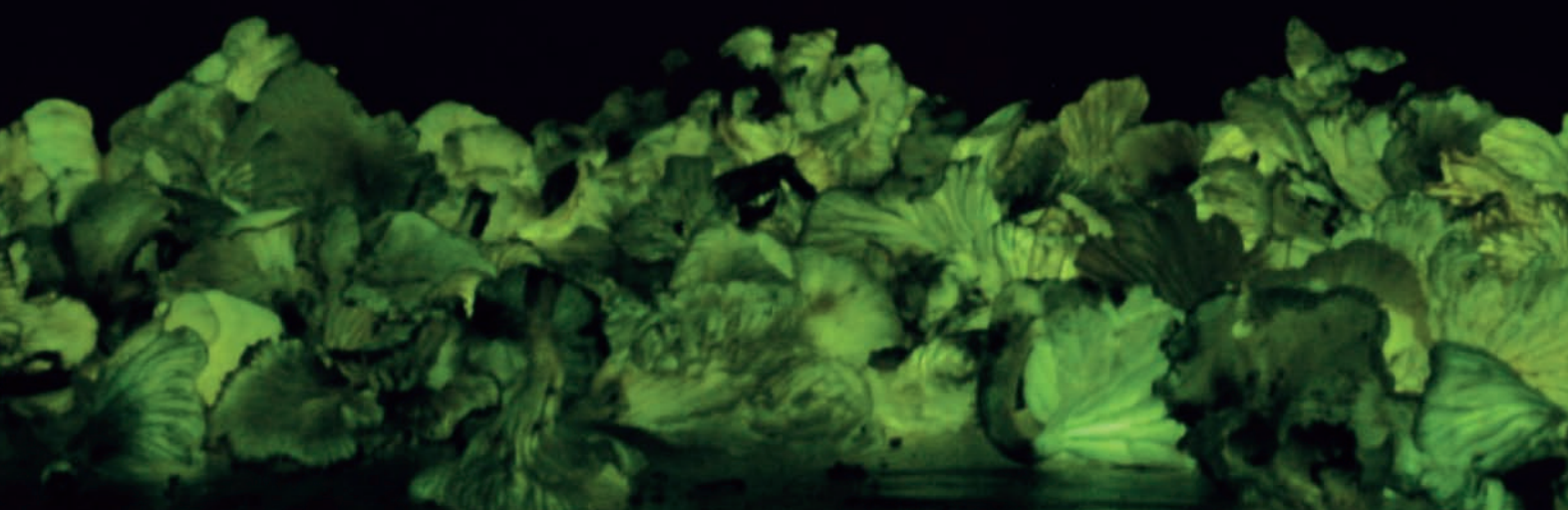
Living lights

Published in February 2010

In the cartoon movie *A Bug's Life* all the inner lighting for the ants' nest comes from luminous mushrooms. "There's a large degree of poetic license in the work," comments Cassius Stevani, from the Chemistry Institute of the University of São Paulo (USP), "but in essence it's true." There really are mushrooms that emit light, or are bioluminescent, and many ants grow fungi in their nests – but not of this type. Stevani is putting a lot of effort into understanding the chemical mechanism that generates this luminosity and what its function in the organism is. On the way, he has already discovered a practical use: detecting metal contamination in the soil.

It only took 5 years for Stevani and his colleagues to discover 12 species of luminescent fungi in Brazil. Among them are the Amazonian Mycena lacrimans, found by Ricardo Braga-Neto from the National Research Institute of the Amazon (Inpa), and a species that looks like an inverted umbrella that grows at the foot of palm trees, like the piaçava [piassaba], or the babaçu, in Piauí. Worldwide there are 71 species, according to a review article written by Stevani in collaboration with North American biologist, Dennis Desjardin, from the São Francisco State University, in California, which in March will grace the front cover of the journal *Mycology*. "There must be many more species to discover," the chemist imagines, "that have not yet been described because they're difficult to find; few people walk around in the forest without a torch on a moonless night."

Until 2002 there were no reports of bioluminescent fungi in Brazil; or rather, there was one species, described in the nineteenth century by Britain, George Gardner, the scientific name of which was *Agaricus phos-*



Mechanism that makes mushrooms glow leads to a method for detecting contamination | Maria Guimarães

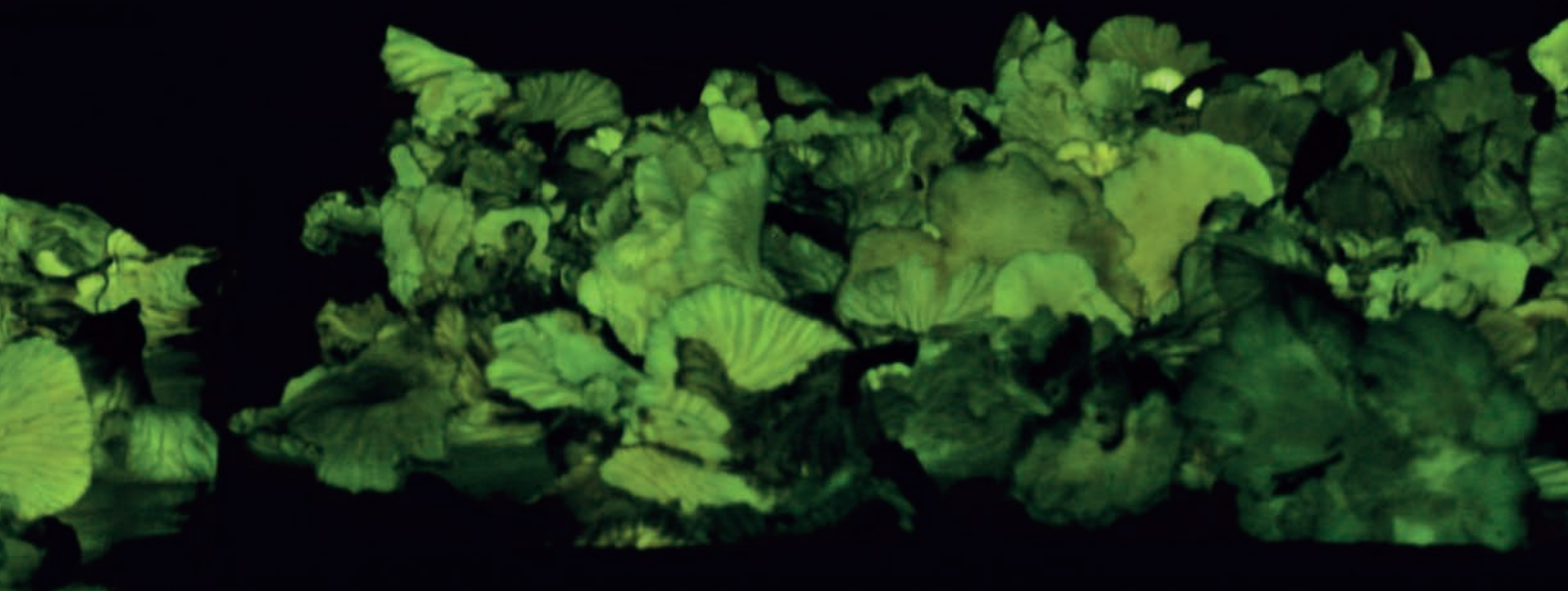
phorescens (later renamed *Pleurotus gardneri*), but today fungi specialists question this classification, based on similar species in Europe, and it was difficult to correct the mistake because the only preserved sample is in a herbarium in England.

A mushroom that seems to be of the same species was recently found glowing at the foot of a piassaba palm tree by North American primatologist, Dorothy Fragazy, who was out later than usual at the end of a day she had spent looking for monkeys in Piauí. Fascinated, she showed her photos to a fellow countryman at the Botanical Gardens in New York, who got in touch with Dennis Desjardin, considered to be one of the greatest specialists when it comes to identifying these organisms. He, in turn, advised Stevani. The Brazilian only had to search the Internet to discover that Dorothy was in Brazil for some work she was doing in collaboration with primatologist, Patrícia Izar, from the Psychology Institute at USP – whom he immediately contacted in his search for a the whereabouts of the mushroom. It is one of these stories of chance, in which information needs to go round the world before arriving back in almost the same place.

It turned out well: the owner of the property where Dorothy and Patrícia were working, Marino Gomes de Oliveira, dried 4 kg of the glowing mushroom in the sun and sent them to Stevani. Now the researchers are close to correcting the identification, with the detailed examination of the mushrooms by mycologists (fungi specialists) Marina Capelari, from the Botanical Institute of São Paulo, and Desjardin. He has dedicated himself to exploring little known forests all over the world, including in Brazil, and says that the pioneering efforts of his group have been responsible for many discoveries. "Recently I led an expedition to an island in Micronesia, in the Pacific, where mushrooms had never before been documented; of the 128 species we found 7 were luminescent," he says, making it clear that glowing fungi are in the minority.

Brazil is promising because it has an immense forest area, the fungi of which have not yet been studied, says Desjardin. "We know very little about Brazil's mushrooms, so we hope to find many new species, whether luminescent or not." He explains also that in order to find luminous fungi you have to think about it. Most of the mycologists who study fungi diversity describe mushrooms during the day (when they also emit light, but the researcher cannot see it) and dry them immediately to preserve them; they need to be examined first in the dark to determine if there is

PHOTOS CASSIUS STEVANI/USP



"Pleurotus" gardneri: rediscovered in Piauí

THE PROJECTS

1. Study of the bioluminescence of fungi and its applications in environmental chemistry
2. Bioluminescence and the pharmacological activity of mushrooms

TYPE
1. Regular Research Project Aid
2. Young Researcher

COORDINATOR
Cassius Stevani – IQ/USP

INVESTMENT
1. R$ 328,413.09
2. R$ 457,741.18

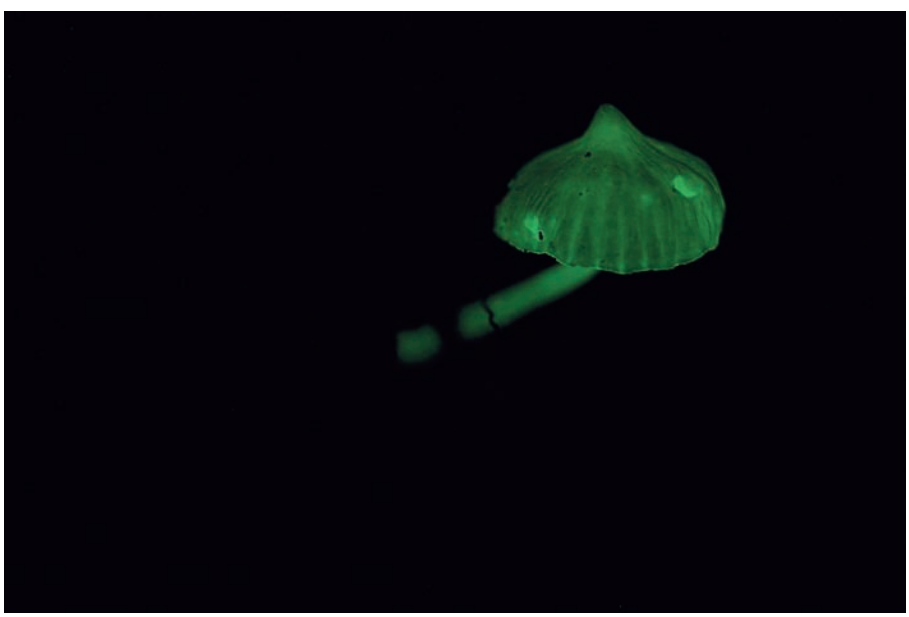
any luminescence and only then should they be dried. "Because of this I guess that several rare tropical fungi are luminescent, but we've not yet noticed it."

Despite being little known, there have been reports of luminous mushrooms for a long time. Aristotle, the Greek philosopher, was the first to report the phenomenon more than two thousand years ago, when he described the living glow and decided that it was different from fire. However, scientific studies on this phenomenon only started in the 1950s and only now are they beginning to contribute to our understanding of the bioluminescence in these organisms that are specialists in decomposing wood and other types of organic matter.

Signaling – Stevani's interest in fungi grew out of his previous work with fireflies and other insects. In 2002, during a trip for collecting material with Etelvino Bechara, a renowned specialist in the bioluminescence of fireflies, now at the Federal University of São Paulo (Unifesp), he took advantage to look for the mushrooms that Bechara had talked to him about. He found them: while he was looking in the dark at an area of damp vegetation close to a waterfall in the middle of the Cerrado [scrubland, savannah vegetation] in Mato Grosso do Sul, he saw a different green light that was constant, unlike the flashing light of the fireflies.

They were mushrooms and gave rise to the project that the researcher from USP became involved with as from 2002 with the help of FAPESP and its Young Researcher program. Even before the work began, the luminous fungi proved not to be restricted to Mato Grosso do Sul. During fieldwork in the State Tourist Park of Alto Ribeira (Petar), in the south of São Paulo state, ecologist João Godoy, now a professor at the São Paulo School of Engineering, was led by his forest guide to a luminous fungus. Surprised, he told his chemist friend, who can now concentrate his field activities in the Petar, which is closer to his laboratory.

Some of these species are helping unveil the minute details of the bioluminescence of fungi and for this Stevani relies on the help of three PhD students, funded by FAPESP. By means of exhaustive chemical trials PhD student, Anderson Oliveira, analyzed three



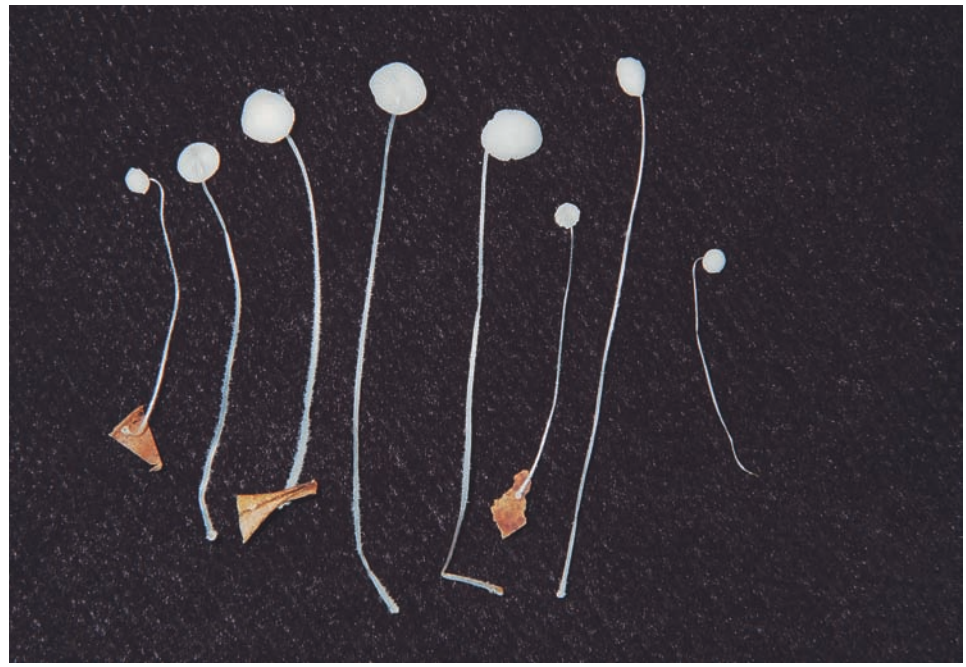
Mycena luxaeterna: light concentrated on the stalks, or stipes

species from the Petar's Atlantic rainforest – *Gerronema viridilucens, Mycena lucentipes* and *Mycena luxaeterna* –, as well as the *"Pleurotus" gardneri* fungus, found in a region of the Cerrado in the Piauí municipality of Gilbués. In an article published in 2009 in *Photochemical & Photobiological Sciences*, the results show that the light production mechanism is similar to that seen in fireflies and in bioluminescent bacteria: enzymes called luciferases oxidam, a substance – or substrate, as chemists prefer to call it – known as luciferin that releases energy in the form of light.

Oliveira used the very latest equipment in chemistry labs, but the basis of the trial for characterizing the enzymatic reaction was discovered more than a century ago. In 1885, French physiologist, Raphaël Dubois, crushed the luminous organs of a *Pyrophorus* firefly and mixed them with cold water. The solution gave off a green glow which little by little faded away. It was luciferin being consumed by the chemical reaction, he concluded. Then Dubois heated a similar solution, disintegrating the enzymes present that are sensitive to heat. On mixing the two solutions – the cold one, where the enzymes survived but no longer with luciferin, and the hot one that only had luciferin –, he saw the mixture emit light. This story is in the book, *Bioluminescence*, published in 2006 by Japanese pharmacist, Osamu Shimomura, a researcher in the Marine Biological Laboratory in Woods Hole, in the United States.

Shimomura won the Nobel Prize in Chemistry in 2008, precisely for his studies on bioluminescence: he isolated the green, fluorescent protein (GFP) from jellyfish, which indicates the activity of specific genes when attached to the DNA of an organism studied in the laboratory. The luminous protein has become essential in many genetic laboratories, an aspiration that is not far from Stevani's mind, given that bioluminescence mechanisms are similar, even among very different organisms.

Mycena fera: mushrooms glow all the time, but are only seen in the dark



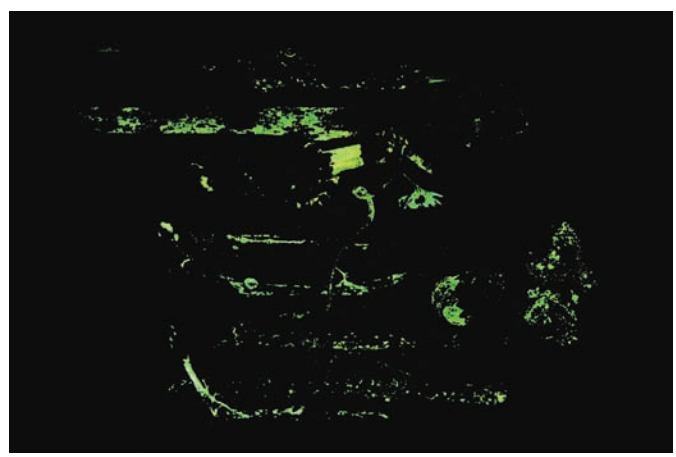
This does not mean, however, that the chemical composition of luciferin is similar in insects and fungi. "Luciferin is the name we give to any substrate that is luminescent, but the luciferins of different organisms may be completely different molecules," explains Stevani. All the fungi studied by his group, however, shine by means of the same substrates and the same enzymes, suggesting a common origin for all of them. However not all bioluminescent fungi are close relatives, warns Desjardin. "Today, we know that there are four families of fungi with bioluminescent species, but they don't always have the same close relationship between them," he says. "Some shining species of *Mycena* are more like species that don't shine than other shining species of the same genus."

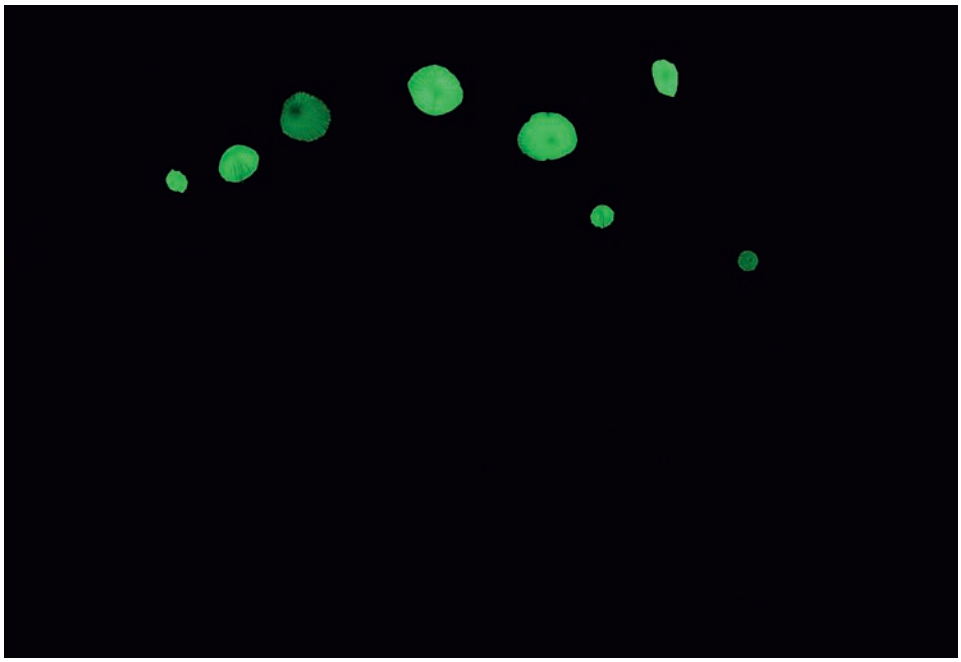
The group from USP is now hunting down the structure of the molecule that makes these tiny mushrooms, sometimes only 0.5 cm in circumference, resemble glow-in-the-dark star stickers, stuck to the trunk of a tree, or as if they were sprinkled in the midst of the leaves that cover the forest floor. Unlike fungi, which produce their own light, glow-in-the-dark stars are phosphorescent stickers that store environmental light and consequently only shine at night, creating constellations in the bedrooms of children of all ages. So far, Oliveira has managed to separate from the fungus extract a solution containing luciferin – it shines when mixed with an enzymatic solution. However, the substance must be in very low concentrations, because chemist Antonio Gilberto Ferreira, from the Federal University of São Carlos (Ufscar), has not managed to detect it by the nuclear magnetic resonance of protons. "We need to extract a larger amount, or use more sensitive equipment," plans Stevani.

The chemist from USP started on this undertaking out of pure scientific curiosity, but considers it essential to find practical uses that bring benefits for other researchers and for society. He seems to be on the right track: the glow of the *Gerronema viridilucens* fungi may help detect high levels of soil contamination by various types of metal, as Luiz Fernando Mendes, another PhD student of Stevani's, showed in an article going to press in *Environmental Toxicology and Chemistry*.

Biological sensors - Mendes grows the fungus on 35 mm diameter glass plates, on a gelatin base of algae, known as agar, the most commonly used culture medium in biological laboratories. After growing for 10 days, the fungi still do

Stems covered by hyphas that are invisible in daylight





Mycena asterina: luminescence restricted to the cap of mushrooms

not attain the shape of a mushroom. In this phase, they comprise microscopic filaments, hyphas, which represent the major part of the life cycle of any fungus and, in some species, they also produce the green glow. The researcher measures the luminosity emitted by each of these plates and deposits on them a small soil extract sample to be analyzed. After 24 hours in a cold chamber the fungus starts emitting less light if the sample is contaminated, which the chemists interpret as a form of damage that the organism has suffered.

Mendes got graphs that represent the intensity of light emitted in the presence of different concentrations of 11different metals – calcium, sodium, magnesium, cadmium, cobalt, manganese, potassium, lithium, zinc, copper and nickel – and that indicate the toxicity of the sample analyzed. The work has already provided a patent registered in Brazil on the use of fungi in environmental toxicity trials. It is enough just to measure the intensity of the light that emanates from the fungus to estimate how much of these metals is in a form that can be absorbed and used by living beings. "This is not a matter of measuring the total concentration of chemical substances; that would have no biological significance and be of no practical use," points out Stevani. The problem is that *Gerronema viridilucens* is not very sensitive, perhaps precisely because it lives in the soil and is adapted even to adverse conditions. "What matters is that the biotrial works; now we need to find more sensitive species that can be tested in the same way," says the chemist.

Strategies - Because it consumes oxygen in its chemical reactions, bioluminescence may be able to perform an antioxidant role that would protect fungi and other organisms, even fireflies, from reactive species produced from the oxygen consumed in breathing. This protection of the organism possibly explains the benefits of glowing in the middle of the forest. However when it is necessary to take up arms against intense oxidative stress, Stevani's group has shown that the organism of fungi favor more specialist reactions in accomplishing this function, and switches off its luminescence. This is what the as yet unpublished work of Olívia Domingues indicates; she is also a PhD student of Stevani's. She saw that in the presence of metals in high concentrations cells give preference to using the co-enzyme, NADPH, for producing reduced glutathione, which avoids the harmful action of the metals. As reduced glutathione competes for resources with the enzymes that produce the luminescence, little by little the fungus switches off. That is why the fungi in Mendes' bio-trials lost their luminosity in soil contaminated by metals.

Olívia's results help explain why bioluminescent fungi serve as a toxicity bio-trial, but do not explain the benefit to the fungus in emitting a greenish glow. Stevani is betting on ecological hypotheses, showing photographs of flies landing on mushrooms. Like a lamp around which various insects fly, the green glow perhaps helps attract insects. It may seem that there is no advantage in announcing your presence to the hungry hoards that are on duty, but the function of mushrooms in the life cycle of fungi is ephemeral, like the fruit of trees: when an animal eats part of the mushroom it takes away its spores, microscopic structures that are going to generate new fungi if they are deposited in suitable locations. Or perhaps the light is a danger signal in the case of poisonous mushrooms, as happens with brightly colored venomous animals. "What isn't probable is that the bioluminescence of fungi has evolved to illuminate anthills or to serve as an indication of the flight path, as in *A Bug's Life*," he jokes.

The chemists' discoveries make it clear that many mysteries will remain lost among the leaves until more biologists and chemists resolve to put out their torches and gaze at the darkness of the forest, which is sometimes sprinkled with green. ■

Scientific articles

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2. MENDES, L. F. Stevani, C. V. "Evaluation of metal toxicity by a modified method based on the fungus Gerronema viridilucens bioluminescence in agar medium." **Environmental Toxicology and Chemistry.** v. 29, p. 320-26. 2010.
3. OLIVEIRA, A.G. AND STEVANI, C.V. "The enzymatic nature of fungal bioluminescence." **Photochemical & Photobiological Sciences.** v. 8, p. 1.416-21. Oct. 2009.