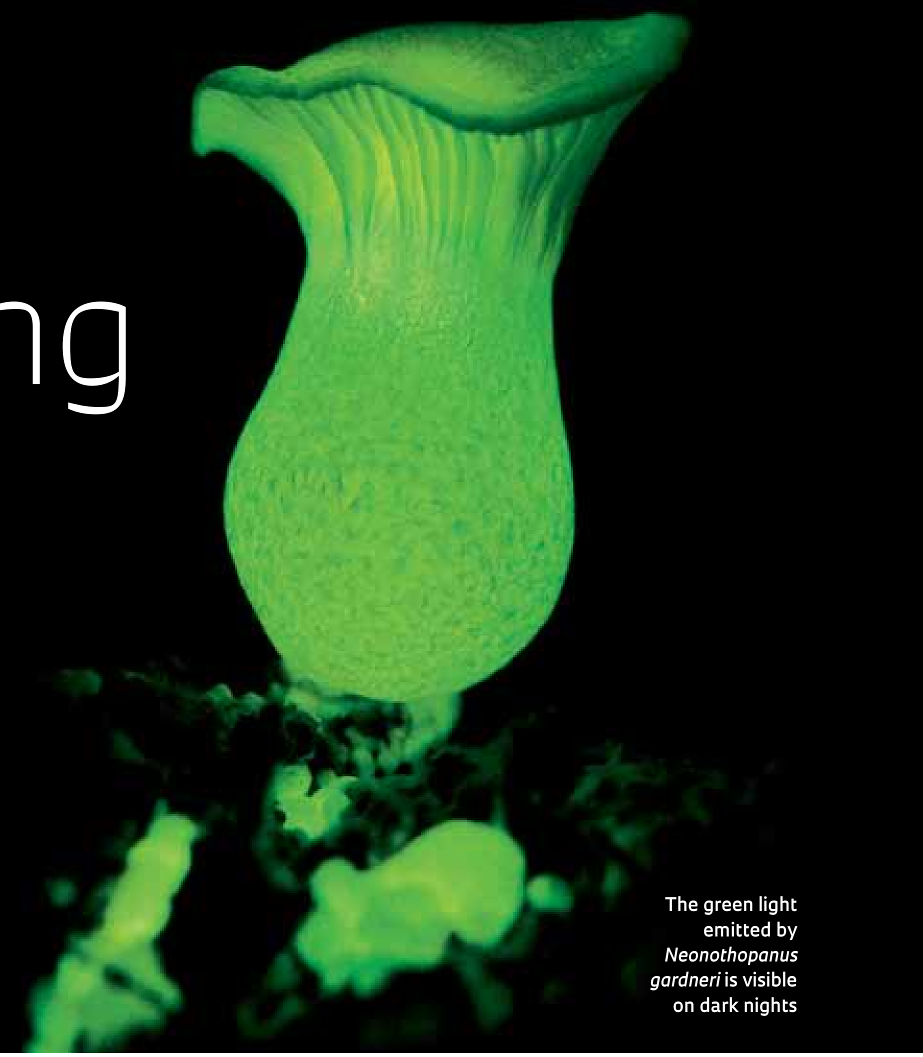
Natural lighting

The chemical process underlying the bioluminescence of mushrooms is recyclable and flexible



The green light emitted by *Neonothopanus gardneri* is visible on dark nights

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A street illuminated by bright trees, instead of light posts. This sounds like the makings of a dream but does not seem to be impossible to chemist Cassius Stevani, a professor at the University of São Paulo Chemistry Institute (IQ-USP). "But we have to be careful, as we do not want the natural forest to emit light at night," he warns. Although it is beyond the realm of reality, this science fiction-inspired scenario is based on Stevani's research on bioluminescent mushrooms, principally the species *Neonothopanus gardneri*, which grows in the Mata dos Cocais forest in the state of Piauí (*see* Pesquisa FAPESP *Issue No. 168*). He and a number of collaborators, mostly in Russia and Brazil, have just unlocked the mystery behind an important part of the chemical reaction that gives these mushrooms their green glow, as reported in the article published on April 26, 2017 in the journal *Science Advances*.

An important contribution of the research was discovering that hispidine, a molecule with pharmacological properties that is found in most plants, is a precursor of luciferin, a substrate that is essential for light production in mushrooms. Hispidine is also found in non-luminescent mushrooms, in which it is responsible for their orange color and for protecting them against damage caused by sunlight.

According to the sequence of chemical reactions demonstrated by the group of researchers, luciferin reacts with oxygen via the action of the enzyme luciferase and produces excited oxyluciferin, which, when it decays into the ground state, emits a photon—and therefore light. The oxyluciferin is then converted into caffeic acid through the action of another enzyme. This is another important finding because caffeic acid was already known to be a precursor of hispidine. Stevani explains that this is how the cycle comes full circle: "The molecules involved in bioluminescence are recycled, which explains the small amount of hispidine that is found in mushrooms: it is constantly being formed, converted, and then recycled – in this way, the bioluminescence cycle continues." As this process consumes oxygen, it could also provide a way for the mushroom to minimize damage from oxidative stress.

Trees and other plants also produce caffeic acid, and this is the source of the playful idea to use genetic manipulation to give them the ability to produce the enzymes needed to complete the reaction and glow; "Luminescent orchids could also be produced for the ornamental plant market," the chemist suggests. U.S. biochemist Hans Waldenmaier, who

These altered images illustrate what the mushroom would look like if it produced alternative substrates

PHOTOS CASSIUS STEVANI / IQ-USP

completed his doctorate under Stevani in 2016, plans to set up a company to produce bioluminescent plants in the United States. The goal is not just decorative. "Maybe one day it will be possible to use this system as a reporter for the biological processes of plants and apply the knowledge to human health problems," says the IQ-USP professor. The use of fluorescent proteins as genetic markers, or reporters, earned Osamu Shimomura, Roger Tsien and Martin Chalfie the 2008 Nobel Prize for Chemistry, precisely because of the importance of this method in visualizing biochemical processes. In their case, a fluorescent protein produced by jellyfish was used, which is widely used in laboratories around the world.

PRODUCTIVE CHEMISTRY

The results reported in the *Science Advances* article arose from the collaboration between Stevani and the Russian chemist Ilia Yampolsky of the Institute of Bio-organic Chemistry in Moscow, a partnership that emerged in an unusual way. When Stevani learned from students coming back from an international congress that Yampolsky was looking to characterize the molecules responsible for bioluminescence in mushrooms, the Brazilian researcher contacted him to propose working together; however, he contacted him too late. The results of the study had already been submitted for publication, based on a mushroom very similar to the Brazilian mushroom, *Neonothopanus nambi*, which grows in Vietnam. In the competitive world of academia, being scooped by a researcher who began working in the field only recently could be cause for spite and enmity. In fact, the opposite occurred. To obtain the results presented in *Science Advances*, each scientist contributed his expertise—for the Russian, it was in the synthesis of organic compounds, and for the Brazilian, it was in chemical mechanisms. In São Paulo, others also contributed – chemists Erick Bastos and Paolo di Mascio of the IQ, Anderson Oliveira of the Oceanographic Institute, and pharmacists Felipe Dörr and Ernani Pinto of the School of Pharmaceutical Sciences, all of whom are at USP.

In addition to clarifying which molecules contribute to the bioluminescence reaction, the researchers found that luciferase is versatile. Yampolsky synthesized variations of luciferin that, upon reacting with luciferase, also generate light. As these molecules are not naturally produced by the mushrooms, the reaction was produced inside an apparatus, known as the luminometer, which detected the presence of light. The key difference is that the light produced in this way would have a wavelength different from the green observed in nature. If the reaction did occur in nature, the mushrooms would emit other colors, such as those shown in the altered images that accompany this article: "poetic license", in the words of the Brazilian chemist.

From pure chemistry to fiction and technological applications, Stevani also dabbles in biology by way of his investigations into the ecological significance of mushroom luminescence. The results obtained by Waldenmaier for his doctoral thesis are still being prepared for publication, but it can already be said that the field experiments he conducted and the accompanying videos suggest that brightness attracts insects and leads to the creation of a unique miniature ecosystem. Mushrooms appear to be a meeting point for fireflies, who usually visit them in pairs. Bush cockroaches (*Ellipsidion humerale*) eat the mushrooms and are hunted by spiders. All of them, Stevani suggests, are attracted by the light that spreads much farther than scent in the forest environment. While this is all happening, animals become covered with spores and help spread the mushrooms. Since they grow near the ground where it is more humid, there is no wind to disperse their reproductive spores. Through collaboration, everyone wins. ■ **Maria Guimarães**

Project

Fungal bioluminescence: species survey, mechanistic study & toxicological assays (No. 13/16885-1); **Grant Mechanism** Regular Research Grant; **Principal Investigator** Cassius Vinicius Stevani (USP); **Investment** R$ 183,183.40 + US$ 58,141.94.

Scientific article

KASKOVA, Z. M. *et al.* Mechanism and color modulation of fungal bioluminescence. **Science Advances**. April 26, 2017.