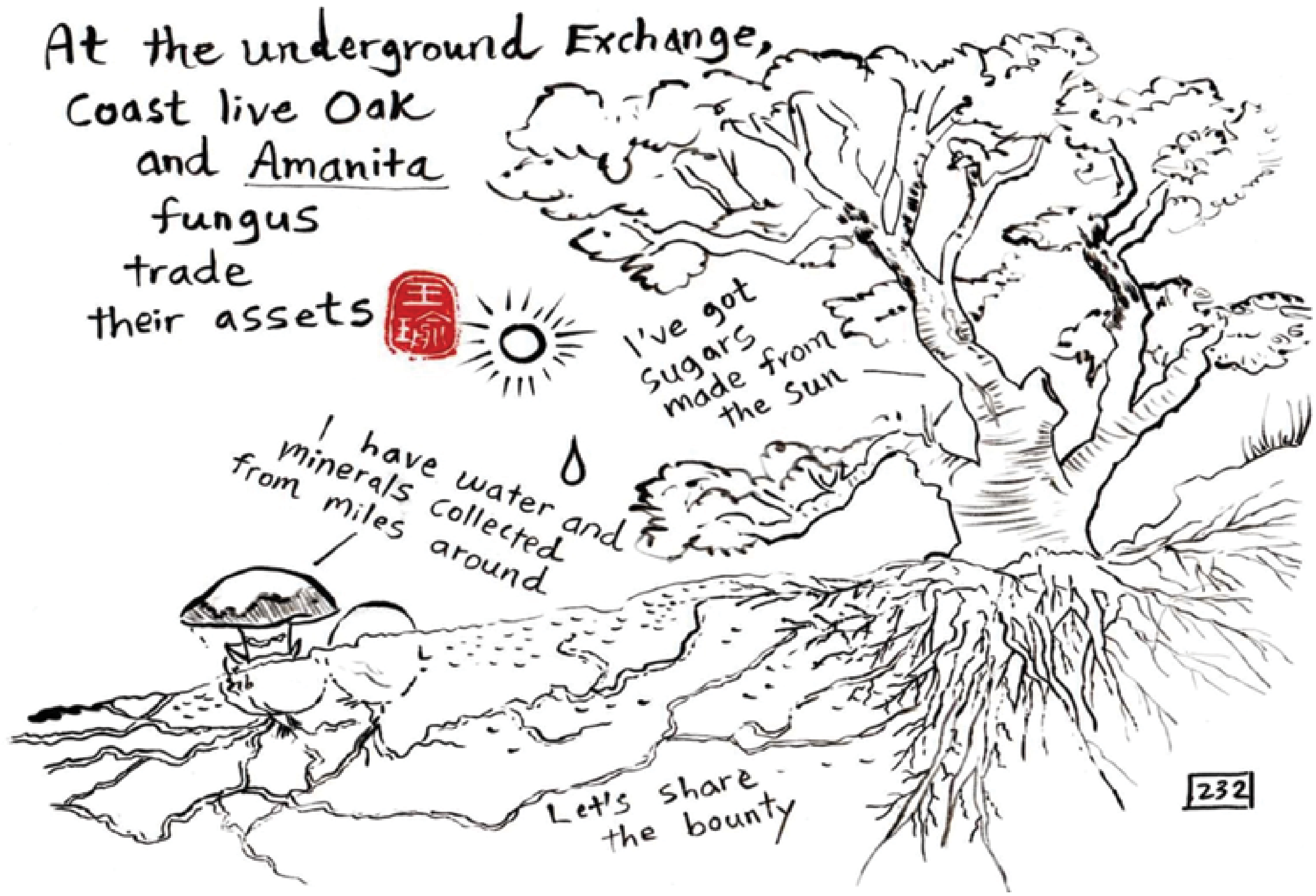

"Plants form a single mycorrhizal type … they are either AM or EM. This is what we've all been taught. But is this true?"

Dual-Mycorrhizal Plants or Dueling Mycorrhizal Fungi: How Common Are Dual-Mycorrhizal Associations?

Britt A. Bunyard

"Dual-mycorrhizal plants are more common than previously thought,"
-from Teste et al., 2020; *New Phytologist* 225: 1835–1851.

Although not entirely understood, it is now well known that most plant groups (including all tree groups, so far as is known) form symbiotic associations at their roots with fungi. These mycorrhizal associations are obligate to the plant as well as fungus. The plant benefits by way of vastly increased absorptive surface area, with the fungus doing much of the work in bringing in moisture as well as vital nutrients like phosphorus and nitrogen. The fungus, not being an autotroph, benefits by way of the plant supplying much if not all of its carbohydrate needs.

The mycorrhizal lifestyle has evolved several different times within the Fifth Kingdom. We categorize mycorrhizal symbioses, in general, into two major groups defined more by the physiology of the relationship than on the taxonomy of the fungus. Mushroom-forming fungi that we are more familiar with form ectomycorrhizal associations, or EM. The majority of fungi form arbuscular mycorrhizal associations, or AM (when I was a student we called them endomycorrhizals). All mycorrhizal fungi, as the name implies, are associated with—indeed grow within the tissues of—their host plant's root. EM fungi do not penetrate the cells of plant hosts. AM fungi do penetrate their host's cells, forming arbuscules (or some

Text continues on page 35.

EXAMPLES OF CONFIRMED PLANT SPECIES WITH DUAL STATUS								
Family	Genus	Number of confirmed species	Spp1	Spp2	Spp3	Spp4	Spp5	Spp6
Adoxaceae	*Viburnum*	1	*Viburnum acerifolium*					
Asparagaceae	*Thysanotus*	2	*Thysanotus juncifolius*	*Thysanotus patersonii*				
Asteraceae	*Gnephosis*	1	*Gnephosis tenuissima*					
Asteraceae	*Leptorhynchos*	2	*Leptorhynchos squamatus*	*Leptorhynchos waitzia*				
Asteraceae	*Millotia*	1	*Millotia muelleri*					
Asteraceae	*Podotheca*	1	*Podotheca angustifolia*					
Asteraceae	*Pogonolepis*	1	*Pogonolepis muelleriana*					
Asteraceae	*Rutidosis*	1	*Rutidosis leptorhynchoides*					
Asteropeiaceae	*Asteropeia*	3	*Asteropeia densiflora*	*Asteropeia micraster*	*Asteropeia multiflora*			
Betulaceae	*Alnus*	7	*Alnus incana*	*Alnus rubra*	*Alnus sinuata*	*Alnus viridis*	*Alnus mandshurica*	*Alnus hirsuta*
Betulaceae	*Betula*	4	*Betula lutea*	*Betula papyrifera*	*Betula populifolia*	*Betula pumila*		
Casuarinaceae	*Allocasuarina*	6	*Allocasuarina muelleriana*	*Allocasuarina verticillata*	*Allocasuarina humilis*	*A. littoralis*	*Allocasuarina torulosa*	*Allocasuarina fraseriana*
Casuarinaceae	*Casuarina*	5	*Casuarina equisetifolia*	*Casuarina cunninghamiana*	*Casuarina glauca*	*C. junghuhniana*	*Casuarina obesa*	
Cistaceae	*Fumana*	1	*Fumana procumbens*					
Cistaceae	*Helianthemum*	2	*H. chamaecistus*	*H. ovatum*				
Cornaceae	*Cornus*	2	*Cornus racemosa*	*Cornus officinalis*				
Cupressaceae	*Chamaecyparis*	1	*Chamaecyparis lawsoniana*					
Dipterocarpaceae	*Marquesia*	1	*Marquesia acuminata*					
Dipterocarpaceae	*Monotes*	1	*Monotes kerstingii*					
Dipterocarpaceae	*Shorea*	3	*Shorea robusta*	*Shorea teysmanniana*	*Shorea balangeran*			
Elaeagnaceae	*Shepherdia*	1	*Shepherdia canadensis*					
Fabaceae	*Acacia*	24	*Acacia stricta*	*Acacia rigens*	*Acacia ulicifolia*	*Acacia linifolia*	*Acacia obtusifolia*	*Acacia suaveolens*
Fabaceae	*Afzelia*	3	*Afzelia pachyloba*	*Afzelia africana*	*Afzelia bipindensis*			
Fabaceae	*Anthonotha*	1	*Anthonotha fragrans*					
Fabaceae	*Berlinia*	1	*Berlinia sp.*					
Fabaceae	*Brachystegia*	2	*Brachystegia spiciformis*	*B. cynometroides*				
Fabaceae	*Chorizema*	2	*Chorizema cordatum*	*Chorizema diversifolium*				
Fabaceae	*Dicymbe*	2	*Dicymbe corymbosa*	*Dicymbe altsonii*				
Fabaceae	*Didelotia*	1	*Didelotia africana*					
Fabaceae	*Dillwynia*	3	*Dillwynia floribunda*	*Dillwynia hispida*	*Dillwynia parvifolia*			
Fabaceae	*Eutaxia*	1	*Eutaxia diffusa*					
Fabaceae	*Gastrolobium*	2	*Gastrolobium capitatum*	*Gastrolobium celsianum*				
Fabaceae	*Gilbertiodendron*	2	*Gilbertiodendron dewevrei*	*Gilbertiodendron sp.*				
Fabaceae	*Gompholobium*	5	*G. marginatum*	*G. tomentosum*	*G. venustum*	*G. villosum*	*Gompholobium latifolium*	
Fabaceae	*Jacksonia*	2	*Jacksonia floribunda*	*Jacksonia scoparia*				
Fabaceae	*Julbernardia*	1	*Julbernardia seretii*					
Fabaceae	*Mirbelia*	3	*Mirbelia dilatata*	*Mirbelia rubiifolia*	*Mirbelia rubiifolia*			
Fabaceae	*Oxylobium*	4	*Oxylobium ellipticum*	*Oxylobium linariifolium*	*Oxylobium capitatum*	*O. lanceolatum*		
Fabaceae	*Pericopsis*	1	*Pericopsis angolensis*					
Fabaceae	*Platylobium*	1	*Platylobium obtusangulum*					
Fabaceae	*Pultenaea*	5	*Pultenaea daphnoides*	*Pultenaea mollis*	*Pultenaea obovata*	*Pultenaea scabra*	*Pultenaea elliptica*	
Fabaceae	*Viminaria*	1	*Viminaria juncea*					

Table continues on page 34.

Table 1. Confirmed dual-mycorrhizal plants (after Teste et al., 2020; *New Phytologist* 225: 1835–1851). This list includes 89 plant genera within 32 families confirmed as dual-mycorrhizal plants, based on published records showing arbuscules/coils for arbuscular mycorrhizas and Hartig net/transfer cells for ectomycorrhizas. Many of these plant families include common forest tree species, but remain poorly studied as far as mycorrhizal status goes. While these genera contain more than 7,000 species in total (the vast majority—nearly 85%—are woody taxa), only a small proportion (238 species) have confirmed dual-mycorrhizal status. Many times this number are presumed, but not confirmed, to be dual-mycorrhizal (see Table 2).

Table continued from page 33.

EXAMPLES OF CONFIRMED PLANT SPECIES WITH DUAL STATUS								
Family	**Genus**	**Number of confirmed species**	**Spp1**	**Spp2**	**Spp3**	**Spp4**	**Spp5**	**Spp6**
Fagaceae	*Fagus*	1	*Fagus grandifolia*					
Fagaceae	*Quercus*	6	*Quercus rubra*	*Quercus agrifolia*	*Quercus imbricaria*	*Quercus alba*	*Quercus coccinea*	*Quercus palustris*
Goodeniaceae	*Brunonia*	1	*Brunonia australis*					
Goodeniaceae	*Scaevola*	1	*Scaevola crassifolia*					
Haloragaceae	*Gonocarpus*	1	*Gonocarpus mezianus*					
Myricaceae	*Myrica*	2	*Myrica californica*	*Myrica cerifera*				
Myrtaceae	*Agonis*	1	*Agonis flexuosa*					
Myrtaceae	*Baeckea*	2	*Baeckea behrii*	*Baeckea crassifolia*				
Myrtaceae	*Callistemon*	1	*C. macropunctatus*					
Myrtaceae	*Calothamnus*	2	*Calothamnus quadrifidus*	*Calothamnus sanguineus*				
Myrtaceae	*Calytrix*	1	*Calytrix tetragona*					
Myrtaceae	*Eremaea*	2	*Eremaea asterocarpa*	*Eremaea pauciflora*				
Myrtaceae	*Eucalyptus*	25	*Eucalyptus diversicolor*	*Eucalyptus marginata*	*Eucalyptus todtiana*	*E. incrassata*	*Eucalyptus dumosa*	*Eucalyptus calophylla*
Myrtaceae	*Kunzea*	2	*Kunzea parvifolia*	*Kunzea ericoides*				
Myrtaceae	*Leptospermum*	5	*L. juniperinum*	*L. liversidgei*	*L. scoparium*	*L. myrsinoides*	*Leptospermum nitidum*	
Myrtaceae	*Melaleuca*	6	*Melaleuca lateritia*	*Melaleuca scabra*	*Melaleuca systena*	*Melaleuca uncinata*	*Melaleuca decussata*	*Melaleuca leuropoma*
Myrtaceae	*Pericalymma*	1	*Pericalymma ellipticum*					
Myrtaceae	*Pileanthus*	1	*Pileanthus filifolius*					
Myrtaceae	*Psidium*	1	*Psidium cattleyanum*					
Myrtaceae	*Thryptomene*	1	*Thryptomene calycina*					
Myrtaceae	*Tristania*	1	*Tristania beccarii*					
Nyctaginaceae	*Pisonia*	1	*Pisonia grandis*					
Oleaceae	*Fraxinus*	1	*Fraxinus uhdei*					
Oleaceae	*Syringa*	1	*Syringa pekinensis*					
Oweniidae	*Owenia*	1	*Owenia sp.*					
Phyllanthaceae	*Uapaca*	8	*Uapaca staudtii*	*Uapaca guineensis*	*Uapaca somon*	*Uapaca staudtii*	*Uapaca bojeri*	*Uapaca ferruginea*
Pinaceae	*Pinus*	1	*Pinus muricata*					
Pinaceae	*Pseudotsuga*	1	*Pseudotsuga menziesii*					
Pinaceae	*Tsuga*	1	*Tsuga heterophylla*					
Polygalaceae	*Coccoloba*	1	*Coccoloba warmingii*					
Ranunculaceae	*Pulsatilla*	1	*Pulsatilla patens*					
Rhamnaceae	*Cryptandra*	2	*Cryptandra arbutiflora*	*Cryptandra tomentosa*				
Rhamnaceae	*Pomaderris*	5	*Pomaderris apetala*	*Pomaderris aspera*	*Pomaderris elliptica*	*P. eriocephala*	*Pomaderris obcordata*	
Rhamnaceae	*Spyridium*	3	*Spyridium parvifolium*	*Spyridium vexilliferum*	*Spyridium globulosum*			
Rhamnaceae	*Stenanthemum*	1	*Stenanthemum notiale*					
Rhamnaceae	*Trymalium*	2	*Trymalium floribundum*	*Trymalium d'altonii*				
Rosaceae	*Adenostoma*	1	*Adenostoma fasciculatum*					
Rosaceae	*Crataegus*	1	*Crataegus monogyna*					
Salicaceae	*Populus*	6	*Populus fremontii*	*Populus nigra*	*Populus tremula*	*Populus deltoides*	*Populus grandidentata*	*Populus tremuloides*
Salicaceae	*Salix*	6	*Salix glauca*	*Salix humboldtiana*	*Salix nigra*	*Salix nigricans*	*Salix sitchensis*	*Salix alba*
Sapindaceae	*Dodonaea*	1	*Dodonaea viscosa*					
Sapotaceae	*Manilkara*	1	*Manilkara sp.*					
Sarcolaenaceae	*Leptolaena*	4	*Leptolaena pauciflora*	*Leptolaena multiflora*	*Leptolaena sp1*	*Leptolaena sp2*		
Sarcolaenaceae	*Sarcolaena*	3	*Sarcolaena eriophora*	*Sarcolaena grandiflora*	*Sarcolaena multiflora*			
Sarcolaenaceae	*Schizolaena*	3	*Schizolaena elongata*	*Schizolaena exinvolucrata*	*Schizolaena sp.*			
Thymelaeaceae	*Pimelea*	1	*Pimelea spathulata*					
Ulmaceae	*Ulmus*	2	*Ulmus japonica*	*Ulmus laciniata*				

Text continued from page 32.

similar structure) that have intimate contact with the interior of host plant cells. There are some twists on this scheme, and exceptions of course: ericoid mycorrhizas are interesting; then there are associations with *Monotropa* plants and their kin; orchids put their own spin on mycorrhizal associations, etc.

Plants are generally considered to form a single mycorrhizal type. That is, plants are either AM or EM. This is what we've all been taught. Oh, there are a few exceptions, those oddball plants that can form mycorrhizas with both … however, this is a tiny minority group. But is it true? And if it's true, why? Why should plants choose one style of root symbiosis over another? Does anyone look for this stuff in nature?

There most definitely are plants that can form both arbuscular mycorrhizas and ectomycorrhizas, either simultaneously within the same root system or at different life stages or in different environments; we call these "dual-mycorrhizal" plant species. As pointed out, dual-mycorrhizal plants have traditionally been considered uncommon and unusual. But are dual-mycorrhizal plants really uncommon?

To find out, I needed to seek the opinions from well-regarded mycologists on both sides of the aisle—from a field mycologist as well as a molecular mycologist. The opportunity presented itself last summer at the Annual Meeting of the Mycological Society of America. Following the keynote lecture, the audience of academic mycologists applauded and headed for the exits (keen on getting an early place in the queue for the free wine and snacks at the evening's social, just outside). When all of a sudden there ensued a heated debate near the podium. The keynote lecturer thanked those in attendance and made a hasty retreat. The argument continued and I was not surprised to see the two verbal pugilists involved: Drs. Ivanna Forré and Gene Jøkkě. That the two will have at least one confrontation at the annual MSA conference is guaranteed. If you put the two in the same room together, there will be a tussle. Both are legendary brilliant (and headstrong) mycologists. Their annual sparring is equally legendary. Some of their debates have been featured in the pages of FUNGI (e.g., "Whither the field mycologist?" in vol.2 no.3; 2009).

Professor Ivanna Forré, Curator of Mycology Collections at an esteemed museum in the Midwest, and who also serves as Adjunct Professor at an equally well-known university in Chicago, was classically trained in mycology and is world-renowned for her work on mycorrhizal fungi. Doctor Gene Jøkkě, the endowed Chair of the Molecular and Cell Biology Department of a world-renowned university in Europe that everybody knows about is responsible for leading the molecular mycology revolution. But to say that the two great minds don't always see eye to eye would be a vast understatement. While I missed the opening salvos of their contretemps, I was able to record what follows and they have graciously allowed me to share with the readers of FUNGI.

Jøkkě: "… but you're surely not saying that dual-mycorrhizal plants are anything more than an outlier—an anomaly—in nature, are you?"

Forré: "That's exactly what I'm saying."

Jøkkě: "So, poplars and willows… Alders. Eucalyptus. [Counting on his fingers.] That's about it. I cannot even think of others can you?"

Forré: "Yes, many. In fact, it turns out that a lot and maybe the majority of plant groups feature species that are dual-mycorrhizal."

Jøkkě interrupted: "—I mean, real plants, tr—"

Forré cut him off: "Yes trees! And it makes sense that so many groups of plants should form symbioses with many kinds of fungi, as they've been coevolving for a very long time. With the emergence of the first terrestrial plants about 400 million years ago, soil fungi of the Glomeromycotina and Mucoromycotina began to form structures in the roots of early Devonian plants. We can see such structures in fossils. This has been published. One of these structures resembled arbuscules, forming what is now commonly called arbuscular mycorrhizas."

Jøkkě: "And when did the 'ectos' come along?"

Forré: "Yes, I was getting to that. Ectos … the EM fungi. Well, as the land masses evolved and ecosystems developed, so did other fungi. At about 190 million years ago, multiple groups of saprotrophic fungi, such as brown- and white-rot fungi from the Basidiomycota, Ascomycota, and Endogonales from the Mucoromycotina began to form a new type of association. At first, this was with gymnosperm trees, for example *Gnetum* species. These were the first ectomycorrhizas. But I must point out that key fungal structures such as Hartig nets, commonly characterizing the EM fungi today, were only first seen in fossil records of Pinaceae roots some 50 million years ago. Then of course other mycorrhizal types also evolved later on and within specific lineages of plants, including the orchid and ericoid mycorrhizas. Mycorrhizal symbioses are so widespread today … we could not imagine a terrestrial world without them."

Jøkkě: "It is well recognized that mycorrhizal fungi are invaluable to plants. They are in large part responsible for improving the mineral nutrition of host plants that need to cope with low nitrogen and phosphorus concentrations in soil. Mycorrhizas can also benefit plants by helping them tolerate drought stress, heavy metals, and pathogens, via both nutritional and direct effects. With this we are in agreement. Moreover, we agree that today, most terrestrial plants require an association with at least one type of mycorrhiza to adequately grow and complete their life cycle in natural ecosystems with AM plants being the most common. And I know there are some documented cases of dual-mycorrhizal plants … they are interesting as curiosities but probably not much use to science."

Forré: "Curiosities?! Dual-mycorrhizal plants are more than just curiosities … they are very underappreciated and this probably accounts for why they are so poorly known. They offer great potential in determining which mycorrhizal type provides the greatest benefits or costs to their host plants and the benefits or costs of specialization on one type. They also offer insights into the abiotic factors that 'drive' AM and EM root colonization levels within the same host plant, thus providing evidence of how the two main mycorrhizal types partition both fundamental niches, the root system and soil nutrients. And one more thing, they also highlight the important functions mycorrhizas can play in ecosystems, in particular during rapid abiotic changes and ecological restoration."

Jøkkě: "But surely you agree that some of the published determinations of dual-mycorrhizal types were erroneous …

Text continues on page 38.

PLANT HOST		
Family	**Genus**	**Common Name**
Adoxaceae	***Sambucus***	Elderberry
Adoxaceae	***Viburnum***	Viburnum or Nannyberry
Anacardiaceae	***Rhus***	Sumac
Apiaceae	***Platysace***	
Aquifoliaceae	***Ilex***	Holly
Asparagaceae	***Thysanotus***	
Asteraceae	***Angianthus***	
Asteraceae	***Gnephosis***	
Asteraceae	***Crepis***	
Asteraceae	**Gnaphalieae (Tribe)**	
Asteraceae	***Helichrysum***	
Asteraceae	***Helipterum***	
Asteraceae	***Homogyne***	
Asteraceae	***Isoetopsis***	
Asteraceae	***Lactuca***	
Asteraceae	***Leptorhynchos***	
Asteraceae	***Millotia***	
Asteraceae	***Podolepis***	
Asteraceae	***Podotheca***	
Asteraceae	***Pogonolepis***	
Asteraceae	***Rutidosis***	
Asteraceae	***Waitzia***	
Asteropeiaceae	***Asteropeia***	
Betulaceae	***Alnus***	Alder
Betulaceae	***Betula***	Birch
Betulaceae	***Carpinus***	Hornbeam
Betulaceae	***Corylus***	Hazelnut
Betulaceae	***Ostrya***	Hophornbeam
Betulaceae	***Ostryopsis***	
Bignoniaceae	***Jacaranda***	Jacaranda
Boryaceae	***Borya***	
Brassicaceae	***Cochlearia***	
Buxaceae	***Buxus***	
Campanulaceae	***Campanula***	
Campanulaceae	***Isotoma***	
Campanulaceae	***Lobelia***	
Caprifoliaceae	***Diervilla***	
Caryophyllaceae	***Silene***	
Casuarinaceae	***Allocasuarina***	
Casuarinaceae	***Casuarina***	She-oak
Cistaceae	***Cistus***	Rockrose
Cistaceae	***Fumana***	
Cistaceae	***Helianthemum***	
Cornaceae	***Cornus***	Dogwood

PLANT HOST		
Family	**Genus**	**Common Name**
Cupressaceae	***Chamaecyparis***	Cypress
Cupressaceae	***Cryptomeria***	Japanese Cedar
Cupressaceae	***Cupressus***	Cypress
Cupressaceae	***Juniperus***	Juniper
Cyperaceae	***Kobresia***	Sedge
Dipterocarpaceae	***Anisoptera***	
Dipterocarpaceae	***Hopea***	
Dipterocarpaceae	***Cotylelobium***	
Dipterocarpaceae	***Dipterocarpus***	
Dipterocarpaceae	***Dryobalanops***	
Dipterocarpaceae	***Marquesia***	
Dipterocarpaceae	***Monotes***	
Dipterocarpaceae	***Shorea***	
Dipterocarpaceae	***Vatica***	
Dryopteridaceae	few genera	
Elaeagnaceae	***Elaeagnus***	
Elaeagnaceae	***Shepherdia***	
Ericaceae	***Astroloma***	
Fabaceae	***Acacia***	Acacia, Mimosa
Fabaceae	***Afzelia***	
Fabaceae	***Aldina***	
Fabaceae	***Anthonotha***	
Fabaceae	***Berlinia***	
Fabaceae	***Bikinia***	
Fabaceae	***Brachystegia***	
Fabaceae	***Calliandra***	
Fabaceae	***Cercis***	Redbud
Fabaceae	***Chorizema***	
Fabaceae	***Daviesia***	
Fabaceae	***Dicymbe***	
Fabaceae	***Didelotia***	
Fabaceae	***Dillwynia***	
Fabaceae	***Eutaxia***	
Fabaceae	***Gastrolobium***	
Fabaceae	***Gilbertiodendron***	
Fabaceae	***Gleditsia***	Honey Locust
Fabaceae	***Gompholobium***	
Fabaceae	***Hardenbergia***	
Fabaceae	***Inga***	
Fabaceae	***Jacksonia***	
Fabaceae	***Julbernardia***	
Fabaceae	***Kennedia***	
Fabaceae	***Lonchocarpus***	
Fabaceae	***Mirbelia***	

Table 2. Presumed dual-mycorrhizal plants (after Teste et al., 2020; *New Phytologist* 225: 1835–1851). This list includes 211 plant genera within 67 families that have previously been considered to have a dual-mycorrhizal status, based on published records indicating both arbuscular and ectomycorrhizal fungal colonization of root systems. In most cases, key features were shown e.g., presence of key fungal structures like arbuscules, coils, etc. While published evidence supports these plants as dual-mycorrhizal, Teste et al. point out that many of these are not well documented, and possibly erroneous.

PLANT HOST		
Family	**Genus**	**Common Name**
Fabaceae	***Oxylobium***	
Fabaceae	***Pericopsis***	
Fabaceae	***Platylobium***	
Fabaceae	***Prosopis***	
Fabaceae	***Pultenaea***	
Fabaceae	***Robinia***	Black Locust
Fabaceae	***Tetraberlinia***	
Fabaceae	***Vicia***	
Fabaceae	***Viminaria***	
Fagaceae	***Castanea***	Chestnut
Fagaceae	***Castanopsis***	Chinquapin
Fagaceae	***Fagus***	Beech
Fagaceae	***Lithocarpus***	Tanoak
Fagaceae	***Quercus***	Oak
Fagaceae	***Trigonobalanus***	
Gnetaceae	***Gnetum***	
Goodeniaceae	***Brunonia***	
Goodeniaceae	***Dampiera***	
Goodeniaceae	***Goodenia***	
Goodeniaceae	***Scaevola***	
Grossulariaceae	***Ribes***	Currant, Gooseberry
Haloragaceae	***Gonocarpus***	
Hamamelidaceae	***Hamamelis***	Witch Hazel
Juglandaceae	***Carya***	Hickory, Pecan
Juglandaceae	***Juglans***	Walnut
Lamiaceae	***Thymus***	
Lauraceae	***Ocotea***	
Lauraceae	***Sassafras***	Sassafras
Lythraceae	***Lythrum***	
Magnoliaceae	***Liriodendron***	Tulip Tree
Malvaceae	***Lasiopetalum***	
Malvaceae	***Thomasia***	
Malvaceae	***Tilia***	Linden
Myricaceae	***Myrica***	
Myricaceae	***Morella***	
Myrtaceae	***Agonis***	
Myrtaceae	***Angophora***	
Myrtaceae	***Baeckea***	
Myrtaceae	***Callistemon***	
Myrtaceae	***Calothamnus***	
Myrtaceae	***Calytrix***	
Myrtaceae	***Campomanesia***	
Myrtaceae	***Eremaea***	
Myrtaceae	***Eucalyptus***	
Myrtaceae	***Kunzea***	
Myrtaceae	***Leptospermum***	
Myrtaceae	***Melaleuca***	
Myrtaceae	***Pericalymma***	
Myrtaceae	***Pileanthus***	

Table continues on page 38.

PLANT HOST		
Family	**Genus**	**Common Name**
Myrtaceae	***Psidium***	
Myrtaceae	***Syzygium***	
Myrtaceae	***Thryptomene***	
Myrtaceae	***Tristania***	
Nitrariaceae	***Peganum***	
Nothofagaceae	***Nothofagus***	
Nyctaginaceae	***Neea***	
Nyctaginaceae	***Pisonia***	
Nyctaginaceae	***Torrubia***	
Oleaceae	***Fraxinus***	
Oleaceae	***Olea***	
Oleaceae	***Osmanthus***	
Oleaceae	***Syringa***	
Orobanchaceae	***Melampyrum***	
Orobanchaceae	***Pedicularis***	
Oweniidae	***Owenia***	
Papaveraceae	***Sanguinaria***	
Phyllanthaceae	***Phyllanthus***	
Phyllanthaceae	***Poranthera***	
Phyllanthaceae	***Uapaca***	
Pinaceae	***Abies***	Fir
Pinaceae	***Cedrus***	Cedar
Pinaceae	***Larix***	Larch, Tamarack
Pinaceae	***Picea***	Spruce
Pinaceae	***Pinus***	Pine
Pinaceae	***Pseudotsuga***	Douglas-fir
Pinaceae	***Tsuga***	Hemlock
Platanaceae	***Platanus***	Sycamore
Poaceae	***Festuca***	
Poaceae	few genera	
Polygalaceae	***Coccoloba***	
Polygalaceae	***Comesperma***	
Polygalaceae	***Persicaria***	
Polygalaceae	***Polygonum***	
Proteaceae	***Grevillea***	
Ranunculaceae	***Pulsatilla***	
Rhamnaceae	***Cryptandra***	
Rhamnaceae	***Pomaderris***	
Rhamnaceae	***Rhamnus***	
Rhamnaceae	***Spyridium***	
Rhamnaceae	***Stenanthemum***	
Rhamnaceae	***Trymalium***	
Rosaceae	***Adenostoma***	
Rosaceae	***Cercocarpus***	
Rosaceae	***Crataegus***	Hawthorn
Rosaceae	***Dryas***	
Rosaceae	***Fragaria***	Strawberry
Rosaceae	***Galium***	
Rosaceae	***Malus***	Apple

Table continued from page 37.

PLANT HOST		
Family	**Genus**	**Common Name**
Rosaceae	***Potentilla***	Cinquefoil
Rosaceae	***Poterium***	
Rosaceae	***Prunus***	Cherry
Rosaceae	***Pyrus***	Pear
Rosaceae	***Rosa***	Rose
Rosaceae	***Rubus***	
Rosaceae	***Sorbus***	
Rubiaceae	***Ixora***	
Rubiaceae	***Opercularia***	
Rubiaceae	***Rubia***	
Salicaceae	***Populus***	
Salicaceae	***Salix***	Willow
Sapindaceae	***Acer***	Maple
Sapindaceae	***Dodonaea***	Soapberry
Sapotaceae	***Manilkara***	Sapodilla, Chicle
Sarcolaenaceae	***Leptolaena***	
Sarcolaenaceae	***Sarcolaena***	
Sarcolaenaceae	***Schizolaena***	
Saxifragaceae	***Saxifraga***	
Stylidiaceae	***Stylidium***	
Taxaceae	***Taxus***	Yew
Thymelaeaceae	***Daphne***	
Thymelaeaceae	***Pimelea***	
Ulmaceae	***Ulmus***	Elm
Urticaceae	***Cecropia***	Cecropia
Vitaceae	***Vitis***	Grape

were misdiagnosed?"

Forré: "Misdiagnosis of mycorrhizas and erroneously assigning mycorrhizal types to plant species has become a major concern recently of those who study them. Part of the problem lies in defining what is and isn't a dual mycorrhizal plant."

Jøkkě: "That's nonsense. It should be clear. I mean, we know when a plant is AM or EM, right? So—"

Forré again cut him off: "That's not quite true. There is no one strict definition of what constitutes a dual-mycorrhizal plant, in part because of a lack of clear definitions of what constitutes an AM or EM plant. There is an ongoing debate over whether functional or morphological traits are more diagnostic in making this determination.

Functionally speaking, mycorrhizal symbiosis has traditionally been defined as mostly involving the mutualistic transfer of carbon from plant to fungus and mineral nutrients from fungus to plant, yet some associations have neutral to negative effects on plant growth in spite of nutrient exchange, especially in higher fertility soil. Furthermore, it is not practical to test for nutrient exchange in the field, and fitness effects can never be evaluated on long-lived hosts. How could you thoroughly test long-lived forest trees? You cannot.

Morphologically speaking, researchers look for Hartig net for EM fungi and arbuscules for AM fungi. But this is not easy to do and not always black and white, either. For example, arbuscular mycorrhizas are typically defined by the formation of arbuscules and vesicles, but arbuscules are ephemeral and some AM fungi form neither structure. Creating further confusion, typically non-AM plants can sometimes be infected by AM fungi. In *Salsola*, for example—this is a plant in the Amaranth family—root cell penetration and short-lived arbuscule formation occurs, but the plant is nonetheless considered nonmycotrophic. And what about EM fungi that we are so familiar with? Ectomycorrhizas were first defined by Frank in 1885 on the basis of an ensheathing mantle, and the presence of a Hartig net is commonly considered a defining characteristic. Nonetheless, some authors have considered plants to be EM on the basis of a fungal mantle covering as little as a single epidermal cell. As in arbuscular mycorrhizas, atypical infection of plant species is not uncommon. For example, you are familiar with sedges. These are grass-like plants in the genus *Carex*. Curiously, at least one species of *Cortinarius* mushroom seems to form some sort of association with this sedge. *Carex* is not generally considered to be EM due to the lack of a Hartig net and no self-respecting Cort would be caught dead forming a mycorrhiza with a non-tree, but there it is.

So part of the reason that we don't know of more dual-mycorrhizal plants is that it's hard to catch them in the act … and to know what it is they're doing, when caught."

"With this, many who'd stayed behind to watch the debate (thus, passing on their chance at free snacks at the social), burst into applause!"

Forré continued: "Given the problems in fitting strict definitions of mycorrhizal types to plants, it is not surprising that defining dual-mycorrhizal plants is equally or even more problematic. There are a number of plants that are widely considered to be dual-mycorrhizal."

Jøkkě: "But are these associations actual mutualisms … do they result in positive growth responses in both partners? And arguably, more germane to our discussion, are they plants that we've ever heard of?"

Forré: "Yes and yes! Well-documented examples of dual-mycorrhizal plants—along with documented positive growth responses to both host plant and fungus—include trees familiar to all of us: *Acacia*; *Alnus* which is alder; *Eucalyptus*; *Fraxinus* which is ash; *Populus* which includes poplars, aspens, and cottonwoods; *Salix*, the willows; *Shorea* which is a group of dipterocarps. *Nothofagus* which is the dominant hardwood of many southern hemisphere forests seems to be dual-mycorrhizal. There are pines and oaks, and others. In all, there are something like 110 genera known to host both AM and EM types. Of course some of these are poorly documented or their ecological significance is slight or unknown. Furthermore, there are a number of plants that are often considered as being exclusively EM, despite periodic records of arbuscular mycorrhizas, including species in the Pinaceae and Fagaceae, the family of oaks and beeches. Yes oak … even the Coast Live Oak, *Quercus agrifolia*—familiar to everyone in California—it is a dual-mycorrhizal tree based on scientific study."

"This brought more applause!"

Forré continued: "Of course we know that dual-mycorrhizal plants are not always dualists … they may not be hosting both EM and AM fungi at the same time. Just like the AM trees familiar to us do not host the same mushroom 'flora'—if you want to call it that—throughout their lives. Young forests may host different mushroom species than middle-aged forests, and those may host different mushrooms than old-growth forests of the same tree species. It is not unusual to observe earlier colonization by AM fungi than EM fungi in lab experiments, so it's reasonable to think this happens in nature. Maybe we don't see so many mushrooms in disturbed habitats and with pioneer plants because it's the AM fungi that are there first and they don't make mushrooms, of course."

Jøkkě: "During your otherwise brilliant exposition on dual-mycorrhizal plants, you've still left one obvious question unanswered. That is, why should plants—any plant—want to be dualists?"

Forré: "Why wouldn't they?"

Jøkkě: "Ah-ha, now I've got you!" [Beaming, he took stock of the audience in rapt attention.]

It has been shown that dual-colonization is sometimes inhibitory or has no effect on plant growth relative to a single type of mycorrhiza formation. For example, you mentioned Coast Live Oak, *Quercus agrifolia*. When this species was studied, survival, biomass, and nutrient content were found to be lower in dual-colonized plants than plants colonized with a mixture of AM fungi or a single species of EM fungi. You also mentioned *Eucalyptus*. Similarly, a study looking at *Eucalyptus marginata* found seedlings were larger than nonmycorrhizal controls or AM plants, but dual-inoculated plants were no larger than controls and significantly smaller than AM or EM plants. And *Populus* is famously dual-mycorrhizal. But one study looking at *Populus fremontii* found that inoculation with a mixture of AM fungi appeared to stimulate total plant biomass compared with nonmycorrhizal controls … but a mixture of EM or EM plus AM fungi reduced root growth so much that it was not offset by a stimulation in shoot growth."

Forré: "You are correct. There can be disadvantages to dual-colonization in some cases. But keep in mind, the picture is far from complete. And despite some cases of negative responses to dual-colonization, if you compiled a list of dual-inoculation studies using dual-mycorrhizal plant species, you would find that, overall, there are more frequent positive and neutral effects than negative ones. In most studies, *Populus,* species receive a net benefit as a dualist. The same goes for *Acacia*, *Eucalyptus*, *Fraxinus*, and *Pinus*. They will typically respond positively to inoculations by both AM and EM fungi, suggesting that these genera contain species that benefit from dual-colonization."

Jøkkě: "So it sounds like the important question to ask is not why some plants are dualists, nor why aren't all plants dualists … the question to ask is when should they partner with either AM or EM, or both?"

Forré: "Exactly! Each type of mycorrhiza has well-documented benefits to plants in terms of growth, nutrient acquisition, and protection from pathogens. Therefore, the obvious question concerns why a plant would form associations with both AM and EM fungi simultaneously, consecutively, or in different environments. In certain plant species, a gradual shift from AM- to EM-type dominance occurs over time or along abiotic gradients, yet both mycorrhizal types persist. Why? Though dual-mycorrhizal status is often considered from a plant perspective, it is possible that dual-mycorrhizal status is not driven by plant benefit, but rather by fungal interactions. Maybe it's time to think 'myco-centrically.' Ectomycorrhizal colonization in predominately arbuscular mycorrhizal plants may reflect hyperpromiscuity by fungi—maybe some species just get around more."

"She smiled and paused for laughter."

Forré continued: "It is also possible that AM colonization in typically EM hosts is not necessarily beneficial, but rather represents a relict of the evolutionary past. The EM status has evolved in approximately 30 independent lineages of plants, with all but five being from predominately AM ancestors. If the costs to plants of AM colonization in otherwise EM hosts are low in ecosystems, there may be limited evolutionary pressure to exclude AM colonization following evolution of EM status in plants, whereas the AM fungus may still benefit. Exclusion of AM colonization in pure EM plants may reflect fungal competition rather than plant control, in which case a lack of EM inoculum may 'drive' temporary AM presence. EM fungi may ultimately outcompete AM fungi due to some of the mechanisms shown in interspecific EM fungal competition studies, including mycelial overgrowth, greater scavenging of nutrients in return for plant carbon, or they may just duke it out with them chemically and come out on top."

Jøkkě: "AM fungi do seem to have advantages in some situations."

Forré: "Correct again. Increased nutrient uptake is driven by different mechanisms, which vary with mycorrhizal type. Uptake of mineral nutrients from soil by AM hyphae has been characterized as 'scavenging' … think of it as a physical exploration and uptake of nutrients without changing their chemical form.

By contrast, EM fungi are generally considered capable of 'mining' nutrients … more like releasing otherwise unavailable nutrients by excreting enzymes or low molecular weight organic acids.

This raises the possibility that AM and EM colonization result in complementarity in nutrient acquisition. Scavenging involves fungal hyphae extending many centimeters beyond the colonized root to expand the volume of soil from which nutrients can be absorbed. This mechanism is important in both mycorrhizal types and is considered to be most important for nutrients such as orthophosphate, ammonium, copper and zinc, where low diffusion coefficients limit mobility in soil solutions. Both types of hyphae can transport phosphorus through the soil at rates faster than would occur by diffusion alone. The relative effectiveness of AM and EM hyphae in facilitating nutrient uptake via direct scavenging will depend on proliferation of hyphae beyond the depletion zones that form around roots. In the field, EM hyphae appear better able than AM hyphae to proliferate in nutrient-rich patches, although—as the saying goes—results may vary."

Jøkkě: "Fascinating!"

Forré: "It absolutely is. And there's more. A difference in the propensity to produce exploratory hyphae may be an advantage of EM fungi, even though it comes with increased absolute carbon partitioning belowground. By contrast, retention of nutrients by the fungus to meet its own needs has been demonstrated for both EM and AM symbioses; therefore, the larger proportion of fungal tissue in EM than

AM roots may be detrimental to plants in low-nutrient soils. In many soils, the majority of nitrogen and phosphorus is found in organic forms, with the ratio of organic to inorganic phosphorus increasing with time. EM fungi utilize a range of oxidative and hydrolytic enzymes to break down soil organic matter and release nitrogen and phosphorus in absorbable forms, albeit with lower capability than saprotrophic fungi.

By contrast, whereas AM fungi can take up and transfer nitrogen from organic matter to their host plant, the weight of evidence is that AM fungi take up nitrogen and phosphorus primarily after mineralization by other soil microbes. Indeed, there is increasing evidence that AM hyphae can stimulate mineralization of organic matter by influencing the metabolism of soil bacteria and compete effectively with soil microbes for those nutrients. In studies comparing nitrogen uptake by AM and EM tree species under controlled conditions, the ratio of organic (supplied as an amino acid) to inorganic (supplied as nitrate and ammonium) taken up per unit root surface area was higher in EM species than in AM species. AM trees accumulated six times more nitrogen from inorganic forms than EM trees did, independent of tree size, with no difference in uptake of nitrogen from amino acids. In the field, root exudation in AM trees appears to result in increased inorganic nitrogen in the rhizosphere, whereas the extracellular enzymes stimulated by root exudates in EM root systems resulted in increased availability of amino acids.

Hence, the traditional view is that an AM or AM-dominated dual-mycorrhizal plant may be able to gain access to additional organic nitrogen and phosphorus by allowing colonization by EM fungi, but access to organic nutrients by AM fungi may have been underestimated. Taken as a whole, there is evidence that AM and EM fungi differ in nutrient acquisition strategies, with EM fungi generally having greater capability. But AM fungi may be more efficient, that is, lower carbon costs to the plant per nutrient gain.

And this doesn't even touch on other benefits to plants like conferring drought or flooding tolerance, protection from heavy metals and other abiotic perturbations, or biotic stresses like plant pathogens. AM seem to have the edge in all of these categories, insomuch as we understand these associations. There likely are many other non-nutritional benefits of dual-mycorrhizal habits like lowering costs of seedling establishment where it may be that AM fungi dominate on seedlings but relinquish to EM fungi later in the plant's life. Also, benefits may include greater ability to exploit whole soil depth profiles, greater flexibility with soil nutrient availability through ecosystem development, and greater flexibility for other relevant soil properties like temperature, salinity, and soil composition. There is no doubt much more to the story but we have a paucity of information and this is an area where it's difficult to study."

Jøkkě: "Well as you know, I feel all questions in science can be addressed by looking at the DNA. Can't we simply take samples of roots, run DNA sequence analysis on them and know what's going on … at least know what fungi are there, and be able to say AM, EM, or both?"

Forré: "Identifying mycorrhizal fungi associated with roots of plants using DNA sequencing can certainly have advantages, yet these newer techniques are not currently robust enough to be used on their own to determine dual-mycorrhizal status. These molecular techniques cannot distinguish between superficial colonization of roots or genuine mycorrhizal colonization with key structures. As such, those doing this research today advocate that dual-mycorrhizal status, and single mycorrhizal status for that matter, should be based on the observations of the key structures—arbuscules or coils for AM status and a Hartig net or similar structures for EM status—using microscopes and high-resolution digital cameras. Even indirect observation can be useful, like X-ray microcomputed tomography. That's not to say DNA analysis has no use. Quite the contrary. DNA sequencing could be used as an early detection technique to screen for possible candidates with genuine dual-colonization by both AM and EM before applying any viewing methodology."

Jøkkě: "Well I must say Professor F., although we rarely agree on anything, this has been very enlightening. You're pretty smart for someone who's never run an electrophoresis gel."

Forré: "And I must say that while you still have much to learn, you're pretty smart for someone who's never set foot in the forest or picked a mushroom. Although we have our disagreements, one thing we can agree on is that fungi are really amazing."

Further reading

Teste, F., M.D. Jones, and I.A. Dickie. 2020. Dual-mycorrhizal plants: their ecology and relevance. *New Phytologist* 225: 1835–1851. doi: 10.1111/nph.16190

Martin, F. 2016. *Molecular Mycorrhizal Symbiosis*. Wiley-Blackwell, New Jersey; 528 pp.