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Small Mammal Consumption of Hypogeous Fungi
in the Central Adirondacks of New York

by

Robert T. Meyer
Candidate for Bachelor of Science
Department of Environmental and Forest Biology
With Honors

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APPROVED

Thesis Project Advisor: _____

Alexander Weir, Ph. D.

Second Reader: _____

Thomas R. Horton, Ph. D.

Honors Director: _____

William M. Shields, Ph.D.

Date: _____

Abstract - There have been many accounts of small mammals consuming and dispersing subterranean (hypogeous) fungi, yet few studies have been conducted in the Northeastern United States. For this reason, we analyzed several small mammal species including deer mice (*Peromyscus maniculatus*), southern red-backed vole (*Myodes gapperi*), eastern chipmunk (*Tamias striatus*) and short-tailed shrew (*Blarina brevicauda*) to determine the extent of fungal consumption in the central Adirondack Mountains in New York. Analysis of several other species caught infrequently during the study included smoky shrews (*Sorex fumeus*) woodland jumping mouse (*Napaeozapus insignis*) and northern flying squirrel (*Glaucomys sabrinus*). Examination of 61 fecal samples revealed fungal spores of the hypogeous fungi *Glomus* spp. and Russulaceae with one sample from eastern chipmunk containing *Gautieria* and one sample from flying squirrel containing spores of the family Boletaceae and Elaphomycetaceae not found in other samples. We found Russulaceae spores in 66% of eastern chipmunks and 35.7% of red-backed voles. *Glomus* spores occurred in 35.7% of red-backed voles, 16% of eastern chipmunks, 10% of short-tailed shrews and 5% of deer mice. Comparisons of fungal and insect items to sex, area and species resulted in few statistically significant differences ($p > 0.05$). However, we believe the data shows biological significance of hypogeous fungi thus, wildlife managers should consider mycophagy when addressing the overall health of forest ecosystems in the Northeast.

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Acknowledgments

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Introduction

Mycorrhizal fungi create connections between trees and allow for increased water and mineral uptake into tree roots (Smith and Read, 2008). Many fungi that fruit both above ground (epigeous) and below ground (hypogeous) are able to form mycorrhizal associations. Hypogeous fungi such as truffles typically disperse spores long distance by using small mammals and invertebrates due to the lack of exposure to wind and rain. Evidence supports that certain mammal species are able to detect the odor of underground fungi (Fogel and Trappe 1978). Once found, a mammal eats the fruiting body of the fungus and passes the spores through their digestive tract. The spores are then able to germinate after defecation (Trappe and Maser, 1976) in a new location. Mycophagy, the consumption of fungi, provides mammals with nutrition while the fungus is able to disperse spores across the landscape (Fogel and Trappe 1978). Many mammals that consume fungi have large home ranges that span multiple vegetation types and can thus inoculate early successional areas with hypogeous fungi (Ovaska and Herman, 1986, Ashkannejhad and Horton, 2006). In this way, mammals are able to disperse ecologically important fungi across the landscape.

Interest in these interactions has resulted in many studies conducted in the Pacific Northwest yet these interactions may not be exclusive to that region alone. For this reason, we will determine the importance of mycophagy to mammals in the Northeast by sampling several small mammals in New York State. This research will answer several questions: 1) which of the mammals in the study consume hypogeous fungi, 2) what hypogeous fungi are consumed by mammals and 3) does presence of fungi differ based on species, sex or location?

Field-site Description

The study was conducted in the Huntington Wildlife Forest maintained by the College of Environmental Science and Forestry and located in the Adirondack State Park in Newcomb, New York. Samples taken from small mammals were part of a larger study on mammals on the property. We chose two study sites based on dominant tree cover representative of Adirondack forests; the Natural Area and the Hare Area. The Natural Area consists of 365 ha of old growth forest with dominant tree species consisting of red spruce (*Picea rubra*), Eastern hemlock (*Tsuga canadensis*), yellow birch (*Betula alleghaniensis*) and American beech (*Fagus grandifolia*) with sugar maple (*Acer saccharum*) and red maple (*A. rubrum*) occurring less frequently. The Hare Area, aptly named for its dedication to snowshoe hare habitat, is located in a 60 ha spruce-fir stand with dominant species of balsam fir (*Abies balsamea*), eastern hemlock, red spruce, sugar maple and red maple with American beech occurring less frequently (McNulty et al., 2008).

Methods

Small mammal trapping

Sampling was conducted from July 16th through July 19th in 2007 which allowed us to sample the early stage of annual fruiting fungi (July-October). One small non-folding aluminum and one large non-folding galvanized Sherman trap were used at each sample location and baited with peanut butter, rolled oats and paraffin (ratio of 1:4:2). A 7x7 trap grid with a spacing of 20 meters was used in both areas consisting of 196 traps total. Traps were set in the evening and checked in the morning for small mammals.

Traps captured deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*), Eastern chipmunk (*Tamias striatus*), and short-tailed shrews (*Blarina brevicauda*). We recorded species, sex and location of captured mammals. We collected feces from the floor of the traps and placed them in labeled manila envelopes to air dry. We then cleaned the floor of the trap with a damp cloth to remove excess feces and prevent contamination of feces from other captured individuals. The animal was then marked with an ear tag if the ear's pinna were large enough or marked with Wite-out if the pinna was too small or absent. We then released marked animals on site.

We captured two smoky shrews (*Sorex fumeus*) and one woodland jumping mouse (*Napaeozapus insignis*) which were found in a riparian area as well as one northern flying squirrel (*Glaucomys sabrinus*) captured nearby at Long Lake. The spores found in these samples are worth noting but since these species did not occur in large enough numbers and occurred in a separate habitat type, they will not be included in the statistical analysis.

Fecal analysis

Feces were air dried after collection in manila envelopes and kept in a jar with Drierite desiccation rocks. Analysis began in September of 2013 after it was determined that the spores within the sample were still identifiable after several years of storage. Feces of individual animals recaptured were used unless the animal was caught the previous night.

We weighed feces to 25 mg (Pyare and Longland, 2001) and placed them in a half-dram vial. Then, 1mL of 15% ethanol was added to rehydrate the sample. A clean mall probe was used to break apart large pellets. We used a Vortex mixer (Bohemia) to homogenize

the sample with three pulses for each sample. A pair of smooth-sided tweezers was plunged to the bottom of the vial, closed and withdrawn. The tweezers were then opened onto a clean slide. The sample is then air-dried on the slide to remove the ethanol for approximately 2 minutes. Two slides were made for each sample, one with Melzer's reagent to determine staining characteristics and one with 100% glycerol to compare spores with Melzer's and to preserve the sample. Once dry, we added one drop of Melzer's reagent or glycerol and then placed a cover slip over the sample. We viewed samples under a Nikon E-800 research microscope equipped with Differential Interference Contrast (DIC) optics starting at 40x, frequently switching to 60x and 100x objectives to give more detail for identification of fecal contents (Pyare and Longland, 2001). We analyzed each slide for 15 minutes to control effort per slide and identified fungal spores to family or genus using spore keys (Castellano 1989; Morton and Benny, 1990). We grouped food materials into the following categories: plant material, insect parts, *Glomus spp.* and Russulaceae. A spore was counted as present in a sample if more than three spores occurred throughout the sample. We believe this criterion was sufficient due to the relatively low amount of feces used per sample. Spores that occurred less often were considered to be eaten unintentionally as the animal was foraging on other foods.

Statistical analysis

We summarized our findings by calculating the frequency of occurrence for each food item, for each species. We used a two-proportion test and contingency tables to evaluate differences between each mammal's location and sex using Minitab 16 software. Similarly, contingency tables allowed us to compare species to each other within each

area. We then used Program R for chi-square analysis to determine if the proportion of food items were homogenous with respect to each mammal. For the analysis of chi-square and contingency tables, we used Fisher's exact p-value due to the small sample size collected.

Results

With the exception of woodland jumping mice, samples from all species of mammals contained fungal spores. Spores were determined to be hypogeous based on radial symmetry, a characteristic of hypogeous fungi. The majority of spores observed included *Glomus* and hypogeous members of the Russulaceae family. Several spores of other epigeous fungi were frequently encountered in fecal samples but were ignored for the purposes of this study. We were able to calculate the frequency of occurrence for spores and insect food for mammals caught in Hare and Natural Areas including deer mice, red-backed voles, eastern chipmunks and short-tailed shrews (Fig. 1). Russulaceae was considered to have been eaten unintentionally by short-tailed shrew and deer mouse due to low spore counts in the samples. One eastern chipmunk sample contained large quantities of the hypogeous fungus *Gautieria* but was not included in the statistical analysis due to its presence in only one sample.

Examination of riparian and Long Lake samples showed several different spores than those found in Hare and Natural Areas. The flying squirrel sample revealed fungal spores of the family Boletaceae and Elaphomycetaceae as well as Russulaceae. Smoky shrew samples contained spores of *Ganoderma*, an epigeous fungi common in the Adirondacks. One sample of smoky shrews also contained an unidentified, symmetrical, pitted spore that may be of a hypogeous fungus.

We also calculated the frequency of insect foods in samples for comparison to fungal foods found (Fig. 1). Insect food consisted of integument and wing scales. Samples from all species contained insect food except for the flying squirrel sample. Insect food was most prominent in short-tailed shrew samples although deer mice also contained more insect food than fungal foods. We also encountered plant matter finding that all samples from all species collected contained plant cells, starch or other evidence of plant matter. Species comparisons of different food items were determined based on sex and location (Natural Area and Hare Area). The comparisons of food items for short-tailed shrew based on sex were inconclusive due to the inability to determine sex in the field. Contingency tables for sex and area resulted in Fisher's exact p-values of greater than 0.05 and we therefore reject a difference in fungal spore presence based on sex or area in the four mammal species. Contingency tables of species comparisons to each other resulted in most Fisher's exact p-values greater than 0.05 except for comparisons involving deer mouse or short-tailed shrew where no spores were found (Fig. 1). Chi-square analysis to test homogeneity of the proportions of *Glomus*, Russulaceae and insect food eaten resulted in a value of 0.008. The second chi-square analysis of only *Glomus* and Russulaceae food items resulted in a value of 0.154. This result indicates that food items occurred differently with respect to all foods but detects no difference in fungal food occurrence for the four mammals.

Discussion

Examination of 57 fecal samples of short-tailed shrews, deer mice, red-backed voles and eastern chipmunks revealed hypogeous members of Russulaceae, *Glomus spp.*, and *Gautieria spp.* as well as spores of epigeous fungi. Measurement of plant and insect food

frequency served as a relative measure to compare the presence of fungi (Fig. 1). We found plant matter in all samples of all species suggesting that plant matter is a major part of the mammals' diet during the study period. Insect food also contributed to the mammals' diet, occurring in all species sampled except in the flying squirrel sample, which could be a result of small sample size.

Eastern Chipmunk. We found fungal spores of hypogeous members of Russulaceae most often amongst chipmunk samples (66.6% of individuals). Chipmunk samples also contained *Glomus spp.* (16.6% of individuals) and one sample with spores of the genus *Gautieria*. These data indicate that the majority of sampled chipmunks ate hypogeous fungi during the study period. Comparatively, 33% of individuals contained insect food items, whereas 66% of chipmunks contained Russulaceae spores. Similarly, a study conducted by Wrazen and Svendsen (1978) showed increased fungal consumption by chipmunks in summer months as opposed to fall and spring. They suggested that increased fungal consumption occurs due to the depletion of stored foods and the unavailability of fall mast, thus fungi act as a food buffer when other food items are scarce. However, the previous year was a high mast year with beech mast of over 140 kg/ha alone (Jensen *et al.* 2012) thus the depletion of stored food may not be as severe as other years. The fungi instead, may have acted as a source of water during dry summer conditions (Smith, 1968). These hypotheses have yet to be tested but they indicate the potential of fungi as an important food item for chipmunks in the Adirondacks. Ovaska and Herman (1986) analyzed *Glomus* consumption in Nova Scotia finding *Glomus spp.* in 7.8% of chipmunks, less than the 16.6% of individuals containing spores in our

study (Table 1). It is not certain whether this two-fold difference in *Glomus* consumption is the result of increased *Glomus* abundance or increased selection for *Glomus*.

Results of the contingency analysis comparing sex, location and species resulted in little statistical difference due to a lack of power yet they did suggest possible biological significance. Contingency table analysis in the current study resulted in a Fisher's exact value of 0.088 and a p-value of 0.017 when testing for insect consumption difference between sexes of eastern chipmunks. Since the Fisher's exact value exceeds our set alpha of 0.05 we must reject a difference between sexes. We would like to note however, that the alpha value of 0.05 is an arbitrary value which may not capture biological significance, as may be the case here. Wrazen and Svendsen (1978) also analyzed insect consumption by chipmunks finding that females consumed more insects than males. They suggested that females would consume insects in greater quantities to obtain more protein for gestation. Our results could reflect their findings since the chipmunk gestation period coincided with the time of our sampling.

Southern red-backed vole. Southern red-backed vole samples contained spores of *Glomus*, occurring in 35.7% of individuals. Previous studies have shown 1.3-40% of red-backed voles consuming *Glomus* (Ovaska and Herman, 1986; Maser and Maser, 1988; Jacobs and Luoma, 2008). Our data would suggest that red-backed voles in the study area consume more *Glomus* than other parts of their range including studies conducted near the Northeast (Table 1). The same number of individuals that consumed *Glomus* also consumed Russulaceae (35.7% of individuals), although both fungi were not always found in the same sample. Southern red-backed voles consume Russulaceae fungi across their range (Maser and Maser, 1988; Pastor et al., 1996) but little research

has shown Russulaceae consumption in the Northeast. The current study not only adds to this knowledge but also shows indications of a higher amount of Russulaceae consumption than other studies have shown (Maser and Maser, 1988; Pastor et al., 1996).

Several spores were absent from this study that are worth noting. Much of the literature on Southern red-backed vole mycophagy involves the presence of *Endogone* and *Hymenogaster* and have been noted in the Northeast in previous studies (Table 1). Reasons for *Hymenogaster* absence may include seasonal specificity of particular species (Castellano et al., 1989). *Endogone* species however, fruit year-round and are common throughout the Northern Hemisphere (Trappe et al., 2009). It is more likely that *Endogone*'s absence in the study could be caused by the taxonomic changes to the group since the 1970's causing us to find the same spore but under the different taxonomic name of *Glomus*. Unless spores in previous studies were identified to species it is not possible to determine if the *Endogone* spores found were changed to *Glomus* or if they remained as *Endogone*.

Short-tailed shrew. Food items recorded for short-tailed shrews were consistent with other studies' findings. Short-tailed shrew diets consist mainly of earthworms and insect food (Hamilton, 1941; Whitaker and Ferraro, 1963). Our data showed 62.5% of sampled individuals contained insect integument, wing scales or other insect parts indicating that insect foods are a major component of short-tailed shrew summer diets. We found *Glomus* spores to a lesser extent in 10% of short-tailed shrew individuals. Similar results have been found in Nova Scotia with 16.7% of individuals containing *Glomus* (Ovaska and Herman, 1986; Table 1). Although spores of Russulaceae were found in both short-

tailed shrews and deer mice, they were not counted due to their low abundance in the slide mounts of our samples.

Deer Mouse. We found deer mice consuming less insect food than anticipated. Studies of eastern populations of deer mice show 56-71% of individuals to contain insect material (Hamilton, 1941; Wolff et al., 1985), compared to 31.3% of individuals in the current study. The difference in insect foods may be a result of insect abundance. Since insect abundance was not measured, the cause of this difference remains undetermined.

Glomus was found in 5.5% of individuals suggesting that *Glomus* was not a major food item of deer mice during our study and instead, it seems more likely that plant matter contributed to the bulk of the mammals' diet.

Other species. Flying squirrels, smoky shrews and woodland jumping mice were not captured in sufficient numbers for statistical analysis but the spores found are worth noting. The sample of Northern flying squirrel revealed spores of Boletaceae,

Elaphomycetaceae and Russulaceae, which are similar to those found in Western populations of flying squirrels (Jacobs and Luoma, 2008; Pyare and Longland, 2001).

Although no spores were found in the woodland jumping mouse sample, the presence of *Glomus* in other species and previous reports of *Glomus* consumption by woodland jumping mouse (Ovaska and Herman, 1986) would suggest that they also consume the fungus but we were not able to detect it from just one sample. Lastly, one of the two samples from smoky shrew contained a symmetrical, pitted spore indicating that it may be hypogeous but this remains undetermined until it is identified.

Analysis of short-tailed shrew, deer mouse, eastern chipmunks and southern red-backed voles has shown the small mammals are consuming hypogeous fungi and by doing so

these small mammals are playing a role in dispersal of mycorrhizal fungi. Examination of the smoky shrew, flying squirrel and woodland jumping mouse samples show that more species may be dispersing spores across the landscape as well and require further research. Future studies should also explore seasonal variations and increase sample size to obtain more statistical power. Now that it is certain that small mammal mycophagy occurs in the Northeast, wildlife managers should take into account the health of fungal biota as it could affect the health of forest tree-stands and small mammal populations.

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Appendix

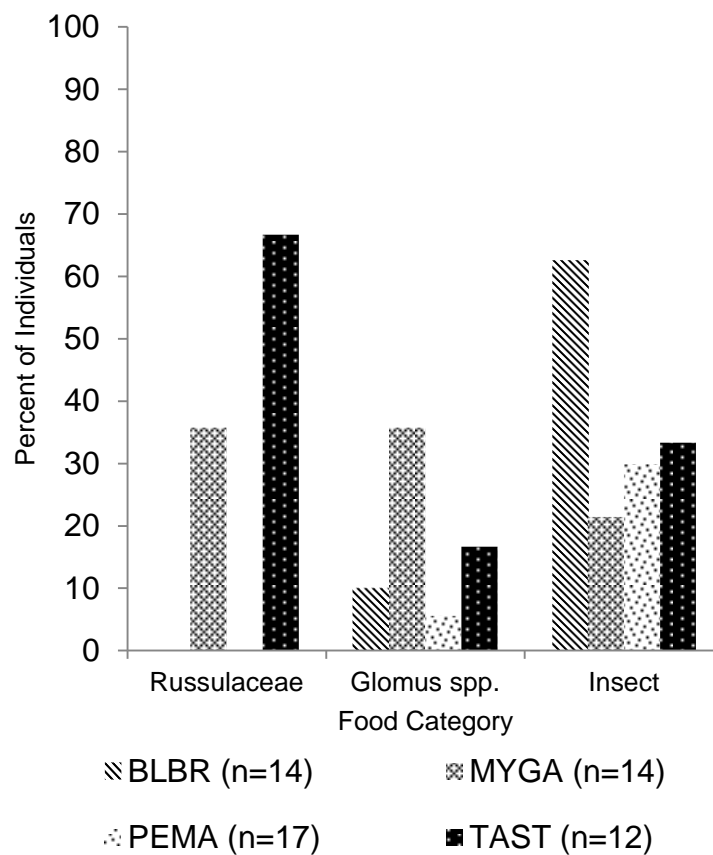


Figure 1. Proportion of food items in the diets of short-tailed shrew (BLBR), deer mouse (PEMA), Southern red-backed vole (MYGA) and Eastern chipmunk (TAST).

Table 1. Comparison of small mammal mycophagy of Northeastern literature to the current study. Species are listed with the frequency of occurrence of each fungal species found in their diet. Note that studies conducted before 1974 that have found *Endogone* could be considered as *Glomus* by the current taxonomic assignment.

Study Location	Season	Mammal Species	Fungal Species (frequency of occurrence)	Source
New York (Hamilton and Newcomb Co.)	Summer	Deer mouse	<i>Glomus</i> (5.5%)	Present Study
		Eastern chipmunk	<i>Glomus</i> (16.7%), <i>Russulaceae</i> (66.7%)	
		Red-backed vole	<i>Glomus</i> (35.7%), <i>Russulaceae</i> (35.7%)	
		Short-tailed shrew	<i>Glomus</i> (10%)	
		Woodland jumping mouse	None	
		Smoky shrew	Unknown Spore	
		Northern flying squirrel	<i>Boletaceae</i> , <i>Elaphomycetaceae</i> , <i>Russulaceae</i>	
New York (Ithaca)	Summer, Fall	Red-backed vole	<i>Endogone</i> (31.4%), some <i>Hymenogaster</i>	Whitaker, 1962
		Short-tailed shrew	<i>Endogone</i> (13.4%), <i>Hymenogaster</i> (1.5%)	
		Woodland jumping mouse	<i>Endogone</i> (69.8%), <i>Hymenogaster</i> (9.4%)	
		Smoky shrew	<i>Endogone</i> (27.7%)	
Ohio	Spring, Summer	Eastern chipmunk	<i>Russula</i> (?)	Wrazen and Svendsen, 1978
Nova Scotia	Spring, Summer	Red-backed vole	<i>Hydnотrya cubispora</i> (15.8%), <i>Glomus</i> (5.3%), <i>Sclerocystis</i> (5.3%), Unknown (21.1%)	Ovaska and Herman, 1986
		Eastern chipmunk	<i>Glomus</i> (7.8%), <i>Sclerocystis</i> (14.1%), Unknown (6.3%)	
		Short-tailed shrew	<i>Glomus</i> (16.7%)	
		Woodland jumping mouse	<i>Hydnотrya cubispora</i> (5.6-30.4%), <i>Endogone</i> (0.5-1.8%), <i>Gigaspora</i> (4.6-17.9%), <i>Glomus</i> (27.3-64.3%), <i>Sclerocystis</i> (19.1-51.8%), Unknown (8.2-12.5%)	