

Hidden within our Botanical Richness, a Treasure Trove of Fungal Endophytes

by A. Elizabeth Arnold¹ Photos courtesy the author.

To anyone with an eye for diversity, Arizona's botanical richness is nothing short of inspiring. Unbeknownst to many botanists, however, is the fact that Arizona's plants harbor upon and within their tissues an even greater richness of fungi. Some fungi associate with plant roots, forming mycorrhizae that increase nutrient uptake, enhance plant water relations, and in some cases protect host plants from disease. Others grow with algae or cyanobacteria to form lichens that occur on exposed surfaces such as bark or, under conditions of high humidity, leaves. Still more fungi can be found as spores or hyphae on the exterior surfaces of plant tissues, as single cells in the nectar of flowers, as agents of decay when plant tissues die, or as pathogens that cause disease in leaves, stems, roots and reproductive organs.

In addition to these relatively well-recognized fungi, yet another group lives in close association with plants and plays an array of ecological roles that, for plants in Arizona, remain largely unexplored. These are **fungal endophytes** — fungi that live within plant tissues such as stems and leaves without inducing symptoms of disease.

Recent studies indicate that Arizona's botanical richness corresponds to a tremendous diversity of fungal endophytes, few of which have been studied in any detail. Endophytes are closely related to many pathogens that infect the same tissues, but probably have evolved into non-virulent inhabitants of plants multiple times across the fungal tree of life. In natural and human-made ecosystems they can protect plants against disease and herbivory, enhance plant growth under challenging abiotic conditions, and provide the raw materials for a remarkable number of applications in biological control, biofuels, and pharmaceutical bioprospecting. To date, however, only a vanishing minority of Arizona's plants have been screened for their endophytic fungi.

The placement of our state at the confluence of several biogeographic and botanical provinces makes Arizona an exceptionally exciting place to study the diversity, ecology, and potential applications of fungal endophytes. Here, I briefly introduce these ubiquitous symbionts of Arizona's plants.

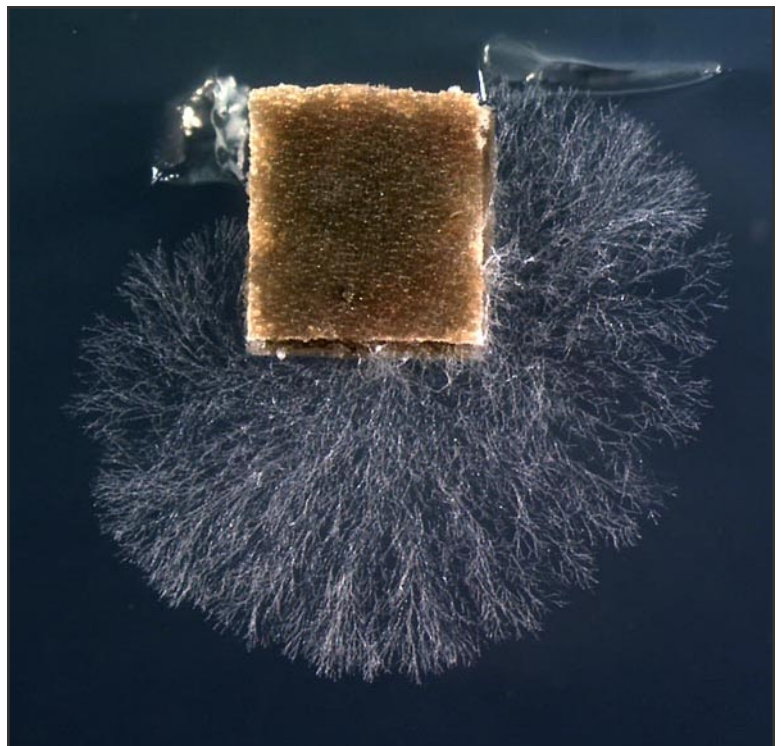


figure 1 Endophytic fungus emerging from a surface-sterilized leaf piece (ca. 0.07x 0.07 inches) on 2% malt extract agar.

Introduction to fungal endophytes

When first introduced in 1866, “endophyte” was used broadly to refer to any organism found within tissues of living plants. Subsequent re-definitions led to confusion regarding the meaning of the term, but modern mycologists generally agree that endophytes are *organisms that colonize internal plant tissues without causing apparent harm to their host*.

Research in my group (<http://www.endophytes.org>) focuses on foliar fungal endophytes – those endophytes that occur inside healthy leaves and other photosynthetic organs. The vast majority of foliar endophytes (hereafter, endophytes) are members of the Ascomycota, the most diverse phylum of fungi.

Endophytes have been recovered from plants in hot deserts, Arctic tundra, mangroves, temperate and tropical forests, grasslands and savannas, and croplands. They are known from mosses and other nonvascular plants, ferns and other seedless plants, conifers, and flowering plants. Every plant species examined to date is host to at least one endophytic fungus, and many plant species associate with hundreds to thousands of endophyte species across their geographic ranges. In Arizona, we have recovered endophytes from hosts as geographically, phylogenetically, and ecologically divergent as mosses along creeks in the Chiricahuas, liverworts from the Tucson Mountains, ferns growing among rocks in oak/juniper woodlands below the Mogollon Rim, ponderosa

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pinus at the top of the Chuska Mountains, cultivated cypresses on the University of Arizona campus, cypresses near burned areas in the Santa Catalinas, and medicinal annuals in the central Navajo Nation — just to name a few. Other researchers have recovered endophytes from a variety of cacti, oaks, and grasses throughout the state.

Because endophytes live within healthy tissue, their presence is unapparent: they manifest no evident, external symptoms or reproductive structures on plant tissues. The tiny, threadlike growth of these fungi (i.e., hyphae) can only rarely be visualized in leaf tissues without chemical fixation — and when observed *in planta* they lack sufficient characters to be identified. Therefore, endophytes typically are studied by culturing: plant tissues are surface-sterilized using chemical agents (e.g., ethanol, sodium hypochlorite) and small pieces are placed under sterile conditions on a nutrient medium. Over time, fungi grow from these leaf pieces into the surrounding medium, and can be isolated in pure culture (Fig. 1).

Typical media include malt extract agar, corn meal agar, potato dextrose agar, and other nutrient sources derived from plants. In some cases antibiotics or other chemical agents are added to restrict bacterial growth and slow the growth of ‘weedy’ fungi. Often, the fungi that are less ‘weedy’ — that is, less cosmopolitan, and more slowly growing — are more interesting for drug discovery and other applications.

The proportion of tissue pieces yielding endophytes in culture is used as a measure of infection frequency, which can be compared among hosts and sites. Because the endophytes found in most plants are horizontally transmitted (i.e., spread contagiously among plants, rather than passed from maternal plants to offspring), older leaves tend to bear more endophytes than younger leaves. Infection frequencies generally follow a broad latitudinal gradient, peaking in moist to wet tropical forests and reaching their lowest in boreal and tundra ecosystems. At smaller scales, though, local factors such as humidity, exposure to UV, and seasonality all shape infection frequencies, with plants in wetter, warmer, and more sheltered places more frequently harboring higher abundances of endophytes.

Once in pure culture, endophytes are examined for reproductive structures such as sexual or asexual spores. However, most endophytes do not readily sporulate in culture, so they lack the characteristics needed to identify them. Accordingly, endophytes often are grouped to “morphotypes” based on whole-colony or vegetative characteristics, and/or are identified using DNA sequence data. Molecular approaches are also being used to highlight

the existence of unculturable fungi, which can be sequenced directly from plant tissues.

Following isolation (Fig. 2), endophytes can be stored as in sterile water and archived in a culture collection. Our laboratory, in conjunction with the Robert L. Gilbertson Mycological Herbarium (part of ARIZ), is developing the needed infrastructure to maintain these important voucher collections. Once safely stored and catalogued, these living cultures — which often can be maintained at room temperature — can be revived for further study or used for DNA sequencing, providing an exceptionally useful resource for future work.

To date, my research group has collected nearly 14,000 cultures of endophytic fungi, of which >4000 come from plants in Arizona.

Distribution and diversity of endophytes

One of the most compelling features of fungal endophytes is their exceptional diversity. This is true both at a global scale, where more than 1 million endophyte species are thought to exist, and at the scale of individual leaves, plants, and locations. Although we do not yet have a definitive understanding of the number of endophytes associated with a single plant or single species — nor can we reliably estimate the number of endophyte species present in Arizona — we now have an accumulation of studies suggesting that their diversity in our state is immense.

For example, to date we have identified 65 species of endophytes among the first 92 cultures we have obtained from *Cupressus arizonica* (Arizona cypress). These fungi, recovered from foliage, are distinct from all fungi recovered previously from twigs or roots of *C. arizonica*. A similar sampling effort that recovered 127 cultures from *Juniperus deppeana* (alligator juniper) has yielded roughly 90 species. In each case, samples were collected from three sites (e.g., the Chiricahua Mountains, Mt. Lemmon, and near Prescott, AZ for *J. deppeana*), and in each case less than 5% of species were found in more than one sampling locality.

Further studies with other hosts in these sites reveal that oaks and pines have similarly diverse endophytes. Some of these endophytes are host-specific, but many are shared among even distantly related hosts. However, endophytes that are common in one host species are often vanishingly rare in others. Moreover, the fungal families we find most frequently in healthy foliage of the cypresses and junipers are distinct from those found in co-occurring oaks and pines — and those endophytes, in turn, differ from those most commonly found in mosses, liverworts, ferns, grasses, and flowering plants such as cacti in our study sites.

Thus far, our surveys indicate that geographic locality matters in terms of species diversity of endophytes, and host taxonomy and locality underlie the composition of

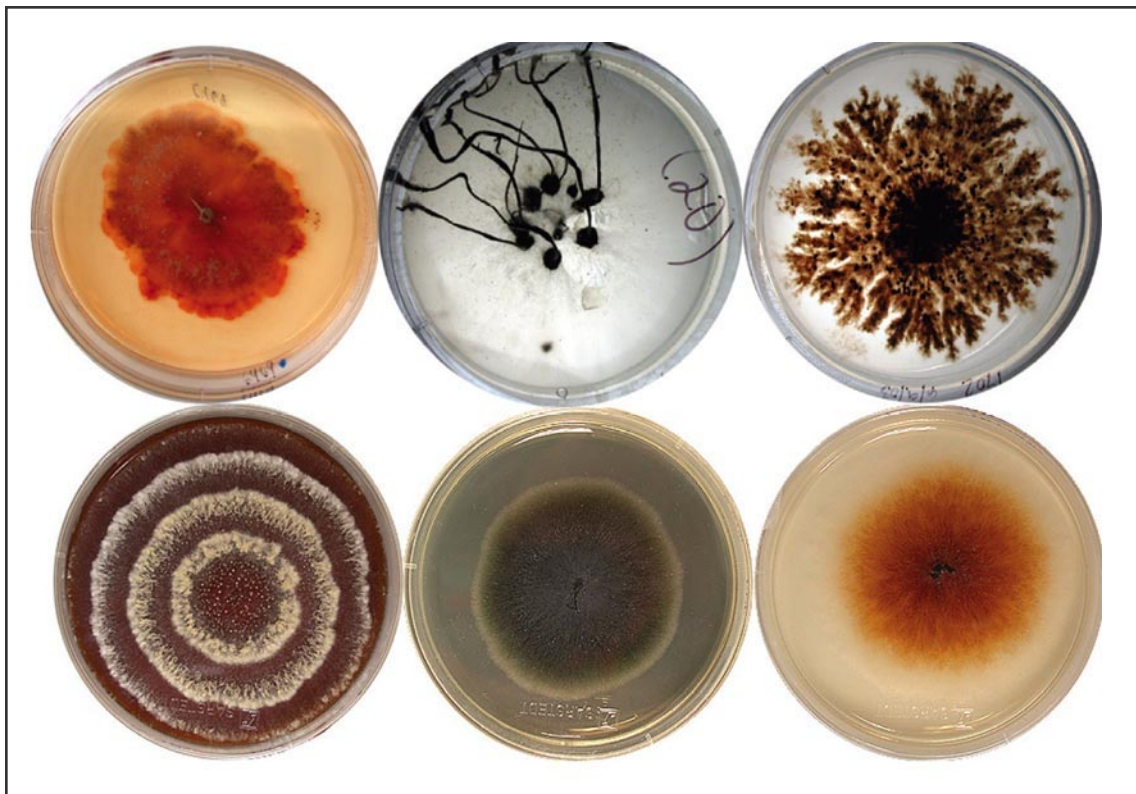


figure 2 Array of endophytic fungi in culture on 2% malt extract agar.

endophyte communities. The movement of plants by humans also plays a role: a study in southern Arizona suggests that non-native trees harbor more abundant but less diverse endophytes, and less specialized endophytes, relative to closely related natives.

Ecology and applications of endophytes

Because most endophytes isolated to date in Arizona represent undescribed species, we are at the tip of the iceberg in terms of understanding their ecological roles. Why do they not act as virulent pathogens of their hosts? Are they distinct from decay fungi that break down leaves and wood? Where do they persist in the environment when not in living leaves? What are the costs and benefits of their presence within host plants?

Each of these questions is a topic of current interest in endophyte biology, and with the exception of pioneering work by a few researchers (*e.g.*, Stanley Faeth, Arizona State University, who has examined endophyte symbioses in Arizona's native grasses and oaks), we know woefully little about the interactions, evolutionary history, and ecology of the endophytes that inhabit our state's native and introduced plants. Studies conducted elsewhere have shown that some endophytes enhance resistance to pathogens and protect plants against insect pests and herbivorous mammals, whereas others increase susceptibility to drought or decrease plants' photosynthetic efficiency. Studies in the temperate zone by researchers such as Regina Redman and Rusty Rodriguez have demonstrated that

some endophytes can confer heat tolerance or salinity tolerance on their hosts.

Together, these findings have inspired collaborations between endophyte biologists and applied scientists working in natural products chemistry, medicinal drug discovery, biological control, and industrial sciences. Endophytes are now recognized as a remarkably promising source of new compounds, new medicines, alternatives to pesticides, and new products for use in bioremediation and biofuels development. Recently, several novel compounds with anti-cancer activity were recovered from an endophyte from Mt. Lemmon; other endophytes in our collection are adept at degrading cellulose, a key for alternative fuel development. Arizona's rich array of endophytes is thus one of our little-known but exceptional natural resources.

Perhaps more than anything, early studies of endophytes in our state and beyond suggest an array of ecological roles, biochemical attributes, and potential uses that are at least as diverse as the endophytes themselves. Far from discouraging, this highlights the draw these fungi have for me as a scientist and a student of Arizona's biodiversity — and the wide-open nature of endophyte biology for those interested in understanding cryptic and previously unexplored interactions between plants and their little-known symbionts.

