MYCOLOGY

THE HIDDEN LIFE OF TRUFFLES

Not just for gourmands, truffles play essential roles in the health of ecosystems

By James M. Trappe and Andrew W. Claridge

IT'S A COOL NOVEMBER DAY NEAR BOLOGNA, ITALY. WE ARE STROLLING THROUGH THE WOODS WITH truffle hunter Mirko Illice and his little dog, Clinto. Clinto runs back and forth among the oak trees sniffing the ground, pausing, and then running again. Suddenly, he stops and digs furiously with both paws. "Ah, he's found an Italian white truffle," Mirko explains. "He uses both paws only when he finds one of those." Mirko gently pulls the excited dog from the spot and pushes through the soil with his fingers. He extracts a yellowish brown lump the size of a golf ball and sniffs it. "*Benissimo,* Clinto," Mirko intones. Though not the finest example of the species, *Tuber magnatum*—which grows only in northern Italy, Serbia and Croatia—Clinto's find will fetch a nice price of about $100 at the Saturday market.

Throughout history, truffles have appeared on the menu and in folklore. The Pharaoh Khufu served them at his royal table. Bedouins, Kalahari Bushmen and Australian Aborigines have hunted them for countless generations in deserts. The Romans savored them and thought they were produced by thunder. Epicures today who prize truffles' earthy aroma and flavors are willing to pay steep prices at the market—the Italian white species has fetched more than $3,000 per kilogram.

Despite humanity's abiding interest in truffles, much about their biology has remained veiled in mystery. Over the past four decades, however, genetic analyses and field experiments have clarified the origins and functions of these organisms. These studies have revealed that truffles play crucial ecological roles in many ecosystems. The findings are informing strategies for conserving some endangered species that rely on these denizens of the underworld.

A FUNGUS AMONG US

TRUFFLES, LIKE MUSHROOMS, are the fruit of fungi. These fleshy organs are temporary reproductive structures that produce spores, which eventually germinate and give rise to new offspring. What sets truffles apart from mushrooms is that their spore-laden fruit forms below ground

IN BRIEF

The truffles that appear on restaurant menus and on the shelves of luxury food purveyors represent only a small fraction of the world's truffle species.

Truffles figure importantly in ecosystems, sustaining both plants and animals.

Recognition of the ecological significance of truffles is aiding efforts to conserve threatened species that depend on them.

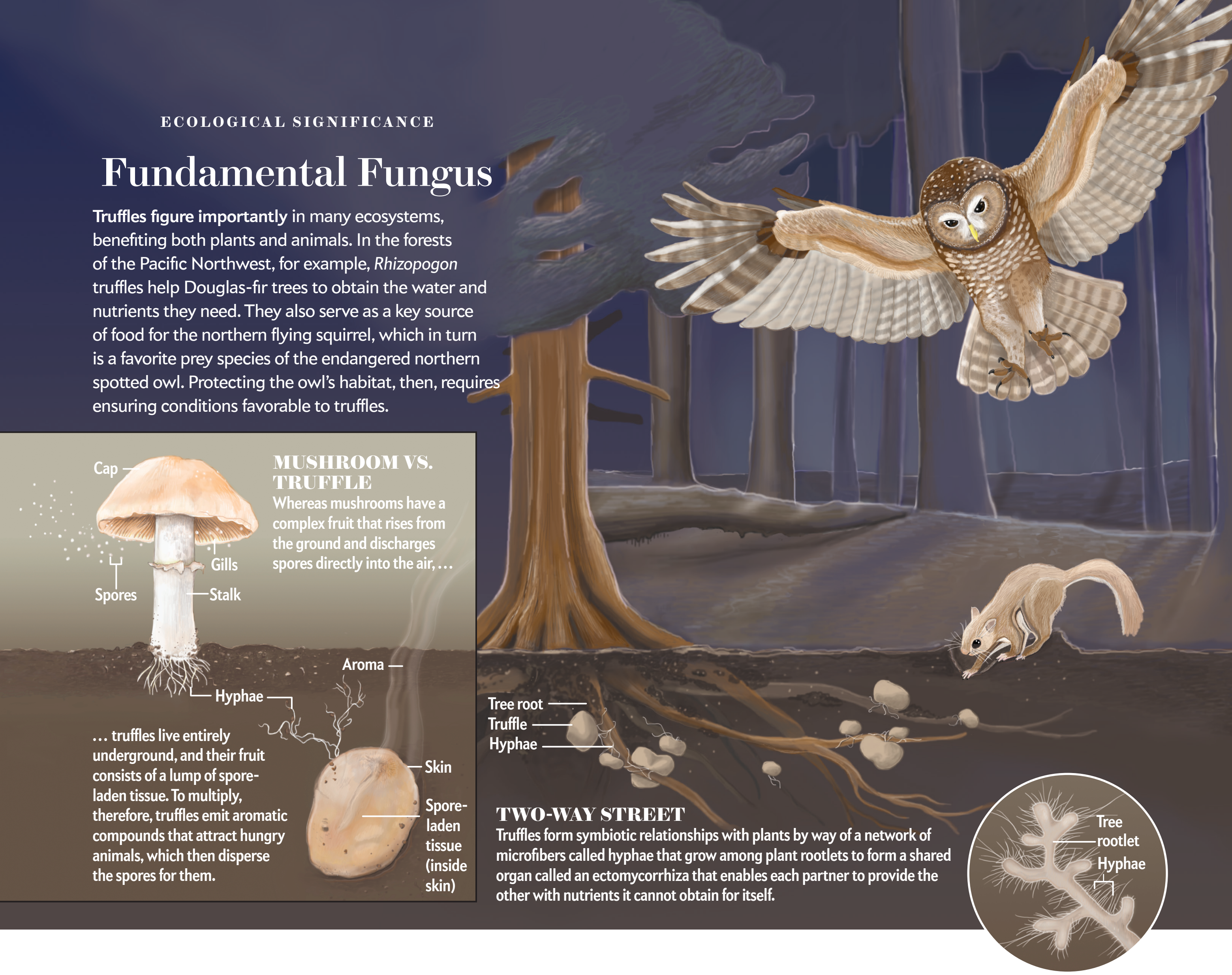
One popular variety of truffle has been successfully cultivated, but agriculture has yet to tame other prized species.

JOERG LEHMANN *Getty Images*

ECOLOGICAL SIGNIFICANCE

Fundamental Fungus

Truffles figure importantly in many ecosystems, benefiting both plants and animals. In the forests of the Pacific Northwest, for example, *Rhizopogon* truffles help Douglas-fir trees to obtain the water and nutrients they need. They also serve as a key source of food for the northern flying squirrel, which in turn is a favorite prey species of the endangered northern spotted owl. Protecting the owl's habitat, then, requires ensuring conditions favorable to truffles.



rather than above. Technically, true truffles are those multicellular fungi that belong to the Ascomycota phylum of organisms and are marketed as food. But there are "false truffles" in the fungal phylum Basidiomycota that function like true truffles. In view of these similarities, we refer to all fleshy fungi that fruit underground as truffles.

Scientific efforts to expose the secrets of truffles date to the 1800s, when would-be truffle growers in Germany asked a botanist, Albert Bernhard Frank, to figure out how the delicacies propagate. Frank's studies revealed that the fungi grow on and into the tiny feeder rootlets that trees use to absorb water and nutrients from the earth. On the basis of those observations, he proposed that the organisms have a symbiotic relationship in which each provides nutrients to the other. He further posited that such relationships between subterranean fungi and plants are widespread and that they shape the growth and health of many plant communities. Frank's theories contradicted conventional wisdom about truffles and other fungi—namely, that they all brought about disease and rot in plants—and drew considerable opposition from his peers. Nearly a century later, scholars finally had definitive evidence, which showed that Frank got the story right.

All truffles and mushrooms produce networks of filaments, or hyphae, that grow among plant rootlets to form a shared absorptive organ known as a mycorrhiza. Thus joined, the fungus provides the plant with precious nutrients and water, its tiny hyphae able to reach into pockets of soil inaccessible to the plant's much larger roots. The plant, in turn, furnishes its consort with sugars and other nutrients that it generates through photosynthesis—products that the fungus needs but cannot produce on its own because it does not photosynthesize. So beneficial is this partnership that nearly all trees and other woody plants require it for survival, as do the associated fungi. Most herbaceous plants (those that do not have a permanent woody stem aboveground) form mycorrhizae too, albeit with different fungi.

Many fungal species, including all the ones that yield truffles, form a variant of the mycorrhiza called an ectomycorrhiza, in which the fungus envelops the feeder rootlets with a protective outer tissue. The diversity of these ectomycorrhizal fungi is impressive: one of us (Trappe) estimates that some 2,000 species are associated with the Douglas fir (an evergreen used for timber and Christmas trees), and probably as many or more fungal species partner only with Australia's eucalyptus

LUCY READING-IKKANDA

trees. Numerous other commercially and ecologically important tree species also rely on ectomycorrhizal fungi. Most of these fungi fruit aboveground as mushrooms, but several thousand species produce truffles.

GOING UNDER

COMPARISONS of the morphology and gene sequences of truffle and mushroom species indicate that most truffles have evolved from mushrooms. But given that truffles require aboveground dispersal of their spores to propagate, why would natural selection favor the evolution of species that hide underground? Consider the reproductive tactic of mushrooms. Although mushrooms exhibit a multitude of structures and colors, they all have fruiting bodies that can discharge spores directly into the air. The airborne spores may then alight nearby or far away to germinate and potentially establish a new colony in association with the roots of a compatible plant host. It is a highly effective approach.

The mushroom strategy is not foolproof, however. Most mushrooms have little defense against environmental hazards such as heat, drying winds, frost and grazing animals. Every day a few spores mature and are discharged. But if inclement weather dries or freezes a mushroom, spore production usually grinds to a halt.

Where such hazards are commonplace, new evolutionary adaptations have arisen. The most successful alternative has been for the fungus to fruit underground. Once the soil is wet enough for the subterranean fruiting body to form, it is insulated from vagaries of weather. The truffle develops with relative impunity, continuing to produce and nurture its spores even when aboveground conditions become intolerable to mushrooms.

At first glance, the truffle's solution might seem facile. Truffles are visibly less complex in shape than mushrooms are. No longer does the fungus need to expend the energy required to push its spore-bearing tissues aboveground on a stalk or develop a cap or other structure for producing and releasing the spores. The truffle is but a lump of spore-bearing tissue, usually enclosed by a protective skin.

The problem is that the truffles cannot themselves liberate their spores, trapped as they are in their underground realm. That feat demands an alternative dispersal system. And therein lies the complexity of the truffle's scheme. Over millions of years, as truffles retreated underground, mutations eventually led to the formation of aromatic compounds attractive to animals. Each truffle species has its own array of aromatics that are largely absent in immature specimens but intensify and emerge as the spores mature.

Of the thousands of kinds of truffles that exist today, only a few dozen appeal to humans. The rest are too small or too tough, or they possess aromas that are unremarkable or downright repugnant. To other animals, however, they are irresistible, their olfactory charms wafting up from the soil. Small mammals such as mice, squirrels and rabbits in the Northern Hemisphere and rat-kangaroos, armadillos and meerkats in the Southern Hemisphere are the main truffle gourmands. But their larger counterparts—deer, bears, baboons and wallabies, among others—also seek out the undercover fungi. Mollusks are attracted to truffles, too. And insects may feed on truffles or lay eggs in them so that their larvae have a ready food source when they hatch.

When an animal eats a truffle, most of the flesh is digested, but the spores pass through unharmed and are defecated on the ground, where they can germinate if the conditions are right. This dispersal system has advantages over the one that mushrooms employ. Feces concentrate spores, in contrast to the more diffuse scattering that occurs with aerial dissemination. In addition, feces are more likely to be deposited in the same kinds of areas where the animals forage for truffles, as opposed to the more random transport of airborne spores. This similarity of environment is beneficial because it increases the likelihood that the spores will land in a spot that has an appropriate plant species with which to establish a mycorrhiza.

Not all truffles rely on scent to attract animals, however. In New Zealand, which lacks native terrestrial mammals, some truffles have evolved rainbow hues that mimic the colors of fruits prized by the local birds. The *Paurocotylis pila* truffle, for one, emerges from the ground as it expands and lies on the forest floor, resembling the plump, red berrylike base of the seeds of *Podocarpus* trees that are a favorite bird food. (Although these colorful fungi do poke above the ground, they are nonetheless considered truffles because their spore-bearing tissues are enclosed in

DEPENDENTS

Truffle Eaters

The animals below are among the many that dine on truffles.


Long-footed potoroo


Meerkat


Great spotted kiwi


Chacma baboon

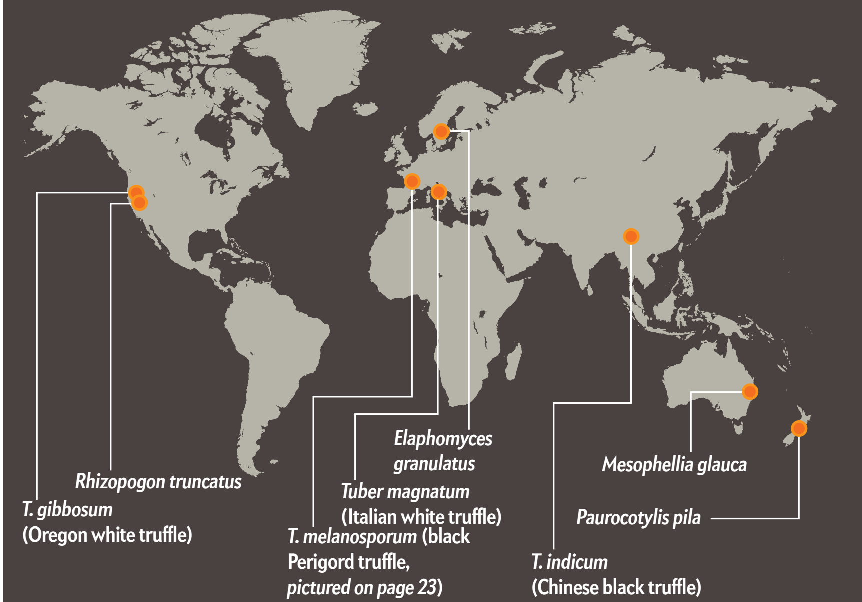

Grizzly bear

DAVE WATTS Alamy (long-footed potoroo); MILLARD H. SHARP Science Source (meerkat); GETTY IMAGES (kiwi); NIGEL J. DENNIS Science Source (baboon); GREGORY OCHOCKI Science Source (bear)

GLOBAL DIVERSITY

Where Truffles Grow

Truffle species number in the thousands; the map shows representative locations where those mentioned in this article grow wild. The highest-known diversity of species occurs in Mediterranean Europe, western North America and Australia—regions mostly characterized by cool, rainy winters and warm, dry summers. With their subterranean lifestyle, truffles are protected from the heat, drought and frost that can occur when the fungi produce their fruit.



M. glauca

R. truncatus

P. pila

a skin, and they thus depend on animals to disperse their spores.)

Yet another dispersal mechanism has evolved with a few groups of truffles, notably members of the ubiquitous Elaphomycetaceae family and the Mesophelliaceae family, which is endemic to Australasia. Their spores mature into a powder rather than a fleshy, spore-bearing tissue. The powder of *Elaphomyces granulatus,* for instance, is enclosed in a thick rind that is eaten by the animal, freeing the spores. Some of the Mesophelliaceae have a similar structure; others, such as *Mesophellia glauca,* possess a powdery spore mass sandwiched between a thin, hard outer rind and an edible inner core.

Even the spores of uneaten truffles can wander. After maturing, truffles decay into a slimy, larva-infested suspension in the soil. Invertebrates feed on this rotting tissue or move through it, picking up spores along the way. Truffle spores also travel when predators capture a small truffle-eating species: owls and hawks may carry rodents full of truffles considerable distances to their nests or roosts, where they eat the prey whole or eviscerate and discard the entrails. Either way the spores return to the soil, where they may give rise to new truffles.

TOGETHER FOREVER

EVOLUTION'S EXPERIMENTS with truffles have been remarkably similar in both the Northern and Southern Hemispheres, despite taking place long after the continents separated. The host plants in these regions are entirely different: whereas pines, beeches and oaks, for instance, partner with truffles in the north, eucalyptus and southern beeches play that role in the south. The truffle and animal species are likewise distinct between hemispheres. Yet the ecosystems and their components—the trees, truffles and animals—function in much the same way.

The greatest known diversity of truffles occurs in temperate areas of Mediterranean Europe, western North America and Australia (although most of Asia, Africa and South America remain unexplored by truffle researchers). These areas have climates with cool, rainy winters and warm, dry summers. Their fungal fruiting seasons are usually spring and autumn, when weather tends to be erratic: some years bring warm, dry spells and others deliver frost; both conditions are inimical to mushrooms. Over time, then, natural selection favored those fungi that sought refuge underground in these regions.

Exactly when the first truffles evolved is uncertain, but scientists have unearthed some clues to their origins. The oldest fossil ectomycorrhizae on record date to around 50 million years ago. And the ancestors of today's pines and other trees with which truffles form essential relationships arose some 85 million years ago. We can assume, then, that truffles emerged sometime between 85 million and 50 million years ago.

Given this long-standing association between truffles and plants, it is no surprise that the fungi figure importantly in the ecology of many habitats. Not only are they essential to the functioning of numerous plant species, but animals have come to rely on them for food. In the U.S. at least one creature, the Western red-

LUCY READING-IKKANDA (*map*); COURTESY OF JAMES M. TRAPPE (*M. glauca*); COURTESY OF MATT TRAPPE (*R. truncatus*); COURTESY OF MICHAEL CASTELLANO AND JAMES M. TRAPPE (*P. pila*) backed vole, depends almost entirely on truffles for sustenance. And the northern flying squirrel, found in North America, eats mostly truffles when available in the wild. On the other side of the globe, in Australia, a marsupial known as the long-footed potoroo subsists on a diet that is about 95 percent truffles. Its fellow marsupials—the other rat-kangaroos and bandicoots—also bank heavily on truffles. And many other creatures the world over routinely supplement their primary food sources with these fungi.

Scientists' developing knowledge of the intimate relationship between truffles, their plant hosts and their animal carriers is guiding the efforts of cultivators and conservationists alike. In the 1980s in Oregon, Mike Castellano of the U.S. Forest Service, Mike Amaranthus of Mycorrhizal Applications and their colleagues began outfitting nursery seedlings with spores of hearty *Rhizopogon* truffle species to help the seedlings withstand drought and other stressful conditions in plantations. Going forward, cultivators could conceivably augment their returns if they substituted gourmet truffles for *Rhizopogon*. For example, Christmas tree farms in the Pacific Northwest could additionally produce the delicious Oregon white truffle, *Tuber gibbosum*. Thus far, however, attempts to inoculate trees with this truffle species have produced inconsistent results.

Meanwhile one of us (Claridge) has used truffles to help determine the population sizes of endangered animals in southeastern Australia—a prerequisite to developing effective protection or recovery programs for these species. He soaked foam pads in olive oil infused with aromatics of the European black Perigord truffle (a favorite of humans) to attract potoroos and other truffle-loving marsupials to stations where they were photographed by motion-sensing digital cameras. The approach detected about 50 times as many of these creatures as had been counted by the traditional method of cage trapping. If the success rates were this high with imported truffle oil, which he used because it was readily available for purchase, what might the figures be once the odors of native Australian truffles are put to the test? That question remains to be answered.

To protect endangered animals that regularly eat truffles, conservationists will have to ensure the availability of their food. This provision applies not only to those animals that depend directly on truffles but also to their predators. Thus, restoring the habitat of the threatened northern spotted owl in the Pacific Northwest requires meeting the needs of the owl's primary prey, the northern flying squirrel, which eats mostly truffles.

FAST FACTS

A Fruit of the Earth

Black Perigord truffles contain androstenol, a sex hormone found in hog saliva. Humans also produce the compound in their sweat glands.

Truffle hunters have long used female pigs to locate the fungi underground, but increasingly they are turning to dogs for assistance because the dogs are more willing than the pigs to accept an alternative food reward for their efforts.

Most commercially available truffle oils are flavored synthetically with laboratory-made compounds such as 2,4-dithiapentane, one of many molecules that give Italian white truffles their distinctive aroma.

Some truffles contain compounds that have potent antituberculosis effects; others exhibit strong anti-inflammatory and anti-oxidant properties.

TAMING THE TRUFFLE

ALTHOUGH RESEARCHERS have learned much about the ecology of truffles in recent decades, the science of growing them has changed little since the 1960s, when French scientists developed a greenhouse technique for adding spores of the black Perigord truffle into the potting mix of oak and hazel seedlings that are later planted in suitable sites to form truffle orchards, or *truffières*. Under ideal growing conditions, the *truffières* can produce a marketable crop in four to five years.

After many failed attempts, similar *truffières* were finally established in the U.S. in the 1980s. Today the most productive truffle grower in North America is Tennessee Truffle, founded by Tom Michaels, who studied with Trappe as a graduate student. Michaels's *truffière* has produced up to 100 kilograms per year of black Perigord truffles since 2008. To get these results, he pays careful attention to the soil, adding lime every year to keep it alkaline and high in calcium. Australia, New Zealand and Argentina now produce Perigord truffles as well.

In stark contrast to the triumphs of Perigord truffle farming, efforts to cultivate the most highly prized truffle species—the Italian white truffle that Mirko and Clinto were hunting, which has an especially intense aroma—have failed. For reasons unknown, this species so far refuses to produce truffles in *truffières*. Sequencing of the complete genome of this king of truffles may yield clues on how to surmount the barrier to producing it on command.

Concurrently, truffles may become more prevalent even without cultivation: as the earth warms, the hotter, drier habitats that many truffles favor will spread, setting the stage for increased production and accelerated evolution. Climate change, then, may yield a benefit for some: more truffles for men and beasts. SA

James M. Trappe is scientist emeritus at the U.S. Forest Service and a professor of forest science at Oregon State University. He has discovered more than 200 new truffle species on five continents. Jim wonders why anyone would go fishing instead of seeking new truffles.
Andrew W. Claridge is a senior research scientist with the New South Wales Office of Environment and Heritage and a visiting fellow at the University of New South Wales in Australia. He has studied interrelationships among mammals and the fungi they eat for more than 20 years. His favorite hobby is fishing.

MORE TO EXPLORE

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