The Nova Sclerotium

Fall 2022

scle·ro·ti·um : the hard dark resting body of certain fungi

Overview

Editor's Note

Tom Clair

Autumn leads to a new school year, end of summer holidays, and the beginning of serious mushrooming. The cooler, wetter conditions encourage the sub-terranean world to make its presence known with mushrooms popping up out of the ground and setting forth their spores. Isn't nature grand! It's also the time when the Nova Scotia Mycological Society holds its foray, where new and experienced mycophiles get together to explore the fungal community in a part of the provincial ecosystem. This year was special because we were finally able to meet in relatively large groups without having to worry too much about COVID-19. Unfortunately, a foray scheduled for Cape Breton developed a scheduling problem with a hurricane named Fiona and had to be cancelled hurriedly. The mainland foray though was held two weeks later near Canning.

This issue of Nova Sclerotium contains a summary of the October 2022 Ross Creek foray that did happen, as well as a species/genus list of what was found. Truffles found themselves in the news in late summer and Nova Scotia Mycological Society Vice-President Dr. Keith Egger offered to write something about the presence of truffles in our region. Who knew? Can we develop an industry here? Finally, mycology in the region is a multi-faceted interest. Foragers, farmers and seekers of medicines also share their passion with scientists who are trying to explain the life cycles and ecological roles of fungi. Maria Roy from Acadia University is working with Dr. Allison Walker on fungi which cause problems to apple growers. She prepared a note to explain her work.

As editor, I'm always on the lookout for articles or stories which might be of interest to fellow fungi aficionados. If anyone has a story to tell or is interested in knowing about some aspect of mycology, please let me know and we'll try to see if can get new information to the rest of our members. Also, if anyone has photos on spectacular specimens they would like to share, send them along to me and we'll see about publishing them.

2022 Fall Foray

Tom Clair

Welcome to the first formal post-COVID foray! Finally, we were able to get together in relatively large numbers to forage and identify fungi in Nova Scotia. This year, the Foray returned to the Ross Creek Centre for the Arts near Canning on the North Mountain, overlooking the Annapolis Valley (https://artscentre.ca/). This site was used in 2019 by the Society, and its familiarity and success at that time made it a logical place to restart the activity on Saturday the 15th of October.

Figure 1: Ross Creek Centre for the Arts

Figure 2: NS Mycological Society members gathered for the Foray

When the planning group visited the site on Friday the 14th to prepare for the 80 members who signed up, they feared that the meet was not going to go well. First, the weather forecast was calling for rain Saturday morning which is never a good thing for encouraging attendance. Secondly, the group looked at two of the three trails which were going to be used and didn't find many fungi at all. It was definitely looking grim for the next day. Luckily, the rain held off, and approximately 70 people showed up for the event.

At 10:30, the participants were rounded up and divided into three groups led by Gavin Kernaghan, Keith Egger, Allison Walker, and John Crabtree, and then went on their way, baskets in hand. Luckily, with more eyes on the ground, and more digging under leaves, the initial fears of a low species count were seen to be groundless. Approximately 40 species were found in an hour and a half of searching. Samples were displayed in the lamella, a Quonset-type shelter which was lined with tables for displaying them.

Figure 3: Lamella shelter lined with tables to display mushroom samples

Figure 4: Mushroom samples laid out for identification

The group leaders plus local experts Scott Cunningham and Ken Harrison Jr. consulted with the collectors and identified most of the samples collected at least to genus level. Of particular interest were *Lepista irina*, a choice edible mushroom and *Sarcodon lanuginosus* which was named by Ken Harrison Sr. His son, Ken H. Jr. also happened to be one of the local experts at the foray. It was clear listening to the conversations going on that the taxonomy of a number of groups is in flux caused by improved information from the use of DNA assessments.

A new feature of the Foray was a display by Amanda Griffin on the use of fungi for dying cloth and other materials as was done in pre-industrial times. Amanda used local fungi which she prepped in the morning to show how various species could colour wool and cotton.

The first post-pandemic Foray was deemed a success. The location worked well with people and the staff and facilities were just right for the group's needs. Some of the organizers stayed at the Centre the night before the Foray and lunches and excellent post-field work mushroom appetizers were served to the participants. All in all, great location, people and fungi! See you next year!

Figure 5: Demonstration led by Amanda Griffin on using fungi to dye cloth

Ross Creek 2022 Taxon List

Keith Egger, John Crabtree, Allison Walker

Species and genus list of collected fungi in NS Fall Foray, 2022. Assembled by Keith Egger with contributions from John Crabtree and Allison Walker.

Amanita muscaria var. guessowii

Armillaria sp.

Cantharellus sp.

Catathelasma imperialis

Cerrena unicolor

Chalciporus piperatus

Coprinus comatus

Cordyceps sp.

Cortinarius armillatus

Cortinarius sp.

Coryceps sp.

Dacrymyces sp.

Entoloma abortivum

Entoloma sp.

Ganoderma applanatum

Gymnopilus sp.

Hydnellum frondosum

Hygrocybe sp.

Hypholoma sp.

Inocybe sp.

Lactarius deterrimus

Lactarius sp.

Leccinum sp.

Lepista irina, a.k.a. Clitocybe irina

Lycoperdon sp.

Marasmius sp.

Mycena sp.

Neolecta irregularis

Pholiota sp.

Pleurocybella porrigens

Ramaria sp.	Sarcomyxa serotina
Rhodocollybia butyracea	Suillus luteus
Russula brevipes	Tricholoma sp. near vaccinum
Russula emetica group	Tricholoma subsejunctum
Sarcodon lanuginosus	Tricholomopsis decora

Truffles in Nova Scotia

Keith Egger

You might be surprised to know that there are truffles in Nova Scotia. But before you go online looking for a trained truffle-hunting pig, these aren't the expensive Black Périgord and White truffles prized in France and Italy – so far as I know these truffles do not occur in Nova Scotia. The truffles found here are unlikely to be eaten at all. If you didn't know that many truffles are unappetising or inedible, then perhaps it would be useful to have a short primer on what a truffle is exactly.

A truffle is a mushroom that fruits underground. In fact, a truffle is less a "thing" than a "strategy". What I mean by that is belowground fruiting truffles occur in many different fungal lineages in both the Ascomycota (sexually-produced spores produced in tiny sacs called "asci") and the Basidiomycota (spores produced on the ends of club-shaped cells called "basidia") – these two lineages of mushroom-producing fungi diverged around 400 million years ago so truffles didn't just evolve once to gave rise to all the truffle-producing fungi we see today but rather below-ground fruiting is a strategy that has evolved many times in many different fungal lineages.

Why would some fungal lineages find an advantage to fruiting belowground? Scientists think that it's an adaptation to drying climatic conditions. As the climate of an area becomes dryer, which has happened innumerable times in many different geographical areas, mushrooms that keep their spores enclosed in a moist fruiting body survive better than mushrooms that release their spores to the air like most aboveground mushrooms. Over time that drives an evolutionary strategy that goes from aboveground fruiting to partially aboveground fruiting (these look like aboveground mushrooms that haven't fully developed, keeping their spore-bearing surface enclosed) to belowground fruiting, as represented in the figure below from Wikipedia.

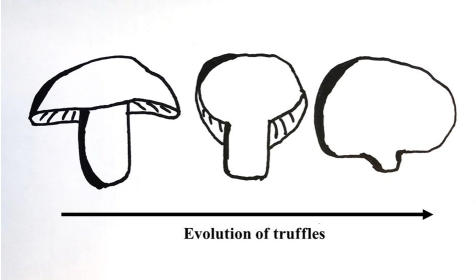


Figure 6: Evolution of Truffles (By Ammontes - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=75035887)

You may notice one major problem with this strategy: if they originally disperse their spores in air then how do they disperse their spores if they are enclosed? It turns out that they take advantage of mushroom-loving animals like rodents to disperse their spores. In order to attract these animals they produce powerful scents when they are ripe – this is the basis of their culinary appeal as the same odors that attract animals are also attractive in truffle-infused food delicacies. This has happened many times in many mushroom lineages leading to a vast number of truffle species worldwide, including the Black Périgord and White truffles in Europe.

By the way, all truffle-producing fungi are thought to form associations with tree roots called mycorrhizas, a mutually-beneficial association where the plant provides sugars that have been produced by photosynthesis while the fungus provides access to nutrients that the tree wouldn't be able to absorb effectively.

What kinds of truffles do we have in Nova Scotia? The most common are species of *Elaphomyces*, called Deer Truffles. Deer truffles look like brownish to black, rounded potatoes.

Figure 7: *Elaphomyces granulatus* found near Weymouth this summer by Matt Smith of Parks Canada

One of the first collectors of *Elaphomyces* in Nova Scotia was Ken A. Harrison, a mycologist who worked at the Kentville Agricultural Station from 1926 to 1966 and who was a prolific mushroom collector while working and after his retirement. I've referenced a scientific article that discusses some of his finds.

As an aside, Deer Truffles are sometimes parasitized by another fungus called *Cordyceps* which is a well-known insect pathogen. What, you might ask, do Deer Truffles and insects have in common that would explain this similarity? Both insects and fungi make their structural framework out of chitin as opposed to cellulose, the main structural component of plants. *Cordyceps* likely recognizes insect exoskeletons and Deer Truffle fruit bodies because they are both made of chitin.

Rhizopogon is another type of truffle found in Nova Scotia. *Rhizopogon* is a diverse genus of truffles that is most closely related to aboveground fruiting mushrooms in the bolete genus *Suillus*. As odd as it may seem, *Rhizopogon*, a belowground fruiting truffle, is more closely related to *Suillus*, an aboveground fruiting mushroom, than *Suillus* is to other boletes such as *Leccinum*, *Tylopilus, Boletus*, etc. We know this from studies of their DNA sequences which prove their close relationship.

Figure 8: *Rhizopogon* species collected by the author in Kejimkujik National Park this summer.

Given that it's not that hard to find truffles in Nova Scotia, why are there so few collections in depositories such as the E.C. Smith Herbarium at Acadia University, which only houses one collection of *Elaphomyces*? This is mostly because finding truffles requires scratching away the surface layers of litter on the forest floor to find the truffles hiding underneath. Raking large areas of the forest floor is damaging to the organisms that live in the soil so is not encouraged. However, one can take advantage of the superb truffle-hunting skills of forest residents. With their sensitive noses held at most a few centimeters above the forest floor, squirrels are extremely adept at finding truffles. One of the surest indicators of their presence is squirrel digs on the forest floor (although squirrels often dig for other reasons, such as caching seed cones). If you find areas where the squirrels have been digging, gently probe the soil around the digs and you may be rewarded with a truffle specimen that was missed. You don't need to dig deep as truffles are usually found within 5cm or so of the surface. And remember to replace the litter and soil to leave as little disturbance as possible. As for the idea that if squirrels can eat a mushroom then it must be safe for people, Japanese squirrels have been observed safely consuming mushrooms that would be toxic to us so don't trust this myth!

If you do find a truffle and want to do your bit for citizen science, I would be most interested in receiving a dried specimen for identification and to add to the E.C. Smith Herbarium at Acadia. Email me at microastro@gmail.com and I'll get back to you with the info I need and an address to send the dried material!

References:

Zhang, B-N and DW Minter. 1988. Elaphomyces spinoreticulatus sp. nov., with notes on Canadian species of Elaphomyces. Canadian Journal of Botany 67: 909-914.

Using Fungal Endophytes to Fight Apple Replant Disease

Maria Roy

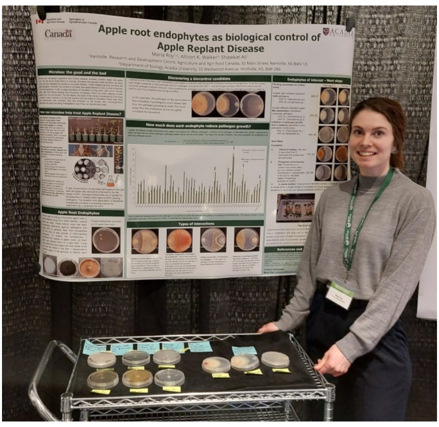


Figure 9: Maria Roy is a Master of Science Candidate at Acadia University

Fungi live everywhere!

Microbes are microscopic organisms that include bacteria, archaea, protists, algae and some fungi. They can live as single cells or in colonies. Microbes have perhaps gained a bad reputation because many human and plant diseases are the result of microbial infection. However, the microbes that cause disease are only a small proportion of microorganisms as a group. In fact, a large population of microbes is living within each of our bodies right now without causing any symptoms of disease. Some are even helping us with digestion and immune function. For this reason, the study of the human microbiome is a hot topic in research today. However, it is not just humans that are hosts to microbes. Plants also have many associations with microbes. Just like probiotics in the human diet, microorganisms associated with plants are being investigated to see if they can benefit their hosts.

Apple Replant Disease

One problem that could potentially be addressed through the use of "good microorganisms" is Apple Replant Disease, or "ARD". ARD occurs when a new apple tree is planted into soil which was previously used to grow apple trees or closely related species, like pears. When the old tree is removed, fungal and fungus-like oomycete pathogens remain in the soil and plant debris. Once the new tree is planted, these pathogens infect it. Some of the newly planted trees die from this infection while others are stunted and produce less fruit than trees grown in soil that had not been used for apple production. But why was the healthy mature tree which grew in the soil apparently unaffected by these pathogens while the newly planted tree is severely damaged? One possible reason could be that the mature trees had time to form mutualistic relationships with beneficial microorganisms before being attacked by pathogens, while the young tree has not yet developed those relationships and is being overloaded by the pathogens that had accumulated in the soil during the old tree's lifetime. Of course, this is likely only part of the answer to a very complicated problem, but it is a good place to start when looking for ways to treat or prevent ARD. Currently, ARD is treated by chemical fumigation of the soil to kill all the microorganisms within it before planting. This treatment works well but the usage of effective chemical fumigants is being increasingly restricted due to their off-target effects in the environment. Consequently, there is a push for an alternative, more eco-friendly treatment to ARD.

How fungi living within plants might be a source for an eco-friendly treatment for ARD

For my Masters research project at Acadia University Department of Biology (Dr. Allison Walker) and the Kentville Agricultural Research Station (Dr. Shawkat Ali), I am exploring the fungi that live within healthy, mature apple tree roots as a potential source for a treatment for ARD. This process of using a living organism to treat a disease or problem is called biocontrol. Specifically, my research is focused on fungal endophytes as a source for biocontrol agents. Endophytes are microbes that live within plant tissues without causing disease. This is reminiscent of how there are microbes in our guts currently that are not causing us no get sick. Keeping with that analogy, some of these microorganisms can also help you defend against pathogenic microorganisms (aka germs). Other microbes might simply live there without causing any effects, while still others are not making you sick now but given the right opportunity, might overpopulate your gut and cause disease. Likewise, although some of these fungal endophytes might be neutral or even opportunistic pathogens if the environment changes or the tree is stressed out for example due to drought, others might be able help the tree defend itself against the pathogens that cause ARD. Endophytes can protect against pathogens by producing chemicals that kill or slow down pathogen growth, taking up space or nutrients that the pathogens need, and/or sending signals to the plant to improve its immune response. Fungal endophytes with these properties are the ones that I am trying to identify with my research.

Finding Fungal Endophytes

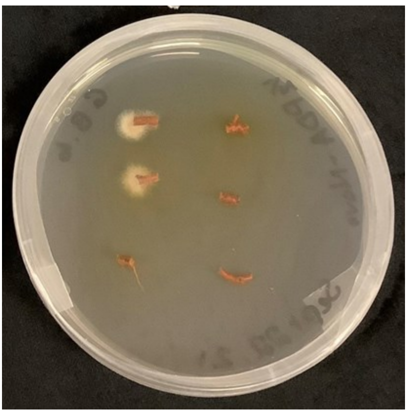


Figure 9: Endophytes growing from inside of roots

with Apple Tree Roots

My first step in finding a biocontrol agent was to isolate different fungi from within healthy, mature apple tree roots. These mature roots had been successfully defending against pathogens that cause ARD so it is possible that they host endophytes that can slow or stop growth of ARD-causing pathogens. To isolate the endophytes, I disinfected the outside of apple roots, cut them open, and plated them on a petri dish. Fungus living within the apple tree root tissue grew from the inside of the root onto the media in the dish. I sequenced a region of the genome that differs among most fungi, in order to identify what type of fungus I was looking at (DNA barcoding).

Can any of these fungal endophytes stop or slow pathogens that cause ARD?

Once I had a collection of fungal endophytes, I wanted to test if they could reduce or interfere with growth of pathogens. To do this, I grew each fungal endophyte on the same petri dish as an ARD pathogen. I could then compare the pathogen's growth in these conditions with the pathogens growth on a plate by itself to see if the endophyte was having any effect.

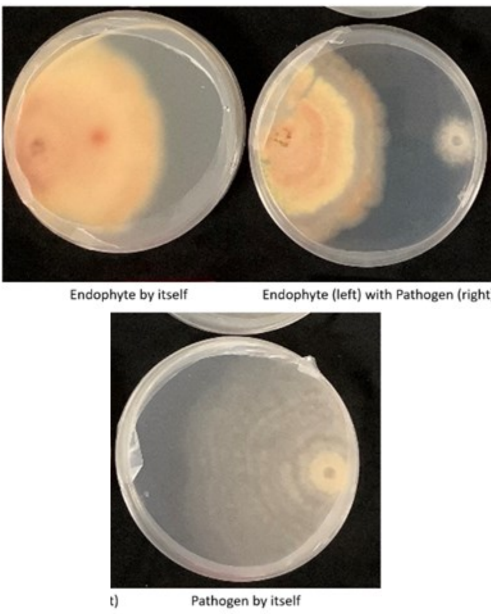
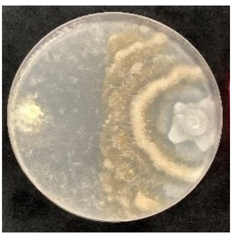


Figure 10: (top left) Endophyte by itself (top right) endophyte on the left and pathogen on the right (bottom) pathogen by itself

In this example, the pathogen on the same plate as the endophyte is growing at a much slower rate than the pathogen growing by itself. This could mean that this endophyte would be a good candidate for biocontrol.

When the fungal endophyte and the pathogen are grown on the same plate, four different things could occur (see table on following page).

The last two types of interactions are the most interesting when it comes to selecting a candidate for biocontrol. A fungal endophyte that can parasitize or inhibit growth of ARD pathogen could

Deadlock at contact: the two competing fungi cannot overgrow each other. They stop once in contact and sometimes have discoloration or deformation of the fungal mycelium at contact. 	**Partial overgrowth of endophyte by pathogen**: the pathogen is likely parasitizing or outcompeting the endophyte. 
Complete overgrowth of the pathogen by the endophyte: what is likely happening here is something called mycoparasitism, where the endophyte curls its hyphae around the hyphae of the pathogen in order to penetrate them. This, in addition to excreting chemicals, can kill the pathogen and allow the endophyte to consume it. 	**Reduction of pathogen growth rate from a distance**: this likely occurs through secretion of anti-fungal chemicals by the endophyte into the media on the petri dish which can reduce the pathogen's growth rate. 

be useful in treating or preventing this disease in young apple trees.

Next steps

Experiments that take place on petri dishes can only tell us so much about how a treatment will play out in the field. However, treating actual orchards takes a lot of time and labour. For this reason, I am using this laboratory work as a screening tool to pick candidate fungi that should be tested further. I tested 48 fungal endophytes against two different ARD pathogens. Now, I can pick the fungal endophytes that performed the best in those experiments and conduct further tests to see what kind of chemicals they are producing and if they survive and protect against pathogens when used as a treatment in living apple trees. This is a process that can take years to yield a product that can be used at a field scale but it is a promising avenue to explore for an eco-friendly treatment to ARD.

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Contact: nsmycologicalsociety@gmail.com
Submissions: novasclerotium.editor@gmail.com

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