Dr. John Klironomos' Presentation,

Fungal-Plant Partnership Reconsidered Presentation

Article by Jason Karakehian

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John Klironomos from the University of British Columbia at Okanagan. Moved from University of Guelpf; Ontario, Canada. Currently has a research group of 40 people. Studies partnerships of fungi.

John's last visit to the BMC was 10 years ago.

Mycorrhizal fungi have been reclassified into different types. The focus of this lecture is arbuscular mycorrhizal fungi – (formerly known as vesicular-arbuscular mycorrhizas, or VAM) classified into the phyllum Glomeromycota. They have a great impact on our ecosystems but are not conspicuous or photogenic. What do they do and how do they interact with plants? Particularly, how might they interact with invasive plants which can make invaded systems even more vulnerable to more invasion – a "scorched earth strategy" (illegal military tactics). John's new website: www.johnklironomos.com

Arbuscular Mycorrhizal Fungi (AM fungi or plural: AMs = arbuscular mycorrhizas) are very common and always live below ground. They produce no fruiting body above ground. Instead, they produce single very large asexual spores which are usually globose and between 50 – 100 µm; their only morphological difference being in their color and size. In fact, you could pick a single spore up with a pair of forceps and with a squeeze you could hear them pop. These are produced at the ends of hyphae in the soil. Formerly, AMs were classified in the Zygomycota along with your typical bread mold Rhizopus stolonifer due to their similarity in producing these spherical asexual reproductive bodies at the tips of hyphae (sporangia) and having mycelium composed of hyphae that are virtually without cross-walls.

There are and estimated two hundred species of AMs and these form relationships with nearly every plant species on earth – approximately 250,000 species of vascular plants! They are not picky either with a low host specificity (AMs can interact with many different plant species). However, they are obligate biotrophs and must feed from living host plants.

To observe the interactions of AMs with the cells of a plant root, a field collected root is first "cleared" with chemicals which remove dark chemical compounds in the root tissues. Very thin sections of the root tissue are then made and treated with fungi specific stains as to not stain the plant tissue. Within the large, boxy plant cells of the root you can now easily observe the narrow hyphae wending their way through around the cells. Frequently, the AM will produce haustoria which are inserted into the cell walls of individual cells – the cell membranes are not penetrated. These appear as branched – tree like structure inside a cell. Highly organized and evolved, this delicate interaction is synchronized by chemical signals between plant and fungus. The arbuscule (branched structure) is the site of exchange where the fungus gets its carbon plant gets its nutrients – mostly phosphorus (highly limiting nutrient in most ecosystems). All fungi, from the fruiting body to the mycelium, are just organized hyphae. Arbuscular mycorrhizas produce arbuscules which are simply branching hyphae, or they might produce swellings called vesicles which store food in the form of lipids. The sheer size of hyphae in the AMs is notable: up to 20 µm, compared with the hyphae of Basidiomycetes for example, which are typically up to 5 µm in diameter. They are very thick near the plant roots to prevent mechanical shearing or attack by insects, bacteria and other fungi which are attracted to the "spill-over" of nutrients produced by the plant and stored underground. From there they branch out into the surrounding soil, becoming narrower and narrower – down to 2 µm at the terminal hyphae. Having no cross-walls in their hyphae, water, nutrients, and thousands of nuclei and other cellular components move freely as if in a pipeline – even having 2 directional movement.

Arbuscular mycorrhizas are very common and cosmopolitan. Whenever we pull a root – we destroy the network, but how do we know which species we've just pulled with our leeks and onions? Morphologically there are very few characters, about 6. If you take every character – size, ornamentation, color, developmental stages, and if you only looked at these you would only end up with 200 morphologically defined sp. It would be like identifying bacteria only by looking at them through the microscope; they all look like commas! To truly understand species molecular methods must be employed. Fortunately AMs can be grown along with plants like leeks in plastic bins in greenhouses. The spores of AM's are not airborne and too heavy to travel easily – so cross contamination of species isn't a huge worry.

Plants benefit from relationships with arbuscular mycorrhizas but many plants will grow just fine without them. These benefits can be bottom – up where the plant gets resources such as increased nutrients and the ability to grow in low nutrient soil. If a plant is growing in soil with plenty of nutrients it may shut down the relationship. Over fertilizing, tilling and fungicides destroy the mycelium.

Top-down benefits enable the colonized plants to be more resistant against grazers and pathogens above and below ground. The physiology of the plant changes in AM relationships and the AM produces compounds in the soil that kill other fungi and physically occupy space to keep unwelcome invaders away. Humans can have a great impact on arbuscular mycorrhizas in our agricultural practices.

In experiments the direction and magnitude of responses vary and adding AMs everywhere and to different plant species sometimes produced negative results. Important factors that govern the success of individual relationships are the "quality" of plant partner (some plants not good partners – don't form partnerships) and the "quality" of fungal partner (some fungi are not great partners but cheaters!) and so matching the right plant to the right fungus local fungi is critical. Plants and fungi local to each other are co-adapted. Furthermore, they must be environmental partners – in high nutrient conditions the fungus evolves to be parasite.

This has an impact even on the scale of your house or garden plants. Potting soils like Promix put fungal inoculum into the soil, but without the time and obligate plant to produce its massive mycelium, the fungus might take nutrients from the plant to establish itself before giving anything back to the plant – depressing the health and appearance of your potted plant for some time. The timing of introduction of plant to fungus is important. AMs are on a continuum of symbiosis from parasitism to mutualism. Symbiosis is not mutualism – mutualism is a kind of symbiosis. In experiments with a plant community – adding 10 random species of AMs provided a maximum benefit after which the benefits plateau.

In an environmental context, how can we predict positive and negative interactions?

To study this, an experiment using Wild Strawberry was conducted. First, three abiotic gradients were established: soil nutrient content (fertilizer added never to daily), soil water content (watered never – daily and soil pH (3.5 – 9.0 adjusted with sulfuric acid and calcium carbonate). Next, plants were grown with no fungi, one commercial species of inoculum and field collected AM fungi. The responses of the plant to these varied conditions were measured by frequency of flowering (species will not flower if stressed). The results showed that the non-AM fungi plants did the best when adequately fertilized (it is a cost to have a partner when adequate nutrients are available). Plants benefit more from AM fungi under extreme conditions but not as much under optimal conditions – so we need to know what conditions we want to grow plant in before we think about how a plant will benefit from AM fungi.

They provide an insurance policy rather against stressful conditions and are a cost under optimal conditions. The AM fungi symbiosis can increase the host plant niche size allowing the plants to grow in a wider range of environments.

Invasive plants such as Alliaria petiolata (garlic mustard), a member of the mostly non-mycorrhizal forming mustard family, can have a profound effect on the mycorrhizal relationships in an ecosystem. This plant produces anti-fungal compounds and can invade understory forests where other invasives typically require and disturbed environments. A. petiolata reduces AM fungi, and so has a negative impact on seedlings such as Acer saccharum, A. rubrum, and Fraxinus americana, all of which are highly AM dependent. Woody, perennial, late maturing forests are highly dependent on mycorrhizas. In trials, the anti-fungal compounds of garlic mustard stick around in the soil and indirectly affect their competitors by knocking out their fungal associates – a scorched earth policy of harming anything that is useful to your enemy.

Exotic plants are more likely to be non-mycorrhizal or the relationship may not be quite as important, whereas native plants benefit more from symbiosis. An experiment of whether exotic invasive plants are less dependent on mycorrhizas was conducted in the field with ten dominant populations of invasive plant species and ten dominant native mycorrhizal species. The soil from these habitats was examined to see how “infective” the soil is – how readily the EM fungi in the soil can colonize a plant. Invasive plants are poor mycorrhizal hosts and reduce inoculum in the soil, making invaded soil less supportive of native plants. This leads to an “invasion meltdown” where invasive organisms make a system even more prone to further invasion. Consider this: for an EM fungus to survive and grow in the soil, it must quickly find a suitable plant partner. The hyphae of germinated spores find their associate by growing toward CO2, which is respired in high amounts by plant roots. An invasive plant communicates CO2 and the germinated hypha grows toward this chemical signal. It has enough energy to grow up to a very lengthy meter and colonize a plant. If it grows toward an invasive plant that is incompatible with, the nascent fungus will die. Now consider a habitat that is dominated by invasive non-mycorrhizal species.

To find your own spores of EM fungi, collect a soil sample from a habitat dominated by native plants. Sieve through 250 µm mesh and 50 µm mesh. Most spores are between 50 and 100 um. Then put what remains in water – dirt will filter down to the bottom and the spores will float. Take a sample from the top part of tube and observe with a dissecting scope.