Hypogeous, Sequestrate Fungi (Genus *Elaphomyces*) Found at Small-mammal Foraging Sites in High-elevation Conifer Forests of West Virginia

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Abstract - Little is known about hypogeous, sequestrate fungi (i.e., truffles) in the eastern United States. Since the fruiting bodies of these fungi are part of the diet of multiple rodent species, filling data gaps is important to understanding more about truffle species distribution and habitat associations. During a microhabitat study on radio-collared *Glaucomys sabrinus fuscus* (Virginia Northern Flying Squirrel) in 2013, we opportunistically sampled truffles at small-mammal digs and scratches within our microhabitat plots. All sampling was conducted within known squirrel-foraging home ranges. We found 3 *Elaphomyces* species: *E. macrosporus*, *E. verruculosus*, and *E. americanum*. Our observations of *E. macrosporus* are the first from West Virginia. Herein, we describe the microhabitat associations for each fungal species. We suggest using small-mammal digs and scratches as potential indicators to opportunistically gather more information on truffle species in coniferous forests of the eastern United States.

Introduction. Biological linkages between small mammals and truffle fungi are well-established (Carey et al. 1999, Hall 1991, Maser et al. 1986, North et al. 1997). Small mammals consume a wide taxonomic variety of truffle species (Johnson 1996). Due to the subterranean nature of the truffles, spore dispersal is dependent on small mammals, such as mice (Cricetidae), voles (Cricetidae), jumping mice (Dipodidae), and squirrels (Sciuridae) (Fogel and Trappe 1978, Luoma et al. 2003, Stephens and Rowe 2020). Truffles are excavated and consumed by small mammals, and the truffle spores are subsequently returned back to the forest floor in their fecal droppings, providing the major mechanism for truffle spore dispersal across the landscape (Johnson 1996, Maser et al. 2008).

Truffle communities and their associations with small mammals in the western United States are well studied (e.g., Fogel and Trappe 1978, Lehmkuhl et al. 2004, Maser et al. 1985, Meyer and North 2005, Trappe et al. 2009). However, in the Appalachian Mountains of the eastern United States, these truffle communities and their associations with small mammals have received less attention and have remained largely speculative. A handful of studies in this region focused on the diets of small-mammal species, including *Glaucomys sabrinus* Shaw (Northern Flying Squirrel), *Myodes gapperi* Vigors (Southern Red-backed Vole), *Neotoma magister* Baird (Allegheny Woodrat), and *Napaeozapus insignis* Miller (Woodland Jumping Mouse), and found these small mammals consumed a variety of truffle species (Castleberry et al. 2002, Mitchell 2001, Orrock and Pagels 2002, Orrock et al. 2003, Weigl et al. 1992). A few other studies assessed truffle communities in high-elevation *Picea rubens* Sarg. (Red Spruce) forests and spruce–northern hardwood forest of the central and southern Appalachian Mountains (Bird and McCleneghan 2005, Ford et al. 2004, Loeb et al. 2000).

Truffle fungi fruit subterraneanly, have patchy distributions across the landscape, and only produce fruiting bodies during certain times of the year, making surveying for them...
challenging (Claridge et al. 2000, Van der Linde et al. 2012). Randomized plots may result in no detections of truffle fungi (e.g., Trapp et al. 2017), and due to the ephemeral nature of truffles, the majority of plots will not contain truffle fruiting bodies (Claridge et al. 2000). Small-mammal digs and scratches are a potential indicator of truffle presence (Castellano et al. 1999). In this study, we report the results of our opportunistic sampling of small-mammal digs and scratches at foraging telemetry points of radio-collared *G. s. fuscus* Miller (Virginia Northern Flying Squirrel) during a microhabitat study of the squirrels in the central Appalachian Mountains of West Virginia (Diggins and Ford 2017). We recorded information about microhabitat characteristics associated with truffle sites with the aim of filling data gaps in truffle species distribution and ecology.

**Methods.** Our study sites were in eastern West Virginia on the Allegheny Mountains and Plateau, a physiographic sub-region of the Appalachian Mountains. Sites occurred in a *Picea abies* (L.) H. Karst (Norway Spruce)–*Tsuga canadensis* (L.) Carrière (Eastern Hemlock) stand at Kumbrabow State Forest in Randolph County, a Red Spruce stand at Snowshoe Ski Resort, and a Red Spruce–Eastern Hemlock stand at Mill Run on the Greenbrier Ranger District, Monongahela National Forest in Pocahontas County (Fig. 1). Norway Spruce occurred in plantations and is not a native species to the Appalachian Mountains. Elevations varied from 950 to 1450 m, and the climate is cool and moist with annual precipitation of 120–150 cm (Stephenson 1993).

We opportunistically sampled for truffles at eighty 1 m x 1 m microhabitat plots. We obtained plot locations from telemetry points of 4 radio-collared Virginia Northern Flying

![Figure 1. Location of microhabitat plots obtained from radio-collared *Glaucomys sabrinus fuscus* Miller (Virginia Northern Flying Squirrel) in West Virginia in summer 2014.](https://bioone.org/journals/Northeastern-Naturalist)
Squirrels (2 males, 2 females) during June–July 2013. We randomly selected 20 foraging points per squirrel for microhabitat surveys. For protocol on trapping, radio-tracking, and microhabitat surveys, see Diggins and Ford (2017). Since there were other mycophagist small mammals potentially present at the study sites (e.g., Southern Red-backed Voles, Woodland Jumping Mice, Allegheny Woodrats, *Tamiascurius hudsonicus* Erxleben [Red Squirrel], and *Peromyscus* spp.), we could not attribute small-mammal digs specifically to Virginia Northern Flying Squirrels, even though the digs fell within foraging plots of the radio-collared flying squirrels. We searched plots for 2 indications of small-mammal foraging for truffles: digs and scratches. We classified digs as distinctive small holes, typically 1–10 cm in diameter and <2 cm deep (Fig. 2). We determined a dig as “fresh” if it did not have debris (i.e., leaf fragments, needles) or spider webs covering it (Castellano et al. 1999, 2003). We classified scratches as a small area where the top layer of humus was scraped away. Scratches were shallower than 2 cm and smaller than 5 cm x 5 cm, as larger and deeper scratches were likely made by non-target species such as *Meleagris gallopavo* L. (Wild Turkey) or *Odocoileus virginianus* Zimmerman (White-tailed Deer) (Elbroch 2003). When evidence of small-mammal digs and scratches (hereafter “digs”) were found, we searched in a 0.5 m x 0.5 m area around the dig for any truffle fruiting bodies. We collected truffles using a destructive sampling technique. Using a standard garden spade, we removed humus down to the mineral layer and exhumed truffle fruit bodies as we dug. After completing our survey, we replaced the humus back into the area we had just excavated. We also searched for truffles within a plot if we saw *Tolypocladium ophioglossoides* (J.F. Gmel.) C.A. Quandt, Kepler, & Spatafora, an Ascomycete fungus that is parasitic on *Elaphomyces* fruiting bodies.
and is known to occur in West Virginia (Fig. 3; Mains 1957). We placed excavated truffles in wax bags and kept them in cool, dry conditions until they were processed, at which time we identified them to species (Castellano and Stephens 2017, Castellano et al. 2012). We compared differences in canopy cover, duff cover, and average organic horizon depth of plots containing truffles and plots without truffles using a t-test in Program R version 3.1 (R Development Core Team 2015).

Results. During our study, we found small-mammal digs at 43 Virginia Northern Flying Squirrel microhabitat plots (53.8% of plots). Of those, we found truffles at 60.5% of the plots with small-mammal digs. We identified 3 truffle species: *Elaphomyces macrosporus* Castellano and Elliot at 13 plots, *E. verruculosus* Castellano at 12 plots, and *E. americanum* Castellano at 1 plot (Table 1). We did not find more than 1 truffle species at any 1 plot. We detected all 3 truffle species at Mill Run, *E. macrosporus* and *E. verruculosus* at Kumbrabow, and only *E. verruculosus* at Snowshoe. The closest trees to the plots with digs were an average distance of 2.13 ± 0.33 m. For *E. macrosporus*, the closest trees were Norway Spruce (46.2% of sites), *Acer rubrum* L. (Red Maple; 30.7%), Red Spruce (7.6 %), Eastern Hemlock (7.6%), and *Betula alleghaniensis* Britt. (Yellow Birch; 7.6%). The closest trees to *E. verruculosus* were Norway Spruce (45.5% of plots), Eastern Hemlock (18.2%), *Prunus serotina* Ehrh (Black Cherry; 18.2%), Red Spruce (9.1%), Yellow Birch (9.1%), and Red Maple (9.1%). The closest tree to *E. americanum* was Red Maple. Eastern Hemlock, Red Spruce, and/or Norway Spruce were dominant in the overstory of 88.5% of plots with

Figure 3. *Tolypocladium ophioglossoides* parasitizing on the truffle of an *Elaphomyces* spp. in a plantation of Norway Spruce and Eastern at Kumbrabow State Forest, Randolph County, WV, in summer 2014.
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truffles. In the remaining plots, these species were dominant in the midstory. There was no difference in canopy cover ($t_{35} = 1.26, P = 0.22$), duff cover ($t_{51} = -1.23, P = 0.23$), or average organic horizon depth ($t_{35} = 1.24, P = 0.23$) between plots with truffles and those with no truffles.

**Discussion.** Knowledge on the distribution and habitat associations of many truffle species in the eastern United States is lacking (Maser et al. 2008). Using presumed foraging sites of Virginia Northern Flying Squirrels, we were able to collect 3 *Elaphomyces* species and gather information on tree associations and microhabitat conditions. Previous surveys in Red Spruce forests of the Appalachian Mountains documented *Elaphomyces* spp. as the most common truffles encountered during surveys (Ford et al. 2004, Loeb et al. 2000, Stephens et al. 2017). Similar to our study, Bird and McCleneghan (2005) only found *Elaphomyces* spp. during surveys at Roan Mountain Highlands, NC. Ford et al. (2004) conducted their surveys in West Virginia in similar habitat types as our study, but they did not record any of the *Elaphomyces* spp. we recorded during our surveys. The difference in species recorded during this study and the study by Ford et al. (2004) was likely due to the lack of taxonomic information for North American *Elaphomyces* species prior to work by Castellano et al. (2012) and Castellano and Stevens (2017).

The *Elaphomyces* species we recorded were more similar to fungal communities in Red Spruce–Eastern Hemlock forests and conifer–northern hardwood forests in the northeastern United States (Stephens et al. 2017) than to the species found in the southern Appalachians (Bird and McCleneghan 2005, Loeb et al. 2000). However, specimens originally identified as *Elaphomyces leveillei* Tul. & C. Tul. by Loeb et al. (2000) were later identified as *E. macrosporus* (Castellano and Stephens 2017). The misidentification occurred because *E. macrosporus* was not a described species at the time of that study. At the time of our survey, *E. macrosporus* was only known from New England. Our observations of this species were the first record for *E. macrosporus* in West Virginia; however, other records have been confirmed since (Castellano and Stephens 2017). This species is associated with Eastern Hemlock (Castellano and Stephens 2017), although 84% of plots in this study with *E. macrosporus* did not have Eastern Hemlock within 10 m of the plot. *Elaphomyces macrosporus* plots were dominated by Norway Spruce (90.9% of plots) or Red Spruce (9.1%). Norway Spruce is not native to the United States and is only found

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**Table 1.** Microhabitat characteristics of the fruiting bodies of 3 *Elaphomyces* hypogeous fungi species found at foraging points of *Glaucomys sabrinus fuscus* (Virginia Northern Flying Squirrel) in West Virginia in summer 2014.

<table>
<thead>
<tr>
<th></th>
<th><em>E. americanum</em></th>
<th><em>E. macrosporus</em></th>
<th><em>E. verruculosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sites</td>
<td>1</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>% canopy</td>
<td>88.5</td>
<td>79.6 ± 5.1</td>
<td>82.5 ± 4.5</td>
</tr>
<tr>
<td>Average organic horizon depth (cm)</td>
<td>8.4</td>
<td>6.2 ± 1.0</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>Microhabitat cover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% duff</td>
<td>84</td>
<td>74.2 ± 7.0</td>
<td>76.6 ± 6.4</td>
</tr>
<tr>
<td>% herbaceous cover</td>
<td>11</td>
<td>9.9 ± 4.9</td>
<td>8.8 ± 3.9</td>
</tr>
<tr>
<td>% woody debris</td>
<td>5</td>
<td>7.3 ± 1.7</td>
<td>6.4 ± 1.5</td>
</tr>
<tr>
<td>% moss/lichen</td>
<td>0</td>
<td>8.2 ± 5.0</td>
<td>7.8 ± 3.8</td>
</tr>
<tr>
<td>% bare ground</td>
<td>0</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Distance to closest tree (m)</td>
<td>1.40</td>
<td>1.95 ± 0.59</td>
<td>2.40 ± 0.38</td>
</tr>
</tbody>
</table>
in plantations. However, Kumbrabow State Forest was historically a Red Spruce forest. Additionally, Eastern Hemlock is present at the site and can be co-dominate with Norway Spruce. Therefore, *E. macroporus* may have been present at the study site prior to logging and subsequently developed associations with Norway Spruce. While our sampling was limited, our findings suggest *E. macroporus* may be associated with other conifers in addition to Eastern Hemlock.

Although a previous study in the southern Appalachians did not find a significant relationship between soil characteristics and truffle presence (Loeb et al. 2000), other studies described deeper organic horizons were associated with higher abundances of truffles (Meyer et al. 2007). Diggins and Ford (2017) reported significantly deeper organic horizons at Virginia Northern Flying Squirrel foraging points where truffles were found versus random points (12.5 ± 0.5 cm at truffle sites vs 8.6 ± 1.0 cm at sans truffle sites). Our data showed no significant difference in organic horizon depth either between sites with or without truffles or between sites with or without digs. These findings could be due to Virginia Northern Flying Squirrels selecting foraging sites with deeper organic horizons versus random plots (Diggins and Ford 2017).

Truffle fungi form mutually beneficial symbiotic relationships with tree roots, and their fruiting bodies are often found within a few meters from trunks of potential host trees (Fogel 1976, Loeb et al. 2000). Tree root systems can extend up to twice the height of the tree as roots forage for nutrients and water, thus while many truffle fruiting bodies can be found close to host trees they can also be found dozens of meters from the tree trunk. Most of our truffle plots were closest to montane conifers, such as Norway Spruce, Red Spruce, or Eastern Hemlock. These tree species were dominant in the overstory or midstory in all of the plots where we found truffles. For several plots, Red Maples were the closest tree. Nonetheless, we doubt that any of these species are associated with Red Maple because previous studies indicated maples do not support truffle fungi in the genus *Elaphomyces* (Trappe et al. 2007).

*Elaphomyces* species are gregarious and will fruit over the course of several weeks or even months (M.A. Castellano, pers. observ.; Fogel 1976). Using small-mammal digs and scratches helped us key into the potential presence of truffles and may be a useful indicator versus random surveys, since small mammals are much more efficient than humans at finding truffles (Pyare and Longland 2001). However, forest composition may limit the use of this technique to find truffles. Our study took place in boreo-montane conifer-dominated forests. We anecdotally observed that digs were difficult to locate in areas with more hardwood trees in the overstory, possibly because the digs were easily covered by deciduous leaf litter.

*Elaphomyces* species are common food items of Appalachian Northern Flying Squirrels (Mitchell 2001, Weigl et al. 1992), as well as other mycophagist small mammals found in these mountains (Orrock and Pagels 2002, Orrock et al. 2003, Stephens and Rowe 2020). Our sampling was conducted at a limited number of sites and only occurred during a short season. However, we detected truffles at 32.5% of our telemetry sites, indicating that assessing truffle communities by locating small-mammal digs was useful. Loeb et al. (2000) detected truffles in less than 10% of their plots, whereas Trapp et al. (2017) did not detect any truffles during their study. Our work expanded the known distributions of these truffle species and their habitat associations in the central Appalachians. Further work needs to occur to understand truffle communities throughout the Appalachian Mountains and how past and current land-use practice may be influencing their distribution across the region.

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Literature Cited


