AN ABSTRACT OF THE THESIS OF

Wesley Colgan III for the degree of Doctor of Philosophy in Forest Science presented on July 30, 1997. Title: Diversity, Productivity, and Mycophagy of Hypogeous Mycorrhizal Fungi in a Variably Thinned Douglas-Fir Forest.

Abstract approved: _____

Randolph J Molina

Hypogeous fungi are a critical component of forest ecosystems world wide. In Pacific Northwest forests, they are the base of the food chain of the threatened Northern Spotted Owl.

As part of The Forest Ecosystem Study (a interdisciplinary study designed to increase development of suitable spotted owl habitat), diversity and productivity of truffles were studied from March 1993 through December 1996 at approximately six week intervals at the Fort Lewis Military Reservation near Olympia, Washington. Half of the stands served as controls, half were assigned a variable density thinning (VDT) treatment. A VDT stand was comprised of a mosaic of 0.16 ha- patches thinned to different densities of standing live trees. To further examine effect of thinning on sporocarp productivity and diversity, this mosaic was stratified into 2 sub-treatments, lightly thinned and heavily thinned areas.

Total standing crop biomass over all seasons was significantly reduced in VDT stands compared to the control stands. However, VDT stands had greater truffle standing

crop during two of the three winters sampled. This suggests that, in the short term, thinning could reduce production overall but enhance production of truffles in winter. Species richness and evenness were highest in the lightly thinned areas within the VDT stands and declined sharply in the heavily thinned areas, compared to the control stands. Two novel truffle taxa collected during this study were described.

A reliable method to assess dietaries of northern flying squirrels (*Glaucomys sabrinus*) and Townsend's chipmunk (*Tamias townsendii*) was developed, and subsequently used to analyze fecal pellets collected from live trapped individuals. Collections from spring 1991 through December 1996 were analyzed to estimate dietary composition. Hypogeous basidiomycetes and ascomycetes dominated chipmunk and flying squirrel dietaries. Plant material was also a dominant component of chipmunk dietaries (both spring and fall) and fall flying squirrel dietaries. Both mammal species consistently found more fungal taxa than mycologists did during the concurrent mycological survey. Thinning did not significantly change the rank order of fungal components in the dietaries of these small mammals.

Diversity, Productivity, and Mycophagy of Hypogeous Mycorrhizal Fungi in a Variably Thinned Douglas-Fir Forest.

by

Wesley Colgan III

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APPROVED:

Major Professor, representing Forest Science

Chair of Department of Forest Science

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Wesley Colgan III, Author

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CONTRIBUTION OF AUTHORS

Andrew B. Carey and James Trappe were involved in the research, interpretation of data, and writing of Chapter 3. James Trappe was involved in the research and writing of Chapter 5.

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DEDICATION

This thesis is dedicated to my mother and father

Peggy and Wes Jr.

who taught me never to quit.

Diversity, Productivity, and Mycophagy of Hypogeous Mycorrhizal Fungi in a Variably Thinned Douglas-Fir Forest

Chapter 1

Forest Fungi and the Northern Spotted Owl

Introduction

The federal listing of the Northern spotted owl (*Strix occidentalis caurina*) as a threatened species has changed management on much of the public land in the Pacific Northwest. The loss of old growth forests on which this species depends has created the need to manage young forests in an effort to balance human needs for wood and retention (and potentially accelerated development) of habitat elements critical to the owl. The Forest Ecosystem Study (FES, Carey *et al.* 1997a) at the Fort Lewis Military Reservation, Washington is a prototype study to determine whether the habitat needs of the owl and the human need for forest products can both be met in a second growth Douglas-fir forest.

The forests at Fort Lewis have been managed exclusively for timber since the fort was established. Two of the study blocks (designated Star and Stellar) were clearcut in 1937. Natural regeneration produced high density stands of small and medium diameter Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). These have not been manipulated since clearcutting. Two other study blocks (designated Farley and Hill) were clearcut in 1927. Stands in these blocks have been lightly thinned twice, 1972 and again in the late

1980's. None of these blocks were shown suitable habitat for spotted owls. While no two Douglas-fir stands are the same, our study sites at Fort Lewis share many attributes of second growth forests across the region. Many of the findings from our studies will have direct applications in forest management decisions throughout the Pacific Northwest.

The use of fungi as a food resource by mammals (mycophagy) has been documented in Pacific Northwest forests (Carey *et al.*, 1992; Carey, 1995; Maser *et al.*, 1978; Trappe and Maser, 1977). However the effect of timber harvest on the dietaries of these mammals has been little studied. Northern flying squirrels, for example, preferentially eats truffles, the fruit bodies of fungi that are mycorrhizal with the conifers that dominate these ecosystems (Trappe, 1962; Molina *et al.*, 1992). Squirrels, in turn, are the preferred prey of the northern spotted owl in much of its range (Carey *et al.*, 1992). This connection between owl, squirrel, fungi, and tree illustrates just one path in the complex of forest food webs. To preserve the spotted owl, forests must be managed for all connections in that web.

My portion of the FES entailed study of short term effects of a variable density thinning on truffle diversity and biomass. Silvicultural manipulations affect mycorrhizal fungi by removing host trees and by altering the structure and microclimate of the habitat; the extent and duration of these effects is unknown. Possible effects include 1) reduced sporocarp production due to removal of mycorrhizal host trees that provide the energy to the fungi for fruiting; 2) reduced sporocarp production due to changes in soil porosity, aggregate stability and temperature, and 3) eventual recovery of fungal sporocarp production due to increased vigor of leave trees, recovery of soil physical characteristics,

and canopy closure that reduces soil temperature. Changes in understory vegetation in thinned areas may also affect sporocarp production.

This study is the first to examine effects of a VDT in a mature second growth Douglas- fir forest on sporocarp production of mycorrhizal fungi and the animals that feed on them. It is also first to integrate data on fungal productivity (as estimated by plot sampling) and side-by-side small mammal dietary data.

Thesis Organization

This thesis is divided into seven chapters. Chapter 2 presents the results of the three year effort to assay the effects of VDT on diversity and productivity of hypogeous fungi and focuses on changes in species composition immediately following thinning. Chapter 3 develops methods used to analyze fecal pellets of mycophagous small mammals. Chapter 4 examines dietaries of arboreal mammals foraging in these thinned and adjoining control stands. Spring and fall dietary composition based on fecal pellet analysis and the fungi found by the concurrent mycological survey are compared. Chapters 5 and 6 describe two new species of hypogeous fungi found during the mycological survey. Chapter 7 summarizes findings of these studies and suggests directions for further research.

Chapter 2

Diversity And Productivity Of Hypogeous Fungal Sporocarps In A Variably Thinned Douglas-Fir Forest.

Wesley Colgan III

Abstract

Although ecosystem management techniques are designed to enhance species diversity in managed forests, no comprehensive study has been conducted to evaluate effects of such techniques on hypogeous fungus diversity and productivity. During this study, truffles were collected in a 55-65 year old Douglas-fir forest from March 1993 through December 1995 at approximately six week intervals. Half of the stands served as controls, half were assigned a variable density thinning (VDT) treatment. A VDT stand is comprised of a mosaic of patches thinned to different densities of standing live trees. To further evaluate the effect of harvesting impacts, this mosaic was divided into 2 sub-treatments, lightly thinned and heavily thinned areas. Truffle standing crop varied greatly, but generally was highest in spring with a smaller peak in the fall. At least some sporocarps were found year round, with winter having the lowest biomass and species richness. VDT stands had higher standing crop during 2 of the 3 winters sampled. This suggests that thinning could enhance production of truffles in winter. Overall standing crop biomass (over all seasons) was significantly lower in VDT stands compared to control stands. Within the VDT stands, species richness and evenness was greatest in the lightly thinned areas and dramatically less in the heavily thinned areas, compared to the control stands. The abundance of *Gautieria* and *Hysterangium* species was lower in thinned stands, while *Melanogaste*r species diversity and productivity was highest in these stands. Although total number of collections (one to several sporocarps of the same species in close proximity to one another

on a single 4.0-m^2 sample plot) was lowest in the heavily thinned areas, number of sporocarps and biomass per collection were greatest. These data suggests that increased fruiting in the heavily thinned areas may have been a stress reaction by these fungi.

Key Words: disturbance, ecosystem management, Gautieria, Hysterangium, Melanogaster, *mycophagy, mycorrhiza, northern flying squirrel, truffles.*

Introduction

Mycorrhizal fungi assist forest trees in the uptake of water and nutrients from the soil and facilitate movement of carbohydrates from host plants into the mycorrhizosphere. This carbon source supports a vast array of microbes, insects, nematodes, bacteria and other soil organisms (Ingham and Molina, 1991; Fogel, 1988). Sporocarps of these fungi are a food resource for many forest mammals worldwide (Chapters 3,4, this volume; Carey *et al.*, 1992; Carey, 1995; Fogel and Trappe; 1978; Maser *et al.*, 1978; Trappe and Maser, 1977; Malajczuk *et al.*, 1987; Hays *et al.*, 1986; Claridge and May, 1994; Mcintire, 1984; Blaschke and Baumler, 1989; Viro and Sulkava, 1985; Launchbaugh and Urness, 1992). Most of the sporocarps consumed by animals in temperate forests are formed by ectomycorrhizal (EM) fungi that form a symbiotic association with the feeder roots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and other members of Pinaceae, Fagaceae, Betulaceae, Myrtaceae and Salicaceae (Trappe, 1962; Molina *et al.*, 1992)

Hypogeous fungi (hereafter referred to as truffles) fungi attract animals to their mature sporocarps by producing aromatic compounds (Fogel and Trappe 1978), some of these are chemically identical to the sex pheromones of their animal consumers (Claus *et*

al., 1981). The animals extract the truffle, consume all or part of it (Fogel and Trappe, 1978; Trappe and Maser, 1977). including spores, bacteria and yeasts that live in the sporocarps (Li *et. al.,* 1986). The spores, yeasts and bacteria pass through the digestive tract unharmed and, along with feces are deposited in new locations (Trappe and Maser, 1976). Rain or snow-melt water may then move the fecal contents into the soil, were they colonize new roots (Trappe and Maser, 1977).

Sexual reproduction (sporocarp formation) of mycorrhizal fungi has a strong seasonal aspect in the Pacific Northwest (PNW) (Fogel, 1976; Hunt and Trappe, 1987; Luoma *et al.*, 1991). Studies of truffle abundance in this region have largely examined different forest types and succesional stages (Amaranthus *et al.*, 1994; Vogt *et al.,* 1981; Luoma *et al.,* 1991; O'Dell *et al.,* 1992). Luoma (1989) and studies in progress (D. Luoma; J. Smith; in Pilz and Molina, 1996) show differences in the EM fungus community structure by comparing stands at ages of 25 and 50 years, and between 80, 160, and 400+ years. Most of these studies have focused on the peak spring and fall fruiting periods of the truffles and other ectomycorrhizal fungi. This approach has left rather gaps in our understanding of the functional importance of many truffle species at other times of the year. For mycophagous mammals that are active year round (e.g. the northern flying squirrel, *Glaucomys sabrinus*) truffles that fruit during winter and summer are critically important. North *et al.* (1997) included winter and summer sampling in old growth, naturally mature, and managed mature forests in the PNW. Hunt and Trappe (1987) and Fogel (1975) documented productivity monthly on sites in western Oregon.

These studies show that there are some species of truffles available year-round in some PNW forests, although lowest diversity and standing biomass occurred in winter.

Little is known of mycorrhizal fungal dynamics during the first 25 years following disturbance. Amaranthus *et al.* (1994) found significantly lower truffle abundance and diversity in 4 to 27 year old plantations (regenerated from clear cuts) than in neighboring mature (180 year-old) forest fragments in the Siskiyou national forest in southwestern Oregon. Waters *et al.* (1994) is the only published paper that has addressed the effects of thinning on the production and diversity of truffles. This study focused on thinned stands in an *Abies* spp. forest at 17 and 20 years post thinning. They found that overall frequency of sporocarps did not differ significantly in these stands. However they did find an association between thinning intensity and frequencies of the most common genera collected in one of their study sites, suggesting that thinning changed the community structure of truffle species fruiting in these stands. The immediate (<5 years) effects of thinning treatments on truffles have not been studied in any comprehensive way.

In addition to performing a myriad of key functions fungi support a number of critical trophic pathways in forest ecosystems. The northern flying squirrel, for example, strongly depends on truffles as the major component of its dietaries Carey *et al.* (1992) and Carey (1994) found that flying squirrel abundance increased with fungal diversity along a north west cline in the North Cascades and Olympic Peninsula in northern Washington to the central Western Cascades, southern Coast Ranges and Klamath Mountains of Southwest Oregon. Waters and Zabel (1995) found flying squirrel densities correlated with sporocarp frequency in fir forests in northeastern California. However we

poorly understand the roll of specific fungal species and the effect of disturbance and forest management (e.g. thinning and tree harvest) and the population dynamics of fungal and small mammal communities.

Intermediate cutting, especially thinning, are frequently proposed as a method of accelerating development of late-seral forest. Long-term effects are thought to be positive. Short-term impacts are on fungi may be negative and thus could have a depressive effect on mycophagists, including the primary prey of the northern spotted owl, the northern flying squirrel. The objective of this study was to document the changes in the productivity and diversity of truffles for the first three years following the installation of a variable density thinning. This study was one of a suite of interdisciplinary efforts of the forest ecosystem study (FES, Carey *et al.* 1997a)

Methods

Study Area

The FES is located on four blocks of forest, 30 km NE of Olympia, WA on the Fort Lewis Military Reservation. The soils of Star and Stellar blocks are classified as Tenino gravelly sand loam. The soils of Hill and Farley blocks are classified as Everett gravelly sand loam. All blocks fall into the Southern Puget Trough physiographic province (Franklin and Dyrness, 1973). The stands are essentially flat to gently rolling, and slopes rarely exceed 15%. Elevation ranges from 100-143m, annual average precipitation is 91 cm.

The forests on the Fort Lewis Military Reservation have been designated as habitat critical to the spotted owl because they constitute the only federal forest stepping stone between the Cascade range to the east and the Olympic Peninsula to the West. Fort Lewis surrounded by urban and agricultural land on the northeast and south, and the Puget Sound on the northwest.

The forests at Fort Lewis are dominated by Douglas-fir. Most of the old growth was cleared in the first half of this century; existing stands regenerated from natural seeding. Farley and Hill blocks were clearcut in 1927 and have been lightly thinned twice (1972 and again in the late 80's). They have almost no remaining coarse woody debris (CWD) or large diameter trees from the original old growth; CWD was intentionally removed from these stands during the early thinning operations. They have developed an extensive understory of *Gaultheria shallon* Pursh., *Berberis nervosa* Pursh., and *Polystichum munitum* (Kaulf.) Presl. Star and Stellar blocks were clearcut in 1937 and had no further silvicultural manipulations prior to the installation of our study. Understories in these blocks were variable and dominated by ground mosses. *Gaultheria shallon* and *Polystichum munatum* occur primarily in scattered canopy openings caused by laminated root rot (*Phellinus weirii* (Murr.) Gilb.). These blocks had considerable residual CWD and numerous large-diameter old-growth trees (approximately 5/ha).

Sampling Considerations

The FES is a 4X4 complete randomized block experiment. Each of the four blocks contains four stands, and each stand was randomly assigned one of four treatments: control

(no treatment), artificial cavities and nest boxes, variable density thinning (VDT), and a combination of artificial cavities, nest boxes, and VDT. Each stand was surveyed and a sampling grid installed. Each grid consists of 64 points aligned in an 8X8 matrix, with points 40 horizontal meters apart. These points serve as trapping stations for arboreal rodents. A buffer zone of at least 80m was maintained between each trapping grid. All concurrent studies used these grid points as reference points. All of the thinning sub-treatments were based on this 40mX40m grid system.

Silvicultural Prescription

The silvicultural prescription for the FES was designed to produce a desired future condition that was based on empirical descriptions of spotted owl habitat and old-growth forests. A goal of this study is to determine if late seral forests can be created while extracting commodities (e.g. timber) from the stands in managed forests. Objectives of this treatment include increasing plant, animal, and functional diversity; increasing structural and spatial heterogeneity, specifically diversity of woody plant species to increase the value of the stands as habitat for the northern spotted owls (Carey et al, 1992; Carey, 1995; Carey and Johnson, 1995). Past management of the Fort Lewis led to monospecific, even-aged, closed-canopy conditions with extensive infestations of laminated root rot (*Phellinus weirii* (Murr.) Gilb.) that created scattered, small, canopy openings. These infection centers were mapped and treatments were devised to promote reforestation of the gaps and to standardize the amount of canopy openings within treatment stands.

A VDT was prescribed to accomplish these goals. All thinning treatments were installed between January and April 1993. An root-rot treatment was installed centered on the *Phellinus* infection centers. Where root rot pockets were ≤15% of the stand the treatment was randomly applied to grid cells for a total of approximately 16% (8 of the 49 grid cells). The root-rot treatment was designed to remove all trees showing low vigor or other evidence of root-rot and to leave all healthy trees (generally ≤40 stems/ha) in these open patches. A heavy thin treatment was assigned to 14 of the 49 grid cells in each thinned stands. This treatment is defined as equivalent to retaining 180 live stems/ha in an average, unthinned, 50-year-old second-growth stand.

These two thinning sub-treatments were underplanted with *Pinus monticola* Dougl. ex D. Don., *Abies grandis* (Dougl. ex D. Don) Lindl., *Thujia plicata* Donn. ex D. Don, or *Alnus rubera* Bong. the fall following the harvest. The balance of the grid cells were assigned a light thin treatment, defined as equivalent to leaving 310 live stems/ha in an average, unthinned, 50-year-old second-growth stand.

Fungal Sampling

Field sampling took place approximately every six weeks from April 1993 through December 1995. At the first sampling, 8 stands (without tree cavities/nest boxes) were sampled; at the second sampling, approximately six weeks later, the other 8 stands (with tree cavities/nest boxes) were sampled. This alternation was continued throughout the study. Fungal sporocarps were collected from each of ten, circular, 4.0-m^2 plots, located at

approximately 10-m intervals along randomly placed transects (modified from Luoma *et al.*, 1991), in each of the control stands of each block at each sampling period. Thinned stands of each block were sampled more intensively to determine if thinning sub-treatments affect sporocarp production differently. Each thinning sub-treatment was sampled by ten randomly placed 4.0 m^2 plots, totaling 30 plots per thinned stand per sampling period.

Epigeous sporocarps (mushrooms) of species eaten by small mammals (primarily the Boletaceae and *Russula* spp.) and other mycorrhizal fungi observed or suspected to be used as food by small mammals were also collected from each plot. Each collection (one to several sporocarps of the same species in close proximity to one another on a single 4.0-m^2 sample plot) was placed in a wax paper bag with a tag recording plot number, stand number, and other pertinent information. Each plot was then raked with hand tools to a depth of at least 5 cm into mineral soil to expose hypogeous sporocarps. Field characteristics of sporocarps were noted (bruising reactions, odor, etc.) for each collection. All plots were marked with a plastic pin flag and the duff was replaced. No plots were sampled twice. All fungal samples were dried the day of collection with a forced air dehydrator set at 49°C (120°F) and then returned to the Forestry Sciences laboratory in Corvallis, Oregon, for identification and weighing to the nearest 0.01g. Voucher specimens are placed in the Mycological Herbarium at Oregon State University (OSC).

Data Retrofitting

Upon evaluating the implementation of the silvicultural prescription, it became apparent that thinning sub-treatments were not uniformly applied to cells as assigned *a*

prori. The evaluation *a posteriori*, however, provided precise data on trees removed and trees retained in each grid cell. For the most part relative density targets (Proportions of grid cells in different classes) were met. We used percentage of basal area removed as the best measure of treatment intensity. This measure was selected over stems retained, relative density, and others, because it measures the degree of disturbance on the grid cells, with emphasis on the dominant trees (the others do not). Cells were categorized into two groups based on basal area removed: lightly thinned (<48% of the B.A. in the grid cell removed) and heavily thinned (≥48% of the basal area in the grid cell removed). Lightly thinned areas averaged 18 % B.A. (SE ± 0.8%) removed (82. % ± 0.8% retained). Heavily thinned cells averaged 73% B.A. (SE ± 1.6%) removed (27% ± 1.6% retained). Each transect in the VDT grid was assigned to one of these new categories. Twelve transects that spanned grid cells of different character were excluded from the analysis.

Analysis

Frequency (presence or absence in 4.0 m^2 truffle plots) was calculated for each species encountered in each thinning sub-treatment and in the control stands. Biomass values were standardized for each transect (10, 4.0 m^2 truffle plots per sub-treatment) to kilograms per hectare (kg/ha) dry weight. Stand level biomass estimates were calculated by weighted mean values (based on percentage of the area in stand occupied by each of the thinning sub-treatments). Values from each pair of stand replicates (stands without nest boxes/cavities were sampled, then 5-6 weeks later, stands with nest boxes/cavities were

sampled), were averaged to estimate standing crop for each sampling period. Biomass data for VDT stands and control stands were compared using analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) with the significance level at 5%. Overall frequency of occurrence of truffles was from each sub treatment and control were compared using Chi square goodness of fit tests with significance level at 5%. Stands with cavities/nest boxes were considered replicates of control and VDT stands without cavities/nest boxes for this study, as they had no effect on mycophagous small mammals during the course of this study (Carey pers. com.). Species/area curves were compiled for the entire collection of samples, for each treatment (VDT and Control), and for thinning sub-treatments.

Species diversity was evaluated by using 2 indices: the Bergen-Parker Index for species dominance based on sporocarp weights in each sub-treatment, and the Margalef's index for species richness based on numbers of sporocarps in each of the sub-treatments (formulae from Magurran 1988). Indices from each sub treatment were compared using Chi square goodness of fit tests with significance level at 5%. Due to uneven numbers of sample transects, Margalef's index was calculated by randomly selecting equal numbers of transect in each category. These two measures were used because indices that attempt to represent both richness and eveness (such as the Shannon Index) often provide no information beyond that provided by richness alone (Magurran, 1988).

Results

During the course of this study, 1786 truffles were collected from 3680 plots (14,720 m^2). Forty-eight species were identified, six being undescribed species, two being undescribed genera. Species frequency over the entire study ranged from 0 to 6.4% of plots in any thinning sub-treatment or control. Four species were encountered only in heavily thinned plots, twelve only in lightly thinned plots, and five species were found only in control plots (Table 2.1).

Table 2.1: Percent frequency of each truffle species found on plots at the Fort Lewis Military Reservation from April 1993 through December 1995, in control stands and thinning sub-treatments.

Taxon	Control	Lightly thinned	Heavily Thinned
Alpova diplophloeus (Zeller & Dodge) Trappe & A.H. Sm.			0.11
Elaphomyces granulatus Fr.	0.11	0.18	0.22
Elaphomyces muricatus Fr.			0.22
Endogone lactiflua Berk. & Broome.	0.98	3.11	2.00
Endogone pisiformis Link:Fr.	0.11	0.24	0.56
Gautieria monticola Harkn.	1.52	0.18	0.11
Gen. & sp. nov. # 1	0.11		
Gen. & sp. nov. #2	0.11		
Genabea cerebriformis (Harkn.) Trappe	0.22	0.06	
Genea harknessii Gilkey			0.11
Genea intermedia Gilkey	0.11		
Glomus macrocarpum Tul. & C. Tul.	0.22	0.12	0.22
Glomus microcarpum Tul. & Tul.		0.12	

Table 2.1 (Continued)

Taxon	Control	Lightly thinned	Heavily Thinned
Glomus mosseae (T.H. Nicolson & Gerd.) Gerd. & Trappe		0.06	
Glomus sp. nov.		0.06	0.11
Hydnotrya variiformis Gilkey		0.36	
Hymenogaster sp.	0.11	0.06	0.11
Hymenogaster sublilacinus A.H. Sm.	0.11	0.24	
Hysterangium coriaceum R. Hesse	1.85	0.42	
Hysterangium crassirhachis Zeller & Dodge	1.41	0.12	
Hysterangium setchellii E. Fisch.	1.52	0.30	
Leucangium carthusianum (Tul. & C. Tul.) Paol.		0.18	
Leucogaster candidus (Harkn.) Fogel comb. ined.	0.11	0.18	
Leucogaster citrinus (Harkn.) Zeller & Dodge	0.22		0.11
Leucogaster gelatinosus Fogel nom. ined.	0.22	0.12	0.11
Leucogaster rubescens Zeller & Dodge	0.22	0.06	
Leucogaster sp. nov.		0.06	
Leucophleps magnata Harkn.	0.33	0.36	
Leucophleps spinispora Fogel	0.11		
Melanogaster ambiguus (Vitt.) Tul. & Tul.		0.18	
Melanogaster euryspermus (Zeller & Dodge) Zeller		0.30	0.33
Melanogaster natsii Wang, Trappe & Castellano, nom. ined.		0.30	0.11
Melanogaster thiersii Wang, Trappe & Castellano, nom. ined.			0.11
Melanogaster trappei Wang, nom. ined.		0.66	0.22
Melanogaster tuberiformis Corda	0.65	0.48	1.67
Melanogaster variegatus (Vitt.) Tul. & Tul.		0.06	

Table 2.1 (Continued)

Taxon	Control	Lightly thinned	Heavily Thinned
Pachyphloeus thysellii Colgan, nom. ined.		0.06	
Radiigera fuscogleba Zeller		0.06	
Rhizopogon hawkerae A.H. Sm.	1.20	0.90	0.78
Rhizopogon rogersii A.H. Sm.		0.06	
Rhizopogon subareolatus A.H. Sm.		0.06	
Rhizopogon villosulus Zeller	0.43	0.24	0.11
Rhizopogon vinicolor A.H. Sm.	6.44	4.37	1.78
Rhizopogon vulgaris (Vittad.) M. Lange	0.11		
Truncocolumella citrina Zeller	0.65	0.18	0.33
Tuber anniae Colgan & Trappe	0.22	0.12	
Tuber gibbosum Harkn.		0.06	
Tuber monticola Harkn.	2.17	1.50	1.56

Overall, 15% of the plots sampled contained $\geq$1 sporocarp (85% had no sporocarps); 18% of plots in control stands contained $\geq$1 sporocarps. Frequency declined to 13% in VDT stands, with 14% in lightly thinned areas and 10% in the heavily thinned areas. Thinning significantly reduced frequency of sporocarps (chi square = 6.22, DF=1, P=0.0134), and heavy thinning markedly reduced frequency of sporocarps (chi square = 22.714, DF=1, P<0.0001).

The total biomass collected was 0.610kg. The largest transect estimate (11.1 kg/ha) was from March 1995, Star block, heavily thinned sub-treatment area. The next largest transect estimate was 5.6 kg/ha from a lightly thinned sub-treatment area in the Stellar

block. Several samples (from all three treatment types) contained no sporocarps. VDT treated stands had significantly lower biomass than the control stands on average (ANOVA Fisher's PLSD, DF=1, P=0.0333; Fig 1).

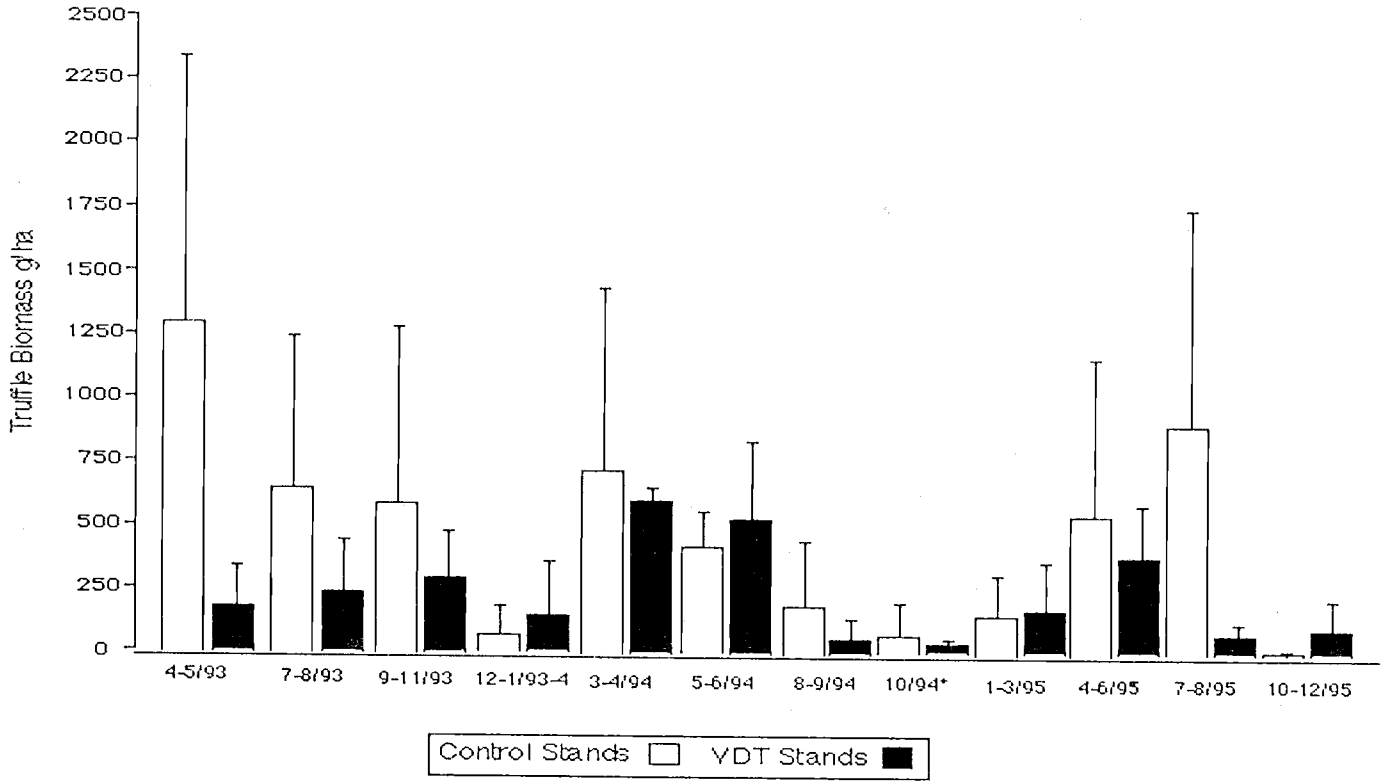


Figure 2.1, Truffle standing crop biomass for the Fort Lewis Military Reservation for control and variable density thinned stands at each sampling period. *12/94 sample was canceled due to snow cover. Bars equal standard error of the mean.

Study blocks did not differ significantly in biomass, and there was no significant interaction between treatment and block. Average standing crop biomass ranged from a low of 0.008 (±0.008) kg/ha in control stands (Oct.-Dec. 1995) to 1.331 (±1.226) kg/ha in the heavily thinned sub treatment (Jan.-March, 1994) (Table 2.2).

Table 2.2: Mean truffle standing crop (kg/ha) for each treatment and sampling period.

	Control			Lightly thinned			Heavily Thinned		
Sample period	Mean	SE	N	Mean	SE	N	Mean	SE	N
4-5/93	1.303	0.629	8	0.320	0.133	16	0.003	0.002	8
7-8/93	0.649	0.229	8	0.471	0.218	14	0.117	0.081	9
9-11/93	0.594	0.333	8	0.528	0.228	15	0.178	0.093	8
12-1/93-4	0.068	0.029	8	0.232	0.139	16	0.056	0.029	5
3-4/94	0.723	0.429	8	0.904	0.285	15	0.618	0.404	6
5-6/94	0.419	0.126	8	0.916	0.404	15	0.582	0.192	8
8-9/94	0.186	0.121	8	0.101	0.062	18	0.040	0.024	8
10/94*	0.694	0.069	4	0.023	0.012	8	0.473	0.445	3
1-3/95	0.155	0.091	8	0.216	0.195	15	1.331	1.226	9
4-6/95	0.551	0.302	8	0.644	0.192	15	0.903	0.673	8
7-8/95	0.896	0.376	8	0.100	0.039	15	0.380	0.163	9
10-12/95	0.008	0.008	8	0.170	0.078	15	0.010	0.010	9
Overall Mean	0.486	0.092	92	0.397	0.061	175	0.397	0.142	90

*12/94 sample was canceled due to snow cover. SE= Standard error of the mean, N=number of transect estimates.

Overall, control stands averaged 0.486 (±0.089) kg/ha while VDT stands averaged 0.233 (±0.034) kg/ha (Table 2.3). Annual variation in standing crop biomass was high, with year 1 control stands averaging almost twice the biomass of any other. Year 2 VDT

stands approached the average found in control stands but then fell off sharply in year 3 (Table 2.3).

Table 2.3: Mean truffle standing crop (kg/ha) by year and stand treatment.

Year	Stand Treatment	Mean Standing Crop	SE
1	Control	0.654	0.192
1	VDT	0.251	0.463
2	Control	0.350	0.109
2	VDT	0.308	0.077
3	Control	0.403	0.149
3	VDT	0.178	0.047
Overall	Control	0.486	0.089
Overall	VDT	0.233	0.034

SE= Standard Error of the Mean

Six species each accounted for ≥5% of the biomass collected and collectively 80% of the biomass in the control stands (Table 2.4); 22 species accounted for the remaining 20%. Five species accounted for 64% of the biomass in the lightly thinned sub-treatment, with 33 species accounting for the remaining 36%. In the heavily thinned areas, 5 species accounted for 85% with one species *Melanogaster tuberiformis* accounting for 59% of the biomass in this sub-treatment. Seventeen other species accounted for the remaining 15%.

Table 2.4: Percentage of total biomass collected for dominant truffle species (accounting for >5% of the biomass collected) for each treatment.

Species	Control	Lightly Thinned	Heavily Thinned
Endogone lactiflua	--	14.78	--
Gautieria monticola	14.74	--	--
Hysterangium coriaceum	12.53	--	--
Hysterangium setchellii	14.58	--	--
Melanogaster euryspermus	--	9.47	6.60
Melanogaster natsii	--	9.43	--
Melanogaster thiersii	--		7.04
Melanogaster trappei	--	11.70	--
Melanogaster tuberiformis	13.08	--	58.88
Rhizopogon hawkerae	7.76	--	6.06
Rhizopogon vinicolor	18.13	18.38	6.22
Total	79.74	63.76	84.80

Overall the heavily thinned sub treatment area showed the greatest dominance by a single species (Bergen-Parker index, Chi Square = 3.125; P=0.022; DF=11; Table 2.5). Overall Margalef's index of species richness was highest in lightly thinned sub treatment (Table 2.6), but the sub treatments were not significantly different.

Table 2.5: Species dominance for each treatment and sampling period. Values for the Bergen-Parker Index using sporocarp weight. Values close to 1 indicate dominance, higher values indicate more evenness

Sample Period	Control	Lightly Thinned	Heavily Thinned
4-5/93	1.95	2.23	2.40
7-8/93	3.09	2.20	2.10
9-11/93	1.64	2.63	2.30
12-1/93-4	1.24	1.19	1.91
3-4/94	1.87	3.57	1.48
5-6/94	2.33	2.85	2.25
8-9/94	1.54	1.22	2.19
10/94*	1.05	1.00	1.04
1-3/95	2.46	1.05	1.09
4-6/95	2.17	2.36	1.19
7-8/95	2.64	3.38	2.26
10-12/95	1.00	3.12	1.26
Overall	5.61	11.93	1.70[1]

*12/94 sample was canceled due to weather; [1]Significantly different (Chi Square P=0.022; DF=11)

Table 2.6: Margalef's index of diversity using sporocarp numbers and number of species (in parentheses) by treatment and sampling period; calculated by randomly selecting equal numbers of transects in each category (higher values indicate greater richness).

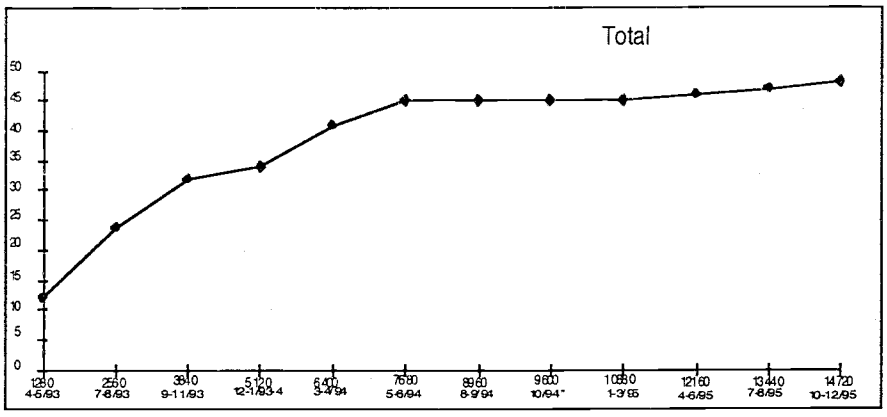
Sample period	Control	Lightly thinned	Heavily Thinned
4-5/93	2.00 (12)	1.90 (9)	1.44 (2)
7-8/93	2.70 (11)	1.57 (6)	1.30 (6)
9-11/93	1.53 (7)	2.11 (9)	2.12 (7)
12-1/93-4	0.00 (1)	0.25 (2)	0.56 (3)
3-4/94	1.63 (6)	2.39 (11)	1.20 (7)
5-6/94	2.03 (10)	1.70 (8)	1.66 (7)
8-9/94	1.52 (5)	2.16 (4)	1.12 (3)
10/94*	1.44 (2)	0.00 (1)	0.28 (2)
1-3/95	0.56 (3)	0.98 (5)	0.64 (3)
4-6/95	2.66 (10)	1.31 (6)	1.46 (5)
7-8/95	2.73 (9)	1.24 (3)	2.09 (6)
10-12/95	NC (1)	1.82 (7)	0.62 (2)
Overall	4.29 (28)	5.38 (32)	3.69 (24)

*12/94 sample was canceled due to weather. NC = Can not be calculated due to small number of sporocarps.

Collections of sporocarps (one to several truffles of the same species in close proximity to one another on a single plot) varied between thinning sub-treatments; 233 collections were found in control stands, 345 collections were found in lightly thinned areas, and 115 collections were found in heavily thinned areas. The largest average number

of sporocarps per collections occurred in the heavily thinned areas, with 2.8 sporocarps/collection, control stands averaged 2.6 sporocarps/collection and lightly thinned areas averaged 2.4 sporocarps/collection. Average biomass per collection was 0.75g/collection, collections in lightly thinned areas averaged 0.810g and collections in heavily thinned areas averaged 1.23g/collection. While frequency and number of collections were fewest in heavily thinned areas, average biomass per collection was significantly higher than control and lightly thinned areas (Fisher's PLSD, DF=2, P-Value = 0.0356, 0.0482).

Species diversity increased rapidly with number of samples then leveled off. Lightly thinned sub-treatment began to level out at 3600m^2 sampled, with 38 species total. Control stands were asymptotic at 2400m^2 sampled, and contained 28 species total. Heavily thinned sub-treatment species composition continued to rise during the entire duration of this study with 22 species total. Figure 2 shows species/area curves for this study. Curves showed classical exponential form reaching an asymptote quickly after reaching a shoulder. as collections continued small numbers of additional species were found as would be expected. For this study, the total species/area curve became asymptotic after 7600m^2 had been sampled. VDT stands did not level off until 5760m^2 and exhibited a slight increase at 9100m^2 (3rd spring post thinning).


Total
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45
40
35
30
25
20
15
10
5
0
1280
4-5/93
2560
7-8/93
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5120
12-1/93-4
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3-4/94
7680
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8960
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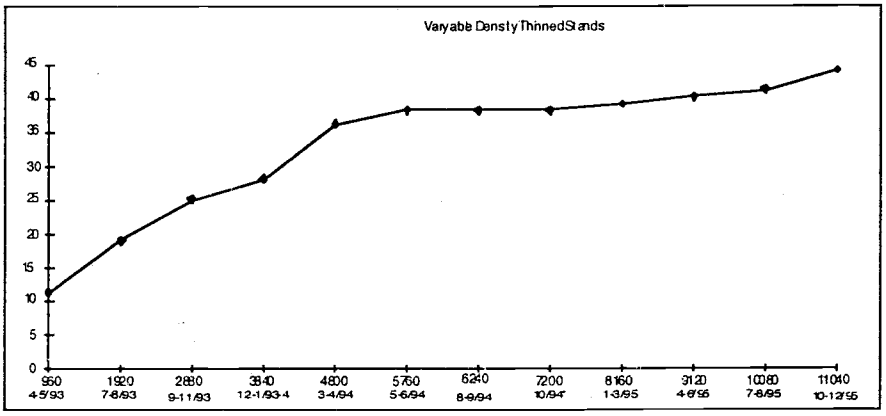
Variable Density Thinned Stands
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40
35
30
25
20
15
10
5
0
960
4-5/93
1920
7-8/93
2880
9-11/93
3840
12-1/93-4
4800
3-4/94
5760
5-6/94
6240
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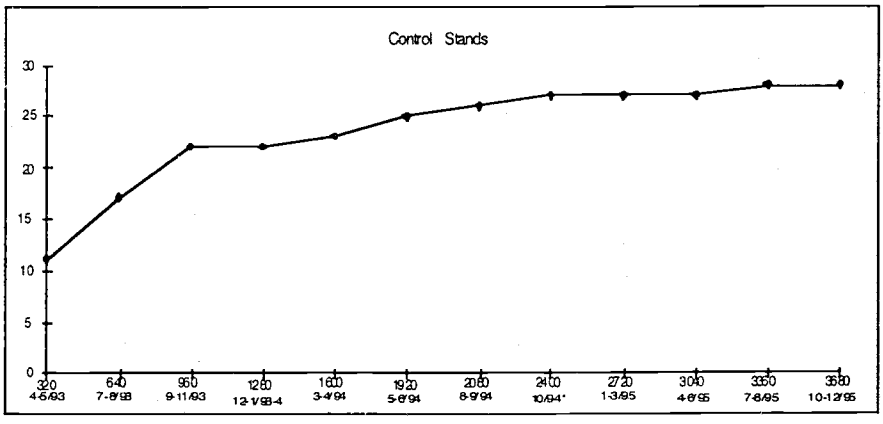
4-6/95
10080
7-8/95
11040
10-12/95


Control Stands
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25
20
15
10
5
0
320
4-5/93
640
7-8/93
960
9-11/93
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1600
3-4/94
1920
5-6/94
2080
8-9/94
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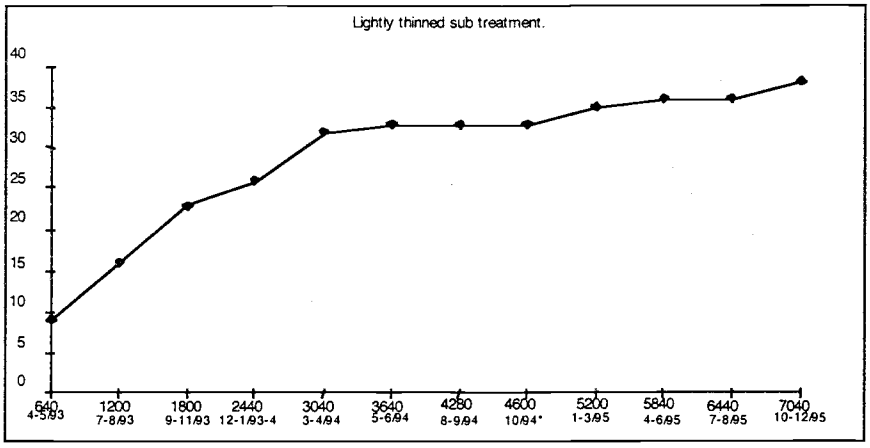
3360
7-8/95
3680
10-12/95

(Continued)



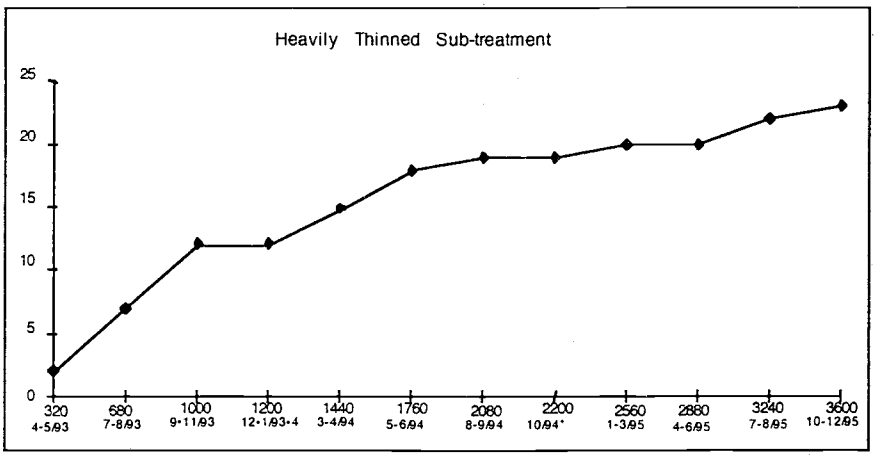


Figure 2.2, Truffle species/area curves for the Fort Lewis Military Reservation: X axis number of species encountered, Y axis, sample dates and cumulative area sampled.

Three truffle genera accounted for >5% of the standing crop biomass during winters at Fort Lewis (Table 2.7). *Endogone lactiflua* and *E. pisiformis* were the most frequently

encountered, while *Rhizopogon* (primarily *R. hawkerae*) contributed most to standing crop biomass.

Table 2.7: Percentage of sporocarps and biomass of truffle genera collected accounting for >5 % winter standing crop.

Group	% of Sporocarps	% of Biomass
Endogone	54%	33%
*Rhizopogon**	35 %	39%
Tuber	6%	7%
TOTAL	95%	79%

*Includes closely related *Truncocolumella citrina*

Discussion

The most striking result of this study are the differences in species diversity in the VDT stands compared to control stands and the shift in species dominance within the lightly thinned and heavily thinned sub-treatments. Diversity of species fruiting increased from 28 species in the control stand to 44 in the VDT stands. This suggests that some species were induced to fruit by the thinning operations. Species immigration is not likely as only three years elapsed during this study. The forests at Fort Lewis were essentially homogenous (within each block) prior to the installation of this study. The stand level treatments were installed randomly, so no species should be absent from any data set. Some of this measured increase may be artificial due to the increased sampling effort in the

VDT stands. It should be noted, however, that there was an increase in diversity even if the excess replication is removed (Table 2.6). Frequency and diversity of sporocarpic *Glomus* species was highest in the thinned stands. This corresponds well with the increase of herbaceous plants and broadleaf shrubs with which *Glomus* species form arbuscular mycorrhizae.

Species dominance shifted most in heavily thinned areas. The genus *Melanogaster* preferentially fruited in VDT stands. Six of seven *Melanogaster* species encountered in this study were found only in the VDT grids. One species (*M. thiersii*) was found only in heavily thinned area. *Melanogaster tuberiformis* accounted for almost 60% of all truffle biomass collected in heavily thinned areas. The overwhelming dominance of this and 4 other closely related species in VDT stands strongly suggests that the thinning promotes sporocarp formation by *Melanogaster* species but not necessarily others. This is particularly interesting as *Melanogaster* is second only to *Rhizopogon* in the dietaries of the mammals at Fort Lewis (chapter 4 this volume). *Melanogaster* is also one of the most nutritious (caloric value/gram of truffle) for small mammals (A.W. Claridge unpubl. data). Waters *et al.* (1994) found a similar increase in *Gymnomyces* sp. in shelterwood stands of *Abies* spp. in northeast California. The increase in productivity of *Gymnomyces* sp. offset the decrease of productivity in *Hysterangium* and *Gautieria* in their study stands.

Contrary to the increase in *Melanogaster*, *Hysterangium* and *Gautieria* declined in frequency and productivity in the VDT stands. *Hysterangium secthelii, H. coriaceum, and Gautieria monticola* accounted for 41.9% of the biomass collected in control stands. These species declined markedly in VDT stands and did not account for a substantial

portion of the biomass collected. None of the three species of *Hysterangium* found during this study occurred in the heavily thinned sub-treatment, *G. monticola* occurred only once. All of these species are known to form dense hyphal mats in Douglas-fir forests (Griffiths *et al.* 1991). These mats are known to host a greater concentration of soil organisms and have substantially different soil chemistry than surrounding "non-mat" soils (Aguileria *et al.* 1993; Griffiths *et al.*, 1990). These mats may occupy up to 28.4% of the forest floor in Douglas-fir forests (Cromack *et al.*, 1979). Many factors could explain the decline of sporocarp production in thinned stands by these fungi. The hyphal mat they form may be more susceptible to mechanical damage by logging operations. The loss of photosynthate due to removal of the host trees may compromise their ability to support large hyphal networks; thereby decreasing the carbohydrate allocated to sporocarp production. Likewise the thinning may have changed the microclimate to the detriment of these species. This closely resembles the trend found by Waters *et al.* (1994). They found relative frequency of *Hysterangium* and *Gautieria* were significantly less in the shelterwood stands 17 and 20 years after timber harvest. This suggests that it may take considerable time for these fungi to produce sporocarps after disturbance. McIntire (1984) found a significant decrease in *Hysterangium* spores in fecal pellets of Siskiyou chipmunks (*Eutamias siskiyou*) in shelterwood stands in southwest Oregon. A study at Fort Lewis found that the amount of forest floor covered by the hyphal mats was significantly lower in the VDT stands (Y. Valochovic, unpub. data). This corresponds well with our observation of sporocarp frequency and productivity. These observations are supported by Aguileria *et al.* (1993) who found *Gautieria* mats only in the rooting zone of the retained old-growth

trees in a 2 year old shelterwood stand in the Oregon Cascades. They reported that mats were scarce in a nearby 11-year-old clear-cut site. Further studies are necessary, but these data suggest that these fungi may be important indicators of soil disturbance in PNW forests.

In a thinning project installed by S. L. Miller, ectomycorrhizal fungi were collected and fruitings mapped in a lodgepole pine forest in Wyoming (S.L. Miller, pers. comm.). His data suggest that removal of host trees and creation of small canopy gaps increased ectomycorrhizal sporocarp production on a very local scale. While there were several species found across all treatments, several species fruited more prolifically in the openings while others only occurred in control and very small openings. His data parallels this study in many ways. *Rhizopogon vinicolor* was the most frequently encountered truffle in control stands and remained high in the lightly thinned and heavily thinned areas as well, but was only 1/3 as frequent in heavily thinned areas as in control (6.4% of plots compared to 1.8). However, *Endogone lactiflua* productivity was greatest in lightly thinned and heavily thinned areas. All of the species that were restricted to one treatment can be considered rare (<5 % of the biomass in the sub-treatment) with the exception of a single collection of *Melanogaster thiersii*. This species was collected only once during the course of this study, as a single cluster in a heavily thinned area.

The decrease in collection numbers combined with the significant increase in biomass per collection suggests that these localized flushes maybe a stress reaction of the fungus (S.L. Miller pers comm.). Many flowering plants will allocate a greater proportion of it's available resources to reproductive structures when under stress. Conceivably the

mycorrhizal fungi could similarly respond to the loss of a host tree due to logging operations. Alternately, these fungi may be responding to reduced competition from *Hysterangium* and *Gautieria* spp. in the heavily thinned areas. Much more research is necessary to test these hypotheses.

The control stands had considerably less standing crop biomass than reported by other researchers. Old-growth and natural mature Douglas-fir forests in the Oregon Cascades averaged 2.3-5.4 kg/ha (Luoma, 1991). Hunt and Trappe (1987) found 2.0 to 3.0 kg/ha in a 35-50 year old Douglas-fir forest in western Oregon. These are substantially more than our study even if winter samples are excluded. Our estimates are similar to those observed by North *et al.* (1997) in natural mature stands (averaged 0.78 kg/ha) in his non-exclosure plots. The carrying capacity of managed forests for mycophagous small mammals requires further study. This question is particularly interesting as North *et al.* (1997) estimated that 60% of all truffle biomass produced in their young managed stands was consumed by mycophagists.

Species area curves are often useful to visualize the species composition of the community and evaluate adequacy of the sample size. All of our curves began to plateau rather quickly, except in the heavily thinned sub-treatment. This suggests that species heterogeneity was greatest in the VDT stands.

Food resources available during times of lowest abundance may be considered limiting to the animals that are active year-round. Truffle abundance during the coldest parts of the winter in the PNW may limit northern flying squirrel densities. North *et al.* (1997) found that consumption of truffles in managed young stands was closest to the

estimated standing crop during winter. That is, truffles were not limiting during times of peak production. In their study, evidence of failed attempts by mammals to enter exclosure plots were only found in those in place over winter.

In this study, winter standing crop of truffles in the VDT stands exceeded control stands in 1993 and 1995. Only a partial data set was collected in winter of 1994, and no firm conclusions can be made as to the winter standing crop for this year. Three genera of truffles accounted for the majority of available truffle resource during winter at Fort Lewis. *Endogone lactiflua* was one of the most frequently encountered species during this study, but it did not account for a substantial portion of the biomass in the control or heavily thinned areas overall. This species and *E. pisiformis* was highest in frequency and standing crop biomass in the VDT stands. Luoma (1991) found *E. lactiflua* to be one of the more frequently encountered, but also concluded that it did not contribute to overall biomass in a meaningful way. The majority of his collections were from mesic study sites. Many of the collections of *E lactiflua* at Fort Lewis occurred during winter months and many were found in or near heavily decayed coarse woody debris (Colgan unpub. data).

Rhizopogon (including *Truncocolumella*) contributed most to standing crop biomass during the winter months. *Truncocolumella* is included with *Rhizopogon*, as its spores cannot be distinguished from those of *Rhizopogon* and *Alpova* in the dietary analyses presented in chapters 3 and 4, and is of similar nutritive value for the small mammals (A. Claridge, pers comm.). Frequency of winter fruiting species in this group did not change substantially in the thinned stands. North *et al.* (1997) suggests that these are some of the more "palatable" (most consumed) species in his study sites. Carey (1995) found

Rhizopogon to be the most frequently encountered fungal spore in feces from flying squirrels in these stands in 1987. This group of truffles is the primary year-round dietary item for mycophagous small mammals at Fort Lewis (chapter 4 this volume). *Tuber* was the third genus to contribute to winter standing crop. Frequency of *Tuber monticola* did not change substantially in the thinned stands. *Tuber gibbosum* was found only once during this study in a lightly thinned area, and was not found in control areas at all.

Although there was an apparent significant decline in truffle standing crop in VDT stands, diversity appeared to increase. Greater amounts of truffle were available in the VDT stands during times of lowest abundance, and some of the more nutritious truffle were most abundant in the VDT stands. Studies on small mammal densities and use of the truffle resources in thinned stands is underway. This multi-disciplinary approach to ecosystem research will allow for a comprehensive evaluation of this ecosystem management technique.

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Chapter 3

A Reliable Method Of Analyzing Dietaries Of Mycophagous Small Mammals

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Abstract

Two methods of analyzing the diets of populations of small mycophagous mammals were compared. For the comparison fecal pellets from 11 northern flying squirrels and twelve Townsend's chipmunks, all caught live, were collected. in one method, pellets from each individual were examined microscopically in the other samples from three or four individuals from each species were pooled and number of slides and fields of view used for each slide were reduced, resulting in less time and effort to collect data. Diet analysis by the two methods did not differ significantly for either mammal species.

Key words: dietary analysis, northern flying squirrel, Townsend's chipmunk, *Glaucomys sabrinus, Tamias townsendii.*

Introduction

A comprehensive understanding of interactions and interdependencies in forest ecosystems is becoming essential as goals of forest management change. Interactions between populations of animals comprising various trophic levels are some of the least understood in Pacific Northwest forests. One food-web linkage now becoming clearer is that between the threatened northern spotted owl (*Strix occidentalis caurina*) and the northern flying squirrel (*Glaucomys sabrinus*). The northern spotted owl feeds primarily on northern flying squirrels over much of it's range (Carey *et al.*, 1992). Northern flying

squirrels feed almost exclusively on fungi, primarily truffles and false-truffles (hereafter referred to as "truffles") over most of their range (Maser *et al.,* 1985; Carey, 1995). Most truffle species form ectomycorrhizae with conifers. The conifers provide the fungi with a source of carbon from photosynthesis. The fungi aid the tree in absorption of water and nutrients from the soil. Flying squirrels and other small mammals eat fungal sporocarps but spores of the fungi pass through the gut of the squirrel intact are excreted to form new fungal colonies in new locations (Trappe and Maser, 1976; Claridge *et al.*, 1992). To rehabilitate forests altered by timber harvest, forest managers must understand and deal with all of these interactions.

The diets of small mammals that feed on fungi (mycophagy) have been studied extensively (Fogel and Trappe, 1978; Maser *et al.,* 1978; Malajczuk *et al,.* 1987; Claridge and May, 1994; Carey, 1995). Stomach contents provide an accurate record of the animals' last meal, and fecal samples from the upper and lower intestine reflect previous meals. However a non-lethal method to assess diet composition of small mammals is necessary for long-term studies. Analysis of the components of fecal pellets allows for repeated capture of individuals in order to measure temporal variation in diet components.

Several techniques have been used to analyze the diet of live-captured individuals. McIntire and Carey (1989) developed the most comprehensive techniques for analyzing fecal collections of mycophagous mammals. They used a compound microscope to identify spores of truffle species and distinguish plant and insect parts and lichen

fragments. They suggested three microscopic mounts per individual and 50 fields of view at 400X magnification per mount to accurately estimate the relative abundance and diversity of components in the diet. Their technique is time-consuming and because taxonomic expertise is difficult to acquire, impractical for large data sets. I therefore conducted this to compare their original method with a truncated version designed to accurately assess the diets of mycophagous small mammal in less time.

Methods

We live trapped small mammals at Fort Lewis Military Reservation, Thurston County, Washington as part of a large integrated ecosystem management experiment. The forests were 55-to 65-years-old and primarily Douglas-fir (*Pseudotsuga menziesii*). Trapping procedure was according to Carey *et al.* (1991). Fecal samples (2-3 fecal pellets) from 11 *G sabrinus* were collected from a single trapping grid in January 1993. Fecal pellets 12 Townsend's chipmunks (*Tamias townsendii*)were collected from a single trapping grid in October 1992. No comparisons are made between the diets of the mammal species as this paper is designed to test sampling methods 1 and 2. Differences between the diets of different mammal species assessed using method 2 will be presented in future publications.

To preserve for microscopic analysis, pellets from *T. townsendii* were preserved with a few drops of formalin, *G sabrinus* pellets were air-dried. Formalin use was discontinued after fall 1992 trapping due to its toxicity and waste disposal problems. Air drying preserves samples adequately but poses additional risk of disease transmission (Hanta virus and others). We have subsequently changed to preserving pellets with a few drops of 70% ETOH to reduce risks of disease transmission to researchers. None of the above methods change the composition of the fecal pellets.

Two analytical methods were compared. The first was similar to the procedure of McIntire and Carey (1989). Pellets were macerated in small vials with 15 drops of deionized water (approximately 4 to 5 times the volume of the pellets) and shaken thoroughly. A pair of parallel-sided forceps was then plunged into the suspension, closed, and withdrawn with a drop of suspension, which was transferred to a microscope slide; the procedure was repeated three times. One drop of Melzer's reagent (iodine, potassium iodide, and chloral hydrate in aqueous solution), and three drops of PVA (poly-vinyl alcohol; Omar *et al.* 1979) were added and the solution was covered with an 22-x22- mm cover slip. Three such slides were prepared for each individual. The slides were left on a warming tray for 12 to 24 hours at 40°C. For each slide, 50 randomly selected fields of view (a total of 150 fields per sample) were then examined at 400X magnification with a compound microscope. Fungal spores were identified to genus according to Castellano *et al.* (1989). Plant fragments and insect parts were identified to lowest possible taxonomic level. Miscellaneous and unidentifiable items occurring in less than 5% of the fields were grouped into one category, "other"

In method 2, the remaining suspensions of fecal samples from the 11 *G.sabrinus* were divided into 3 groups of 3 or 4 individuals; the 12 *T. townsendii* suspensions were divided into 3 groups of 4 individuals. Five slides from each of the pooled samples were then prepared as above (Table 3.1). Twenty-five fields on each slide were examined for the same categories as method one for a total of 125 fields per sample pool. we believe this combination of fields and slides to be an adequate compromise to capture the abundance and diversity of diet items while substantially reducing the person hours required to collect the data. The time required to perform each of the above analysis was recorded.

Table 3.1, Number of samples, mounts, and fields examined for each method and species of mammal.

Species	method	number of samples	# of mounts per sample	# fields per mount	total # of fields
GLSA	1	11	3	50	1650
	2	3	5	25	425
TATO	1	12	3	50	1800
	2	3	5	25	425

GLSA = *Glaucomys sabrinus*, TATO = *Tamias townsendii*

The data were analyzed by multivariate analysis of variance (MANOVA) and Kruskall-Wallis non-parametric T-tests to analyze the mean frequency of occurrence and relative frequency per individual or pooled group for each diet item (fungal genus, plant

part, insect part, etc.). A univariate f test was then performed to test for significant differences between estimates of diet items by the two analysis. After these analyses a Spearman rank order correlation test was performed.

Results And Discussion

The main assumption was that method 1 accurately estimated the true composition of the food items in the fecal pellets , and thus, the resulting means and variances accurately estimates population parameters. Describing the composition of the pellets of each animal would be the best possible procedure if constraints on time and budget were not factors. The relative abundance of each item in the pooled samples was compared to the intensive group. Table 3.2 shows the mean percent frequency of occurrence per slide for diet items in the two species of small mammal with the two methods. Table 3.3 shows the percent relative frequency of each item.

Table 3.2. Mean percent frequency of occurrence and coefficient of variation of fungal spores and other food items in the fecal pellets of the population by sampling method: individual animals (method 1), pooled samples of 3-4 animals (method 2)

	Tamias townsendii				*Glaucomys sabrinus*			
	Freq. (%)		CV%		Freq. (%)		CV%	
	Met. 1	Met. 2	Met. 1	Met. 2	Met. 1	Met. 2	Met. 1	Met. 2
Diet Item	n=12	n=3	n=12	n=3	n=11	n=3	n=11	n=3
Elaphomyces	0.1	0.3	346.4	173.2	4.3	3.7	149.1	128.9
Gautieria	---------	---------	-------	-------	55.9	96.7	87.8	1.6
Hymenogaster	0.3	0.0	248.6	-------	0.2	0.0	3.3	-------
Hysterangium	9.2	27.7	313.1	170.7	33.3	56.0	131.2	15.9
Leucogaster	0.0	0.5	-------	173.2	76.5	96.3	42.0	4.2
Leucophleps	---------	---------	-------	-------	7.2	5.7	316.5	106.4
Melanogaster	8.1	16.8	204.0	82.6	82.5	92.0	34.8	14.1
Leucangium	---------	---------	-------	-------	0.2	0.3	331.7	173.2
*Rhizopogon**	85.0	97.9	36.7	3.8	37.3	50.3	102.2	56.1
Scleroderma	---------	---------	-------	-------	2.4	0.7	331.7	173.2
Tuber	0.6	1.1	346.4	173.2	0.1	0.0	208.8	--------
Plant	35.2	44.0	101.5	34.5	19.4	12.8	67.2	61.6
Insect	8.1	13.3	106.6	64.2	0.2	0.7	237.1	173.2
Epigeous	0.2	0.3	346.4	173.2	---------	---------	---------	--------
Other	1.4	0.0	237.3	-------	2.2	0.4	121.5	0.0
Unknown	---------	---------	-------	-------	1.3	0.0	208.8	--------

* May contain related genera not distinguishable by spores alone.

Table 3.3. Percent relative frequency of fungal spores and other food items in the fecal pellets of the population by sampling method: individual animals (method 1), pooled samples of 3-4 animals (method 2).

	Tamias townsendii		*Glaucomys sabrinus*	
	Met. 1	Met. 2	Met. 1	Met. 2
Diet Item	n=12	n=3	n=11	n=3
Elaphomyces	0.07%	0.15%	1.33%	0.89%
Gautieria	---------	---------	17.31%	23.25%
Hymenogaster	0.20%	0.00%	0.06%	0.00%
Hysterangium	6.21%	13.71%	10.31%	13.46%
Leucogaster	0.00%	0.25%	23.68%	23.15%
Leucophleps	---------	---------	2.23%	1.37%
Melanogaster	5.47%	8.32%	25.54%	22.12%
Leucangium	---------	---------	0.06%	0.07%
*Rhizopogon**	57.35%	48.47%	11.55%	12.09%
Scleroderma	---------	---------	0.74%	0.17%
Tuber	0.40%	0.54%	0.03%	0.00%
Plant	23.75%	21.78%	6.01%	3.08%
Insect	5.47%	6.58%	0.06%	0.17%
Epigeous	0.13%	0.15%	---------	---------
Other	0.94%	0.00%	0.68%	0.10%
Unknown	---------	----------	0.40%	0.00%

* May contain related genera not distinguishable by spores alone.

No statistical differences ($P<0.05$) were found for diet components of Townsend's chipmunks between the sampling methods, except for the single *Leucogaster* spore found in one field of view in one of the pooled samples. This single spore is of little biological relevance as it was rare (<.5%) and it's origin cannot be determined. It may have been

present in the soil and accidentally consumed while attached to the peridium of another truffle, or alternatively, it may have been left over from a previous meal eaten several days or weeks ago (Cork and Kenagy, 1989b). *Rhizopogon* was the dominant diet item for Townsend's chipmunks in both the non-pooled and pooled samples. The coefficient of variation shows the variability for each item in the population of chipmunks. The lack of significant differences may be due on part or in total to large variances for method 1. Any one fecal sample cannot contain a representative sample of the population's dietary. Many commonly consumed items will be absent, thus a large number of samples, much larger than 12 would be necessary to obtain small variances. Pooling fecal samples before analysis, however, reduces the expectancy of zero values for commonly occurring diet items as with means of means under the central limit theorem, the estimates of the average composition based on the pooled samples can be expected to have the lower variances expected for the normal distribution as compared to variance Poisson (rare occurrences) or binomial (present or absent) distributions. To deal with the high variances encountered in this data set a Spearman rank order correlation was performed. The result of this test confirmed the above findings.

Results of the two sampling methods for items in the diet of *G. sabrinus* did not differ significantly ($P<0.05$). *Gautieria*, *Melanogaster* and *Leucogaster* were the dominant food items in this population of mammals for both methods tested. The increase in relative frequency of *Gautieria* (Table 3.3) in method 2 is not statistically significant different. The change of 6.7% in mean relative frequency for this diet component is not biologically

meaningful particularly when the high variability encountered in method 1 is considered. A Spearman rank order correlation test confirmed the above findings

The average time to analyze each individual animal in this study was 55 minutes. This figure includes maceration of the sample, staining, mounting, and recording the data for 50 fields on each of 3 slides. The time to macerate and mount will not change for 3 or 4 animals pooled together. Time savings comes from the fewer number of fields per slide and fewer total preparations. The average time to prepare, stain, mount, and analyze and record data for a pooled sample was 70 minutes for 5 slides, with 25 fields per slide for 3 or 4 animals. Approximate total time to analyze a sample of 12 individual small mammals would be 11 person hours; pooling 3-4 animals together in a single sample reduces this to 3.5 hours.

The people who performed these analysis had a reasonable amount of experience working with fungi prior to this experiment. A considerable learning curve should be expected if persons performing the analysis do not have a background in mycology.

Readers should be aware of limitations of this type of analysis. The accurate assessment of relative abundance of the common (dominant) food items can be assessed with confidence variances around the frequencies of rare items prevent accurate assessment the proportionality and therefore importance of these items to the small mammals. The primary limitation of this type of method is that only at the indigestible portions of the actual items consumed are present. This is not a problem as most truffles are comprised of sterile tissue with spore bearing cells directly embedded or contained in small spore lined chambers (locules). However special concern is necessary as there are some species in the

truffle genera *Elaphomyces* and *Radiigera* that consist of a thick peridium (outer skin) and a powdery gleba (spore mass). Small mammals eat only the peridium and discard the spores; thus, only the spores accidentally consumed can be accounted for in the fecal pellets (Trappe and Maser, 1977; Cork and Kenagy, 1989; Jim Trappe and Wes Colgan personal observations). The same is probably true for the gastromycete species in the genera *Lycoperdon* and *Scleroderma*. Any analysis done only on fecal pellets runs a strong risk of underestimating the relative quantity (and potential importance) of these fungi in the diet, as the soft tissues comprising the peridium would be digested, leaving almost no trace in the fecal pellets. This may be of special concern as these truffles can account for a substantial resource for small mammals in some ecosystems. North (1993) showed that *Elaphomyces granulatus* accounted for 92% of the standing crop of truffles in some sites. If a large proportion of the diet for a population of small mammals consisted *Elaphomyces* , fecal pellet analysis could miss this. Further research is needed to address this potential problem.

Conclusions

Estimating the diet of mycophagous mammals by analyzing fecal pellet is problematic and time consuming. A truncated version of the method used by McIntire and Carey (1989) provides a reliable estimate of diet components in considerably less time. The pooling procedure illustrated above is an adequate alternative when large numbers of samples are to be processed. We believe that the ability to process large numbers of

samples in a rapid and economical way offsets any loss in resolution and/or precision. Indeed, pooling confers an advantage in lowering variances.

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Chapter 4

Dietaries Of Arboreal Small Mammals In A Variably Thinned Douglas-Fir Forest.

Wesley Colgan III

Abstract

Dietaries of Northern flying squirrels (*Glaucomys sabrinus*) (GLSA) and Townsend's chipmunk (*Tamias townsendii*) (TATO) were determined through analysis of fecal pellets collected from live trapped individuals. Animals were trapped on 16 stands of 55-65 year old Douglas-fir forest from fall 1991 through fall 1995. Half of the stands served as controls; half were assigned a variable density thinning (VDT) treatment. A VDT stand is comprised of a mosaic of patches thinned to different densities of standing live trees. Hypogeous basidiomycetes and ascomycetes dominated TATO and GLSA dietaries. Plant material was also a dominant component of TATO dietaries (both spring and fall) and to a lesser extent GLSA dietaries. Both mammal species consistently found more fungal genera than mycologists did during a concurrent mycological survey. Thinning did not change the rank order of fungal components in the dietaries of these small mammals. The decline in abundance and frequency of *Gautieria* and *Hysterangium* and increase of *Melanogaster* in thinned stands recorded by the mycological survey was not reflected in the mammal dietaries. Overall rank abundance of dominant fungal dietary items correlated more positively with frequency of occurrence than with biomass of fungal genera on truffle plots.

Key Words: disturbance, ecosystem management, mycophagy, mycorrhiza, northern flying squirrel, Townsend's chipmunk, truffles.

Introduction

Many small mammals in temperate forest ecosystems consume sporocarps (fruiting bodies) of fungi as a primary food resource (Maser *et al.,* 1978; Hayes *et al.,* 1986; Bennett and Baxter, 1989; Scotts and Seebeck, 1989). Most of these fungi are ectomycorrhizal with a range of plants (Fogel and Trappe, 1978; Claridge and May, 1994) and form hypogeous (underground) fruiting bodies. Hypogeous sporocarps, hereafter referred to as truffles, do not discharge and disperse their spores to the air. Instead, truffles produce odors upon maturity that attract various animals (Fogel, 1975; Fogel and Trappe, 1976). Many mammals excavate truffles from the soil and consume all or part of them. While much of the fungal tissue consumed may be digested (Claridge and Cork, 1994), spores held within the truffle pass through the digestive tract of the mycophagist intact and are voided in the feces of the animal as it moves around its environment (Trappe and Maser, 1976; Maser *et al.,* 1978). Spore viability is assumed to be unaffected by this process, as evidenced either by germination of spores isolated from feces (Trappe and Maser, 1976) or by successfully culturing mycorrhizae on plant seedlings by inoculation with spore-laden fecal pellets (Rothwell and Holt, 1978; Kotter and Farentinos, 1984 a, b; Lamont *et al.,* 1985; Redell and Spain, 1991; Claridge *et al.,* 1992). Some researchers have suggested that passage through the digestive tract of mycophagous animals may be necessary for the germination of spores of at least some truffle species (Lamont *et al.,* 1985; Cork and Kenagy, 1989).

This project is a part of the Forest Ecosystem Study (FES) a multi-disciplinary study designed to enhance structural and species diversity in second growth Douglas-fir forests within the range of the northern spotted owl (*Strix occidentalis caurina*) (Carey *et al.,* 1997a). The long term goal of this study is to provide suitable habitat for spotted owls where current forest conditions exclude them. The primary prey for the northern spotted owl is the northern flying squirrel (*Glaucomys sabrinus*) (Carey *et al.,* 1992; Forsman *et al.,* 1984). This nocturnal squirrel feeds primarily on truffles throughout the year (Maser *et al.,* 1978; Maser *et al.,* 1985; Carey, 1995; Carey *et al.,* 1997b). This connection between owl, squirrel, fungi, and tree illustrates an important path in the complex of forest food webs from below ground to above ground components.

To achieve the long term goal of the study, removal of wood through thinning of managed forests to stimulate understory development should positively affect the food resources of the small mammals; particularly the truffle species that fruit during times of lowest food availability.

To evaluate the effect of this silvicultural manipulation on dietaries of northern flying squirrels (*Glaucomys sabrinus*, GLSA) and Townsend's chipmunk (*Tamias townsendii*, TATO) we examined fecal pellets of live-trapped individuals from October 1991, through November 1995. Our objectives were to describe the dietaries of TATO and GLSA foraging in study stands of the FES and to evaluate effects of thinning on the dietaries. We also wanted to determine if the fungal dietary components corresponded to fungal genera collected during a concurrent mycological survey of the FES. To date no

study has had a comprehensive side by side study of the dietaries of mycophagous small mammals with a mycological survey of hypogeous fungi.

Methods

Study Area

The FES is located on four blocks of forest, 30 km NE of Olympia, WA on the Fort Lewis Military Reservation. The soils of Star and Stellar blocks are classified as Tenino gravelly sand loam. The soils of Hill and Farley blocks are classified as Everett gravelly sand loam. All blocks fall into the Southern Puget Trough physiographic province (Franklin and Dyrness, 1973). The stands are essentially flat to gently rolling, and slopes rarely exceed 15%. Elevation ranges from 100-143m, annual average precipitation is 91 cm. The forests on the Fort Lewis Military Reservation have been designated as habitat critical to the spotted owl because they constitute the only federal forest stepping stone between the Cascade range to the east and the Olympic Peninsula to the West. Fort Lewis surrounded by urban and agricultural land on the northeast and south, and the Puget Sound on the northwest.

The forests at Fort Lewis are dominated by Douglas-fir. Most of the old growth was cleared in the first half of this century; existing stands regenerated from natural seeding. Farley and Hill blocks were clearcut in 1927 and have been lightly thinned twice (1972 and again in the late 80's). They have almost no remaining coarse woody debris (CWD) or

large diameter trees from the original old growth; CWD was intentionally removed from these stands during the early thinning operations. They have developed an extensive understory of *Gaultheria shallon* Pursh., *Berberis nervosa* Pursh., and *Polystichum munitum* (Kaulf.) Presl. Star and Stellar blocks were clearcut in 1937 and had no further silvicultural manipulations prior to the installation of our study. Understories in these blocks were variable and dominated by ground mosses. *Gaultheria shallon* and *Polystichum munatum* occur primarily in scattered canopy openings caused by laminated root rot (*Phellinus weirii* (Murr.) Gilb.). These blocks had considerable residual CWD and numerous large-diameter old-growth trees (approximately 5/ha).

Sampling Considerations

The FES is a 4X4 complete randomized block experiment. Each of the four blocks contains four stands, and each stand was randomly assigned one of four treatments: control (no treatment), artificial cavities and nest boxes, variable density thinning (VDT), and a combination of artificial cavities, nest boxes, and VDT. Each stand was surveyed and a sampling grid installed. Each grid consists of 64 points aligned in an 8X8 matrix, with points 40 horizontal meters apart. These points serve as trapping stations for arboreal rodents. A buffer zone of at least 80m was maintained between each trapping grid. All concurrent studies used these grid points as reference points. All of the thinning sub-treatments were based on this 40mX40m grid system.

Silvicultural Prescription

The silvicultural prescription for the FES was designed to produce a desired future condition that was based on empirically descriptions of spotted owl habitat and old-growth forests. A goal of this study is to determine if late seral forests can be created while extracting commodities (e.g. timber) from the stands in managed forests. Objectives of this treatment include increasing plant, animal, and functional diversity; increasing structural and spatial heterogeneity, specifically diversity of woody plant species to increase the value of the stands as habitat for the northern spotted owls (Carey et al, 1992; Carey, 1995; Carey and Johnson, 1995). Past management of the Fort Lewis led to monospecific, even-aged, closed-canopy conditions with extensive infestations of laminated root rot (*Phellinus weirii* (Murr.) Gilb.) that created scattered, small, canopy openings. These infection centers were mapped and treatments were devised to promote reforestation of the gaps and to standardize the amount of canopy openings within treatment stands.

A VDT was prescribed to accomplish these goals. All thinning treatments were installed between January and April 1993. An root-rot treatment was installed centered on the *Phellinus* infection centers. Where root rot pockets were $\leq$15% of the stand the treatment was randomly applied to grid cells for a total of approximately 16% (8 of the 49 grid cells). The root-rot treatment was designed to remove all trees showing low vigor or other evidence of root-rot and to leave all healthy trees (generally $\leq$40 stems/ha) in these open patches. A heavy thin treatment was assigned to 14 of the 49 grid cells in each

thinned stands. This treatment is defined as equivalent to retaining 180 live stems/ha in an average, unthinned, 50-year-old second-growth stand.

These two thinning sub-treatments were underplanted with *Pinus monticola* Dougl. ex D. Don., *Abies grandis* (Dougl. ex D. Don) Lindl., *Thujia plicata* Donn. ex D. Don, or *Alnus rubera* Bong. the fall following the harvest. The balance of the grid cells were assigned a light thin treatment, defined as equivalent to leaving 310 live stems/ha in an average, unthinned, 50-year-old second-growth stand.

Fecal Pellet Collection

Live trapping of small mammals was performed at Fort Lewis Military Reservation, Thurston County, Washington, by cooperating researchers from the Pacific Northwest Research Station, U.S.D.A Forest Service, Olympia, Washington. Trapping procedure was according to Carey *et al.* (1991). Trapping periods were designated to coincide with mild temperatures in fall after rains had began (fall sample) and in spring prior to summer drought (spring sample) (Carey *et al.*, 1991). Tomahawk traps were opened for 4 consecutive nights, closed the fifth and reopened for 4 more nights the following week. All animals were ear tagged for identification purposes. Traps were baited with a mixture of peanut butter, oats and molasses; bait was replaced each night. Fecal pellets were collected from each individual animal once each trap period. One to 5 pellets were collected from the anus of each animal into a small glass vial. Fecal pellets were collected only on the first capture night for each animal to avoid contamination from bait.

To preserve pellets for microscopic analysis, they were treated with a few drops of 10% formalin, 70% ETOH, or air-dried. Formalin use was discontinued after fall 1992 trapping due to its toxicity and waste disposal problems. Air drying preserved samples adequately but posed additional risk of disease transmission (Hanta virus and others). We subsequently changed to preserving pellets with a few drops of 70% ETOH to reduce risks of disease transmission to researchers. None of the above methods changed the composition of the fecal pellets.

Fungal Sampling

Field sampling took place approximately every six weeks from April 1993 through December 1995. At the first sampling, 8 stands (without tree cavities/nest boxes) were sampled; at the second sampling, approximately six weeks later, the other 8 stands (with tree cavities/nest boxes) were sampled. This alternation was continued throughout the study. Fungal sporocarps were collected from each of ten, circular, 4.0-m^2 plots, located at approximately 10-m intervals along randomly placed transects (modified from Luoma *et al.*, 1991), in each of the control stands of each block at each sampling period. Thinned stands of each block were sampled more intensively to determine if thinning sub-treatments affect sporocarp production differently. Each thinning sub-treatment was sampled by ten randomly placed 4.0 m^2 plots, totaling 30 plots per thinned stand per sampling period.

Epigeous sporocarps (mushrooms) of species eaten by small mammals (primarily the Boletaceae and *Russula* spp.) and other mycorrhizal fungi observed or suspected to be

used as food by small mammals were also collected from each plot. Each collection (one to several sporocarps of the same species in close proximity to one another on a single 4.0-m^2 sample plot) was placed in a wax paper bag with a tag recording plot number, stand number, and other pertinent information. Each plot was then raked with hand tools to a depth of at least 5 cm into mineral soil to expose hypogeous sporocarps. Field characteristics of sporocarps were noted (bruising reactions, odor, etc.) for each collection. All plots were marked with a plastic pin flag and the duff was replaced. No plots were sampled twice. All fungal samples were dried the day of collection with a forced air dehydrator set at 49°C (120°F) and then returned to the Forestry Sciences laboratory in Corvallis, Oregon, for identification and weighing to the nearest 0.01g. Voucher specimens are placed in the Mycological Herbarium at Oregon State University (OSC).

Dietary Analysis

Fecal pellets were examined according to the pooling method outlined in chapter 3, this volume. Briefly, fecal pellet samples from 3-6 animals of each species were randomly selected from the group of animals captured during one trapping period on one trapping grid. Pooled samples were macerated in small vials with approximately 1ml tap water (approximately 4 to 5 times the volume of the pellets) and shaken thoroughly. A pair of parallel-sided forceps was then plunged into the suspension, closed, and withdrawn with a drop of suspension, which was transferred to a microscope slide. The procedure was repeated to total five slides per pooled group. One drop of Melzer's reagent (iodine,

potassium iodide, and chloral hydrate in aqueous solution) was added, and the solution was covered with a 22-x22- mm cover slip. For each slide, 25 randomly selected fields of view were then examined at 400X magnification with a compound microscope. When only one animal was captured in a stand during a trapping period, only 1 slide was prepared; when 2 animals were captured, 2 slides were prepared as above. Presence or absence of dietary items were recorded for each field of view. Fungal spores of hypogeous taxa were identified to genus according to Castellano *et al.* (1989). Spores of epigeous fungi were identified to Family when possible. Plant fragments and insect parts were identified to lowest possible taxonomic level. Miscellaneous and unidentifiable items occurring in less than 5% of the fields were grouped into one category, "unknown".

Analysis

Relative frequency (RF) and standard error of the mean (SE) were calculated for dietary items identified in the fecal pellets for each group of small mammals in each stand type (Control and VDT) for each block for each trapping period. Mean numbers of hypogeous genera encountered in each stand were compared with paired t tests. Relative frequency of truffles (presence or absence in 4.0 m^2 truffle plots) was calculated for truffle genera collected in each stand type (Control and VDT) for the 2 sample dates most proximal to the mammal trapping dates for the 3 springs and falls of concurrent sampling. Biomass values were standardized for each truffle species to kilograms per hectare (kg/ha)

in each stand type. These data were converted to ranks and compared to the rank order of truffle spores in dietaries of TATO and GLSA by the Spearman rank order correlation. Correlation coefficients were compared by paired t tests to determine if truffle frequency or biomass best reflected animal usage of truffles.

Results

Over the course of this study we examined fecal samples from 977 TATO and 446 GLSA. Both TATO and GLSA dietaries were dominated by hypogeous fungi. Dietaries of TATO were comprised of 8 hypogeous basidiomycetes, 7 hypogeous ascomycetes, 2 hypogeous zygomycetes, several epigeous basidiomycete families (most notably the Russulaceae and Boletaceae) and one epigeous ascomycete family (Pezizaceae). Plant and insect material comprised the non-fungal component of the dietaries.

Relative frequencies of dominant dietary components for TATO are listed in Table 4.1. *Rhizopogon* (including related hypogeous genera not distinguishable by spores alone) was the dominant fungus in the dietaries of TATO for all seasons and stands (RF 34.4%, SE 1.2). Plant material accounted for a substantial portion of TATO dietaries (RF 26.8%, SE 1.6). *Leucophleps*, *Hysterangium,* and *Melanogaster* were also major components of the dietaries for TATO in our study. Other hypogeous genera occurred in trace amounts (<5% RF): *Elaphomyces, Endogone, Gautieria, Genabea, Glomus, Hydnotrya, Hymenogaster, Leucangium, Leucogaster, Pachyphleous, Peziza,* and *Tuber.*

Overall fall dietaries of TATO were dominated by *Rhizopogon*, plant material, *Leucogaster,* and *Melanogaster*, with 20 other items occurring in trace amounts. Spring dietaries were dominated by *Rhizopogon*, plant material, *Leucophleps*, *Hysterangium, Melanogaster* and *Genea,* with 16 other items in trace amounts (Table 4.1). *Gautieria* was only present in trace amounts in TATO dietaries throughout the FES.

Overall, GLSA dietaries were similar to TATO dietaries, with the same hypogeous Ascomycetes, Zygomycetes, and epigeous Ascomycete and Basidiomycete families. However in addition to the 7 hypogeous basidiomycetes found in TATO dietaries, GLSA also ate *Scleroderma* and *Radiigera*. Lichen also occurred in the dietaries of GLSA (RF 4.3%, SE=1.0) but not in the TATO. *Rhizopogon* was the dominant component of GLSA dietaries (RF 25.7%, SE 1.5; Table 4.1) compared to TATO. Plant material was a considerably smaller fraction of the GLSA dietaries (RF 6.8%, SE 1.1; Table 4.1). Over the course of the study, GLSA fall dietaries were dominated by *Rhizopogon, Melanogaste*r, *Hysterangium, Gautieria,* plant material and *Leucogaster* (Table 4.1). Spring dietaries also were dominated by *Gautieria, Melanogaster*, and *Rhizopogon* but also included *Leucophleps* as well (Table 4.1).

Table 4.1. Dominant (≥5% RF) dietary items for Townsend's chipmunks (TATO) and northern flying squirrels (GLSA) captured in the FES study stands, fall 1991, through fall 1995.

	Overall				Spring				Fall			
	TATO		GLSA		TATO		GLSA		TATO		GLSA	
Dietary item	RF	SE	RF	SE	RF	SE	RF	SE	RF	SE	RF	SE
*Rhizopogon**	34.4	1.2	25.7	1.5	29.3	1.1	26.7	1.4	39.7	1.7	19.7	4.0
Plant material	26.8	1.6	6.8	1.1	18.8	1.5	6.9	1.6	35.1	1.8	6.8	1.5
Melanogaster	5.7	1.0	16.1	1.6	5.7	1.6	17.6	2.5	5.7	1.3	14.8	2.1
Hysterangium	7.9	1.1	8.6	1.0	8.7	4.2	7.4	1.4			9.7	1.3
Gautieria			9.1	1.2			8.2	1.8			8.2	1.6
Leucogaster									6.7	1.6	5.6	1.4
Genea					5.3	1.3						
Leucophleps	8.6	1.4			12.7	5.6						

RF= % Relative Frequency in the dietary; SE= Standard Error of the mean. * May include related taxa not distinguishable by spores alone

Rhizopogon was the dominant fungal food resource for GLSA and TATO, spring and fall, during the pre-treatment trapping periods (Table 4.2). *Melanogaster, Gautieria,* and *Hysterangium* were also rather frequent components of the small mammal dietaries prior to the thinning operations. *Leucophleps*, *Genia*, and *Hydnotrya* were present in the spring. *Leucogaster* was used during fall of 92 by TATO. During fall 1991 trapping, only 3 TATO fecal samples were available, so they were excluded from the analysis. Rank order of fungal components did not differ significantly between animals foraging in control and pre-thinned stands.

During the three years (3 springs and 3 falls) of concurrent mammal trapping and truffle sampling TATO consumed an average of 5.0 (SE= 0.31) hypogeous genera per stand, GLSA consumed an average of 5.7 (SE=0.44), and mycologists collected an average of 3.3 (SE=0.71) per stand. Mycologists collected an average of 3.8 (SE=0.70) hypogeous genera in thinned stands and 2.7 (SE=0.79) in controls. GLSA consumed an average of 6.4 (SE=0.50) hypogeous genera in thinned stands and 5.1 (SE=0.63) in control stands, but this difference was not significant (t=2.16, DF=5, P.083). However, it should be noted that GLSA dietaries in stands randomly selected for VDT happened to be significantly more diverse than the paired control stands (6.9 hypogeous genera compared to 5.3 in control stands fall 1991, 1992 and Spring 1992, t=-6.63, DF=2, P= 0.022). When dominant dietary components are compared ($\geq$5% RF) the dietaries are essentially identical, with only a slight change in rank order of hypogeous genera (Table 4.2). TATO dietaries in thinned stands averaged 5.1 (SE=0.22) hypogeous genera verses 4.9 (SE=0.50) genera in control stands.

Table 4.2. Rank order of dominant (≥5% RF) dietary hypogeous fungi for Townsend's chipmunks (TATO) and Northern flying squirrels (GLSA) captured in the FES study stands prior to variable density thinning.

	Fall 1991		Spring 1992				Fall 1992			
	GLSA		GLSA		TATO		GLSA		TATO	
Genus	C	T	C	T	C	T	C	T	C	T
*Rhizopogon**	1	1	1	1	1	1	1	1	1	1
Melanogaster	2	3	2	2	+	4	2	4	2	+
Hysterangium	+	5	4	3	4	2	6	+	4	+
Leucophleps	+	+	5	4.5	2	3			+	
Leucogaster	3	4							3	2
Gautieria	4	2	3	4.5		+				
Elaphomyces	+	+		+			4	3		+
Genia				+	3	+		+		
Hydnotrya				+	+	5				
Pachyphleous		+					3	5		
Radiigera		+					5	2		

* May including related taxa not distinguishable by spores alone; C= Stands randomly assigned control treatment; T= Stands randomly assigned VDT treatment (Thinning installed after fall 92 sampling). += Genus present in dietary in trace amounts (<5%RF) (not all trace items are shown). Fall 1991 TATO were excluded due to small numbers of samples collected.

Rank order of dominant dietary items for GLSA and TATO as well as rank order of hypogeous sporocarps collected by the concurrent mycological survey are presented in Table 4.3. While frequency and biomass of *Hysterangium* and *Gautieria* declined sharply in VDT stands, their rank abundance remained relatively high (ranked only behind *Rhizopogon* during spring 1993) (Table 4.3).

All taxa present in dietaries of TATO and GLSA prior to thinning were found in the dietaries of animals trapped in the thinned stands after thinning. The dramatic increase of *Melanogaster* sporocarps found by mycologists (Chapter 2 this volume) was not reflected in the dietaries of TATO or GLSA foraging in thinned stands. *Melanogaster* ranked 2 in GLSA dietaries the spring pre thinning (behind *Rhizopogon*) and ranked: 6,2,2, in the three springs post thinning. While it would appear that there was a decrease in use the first spring after thinning, rank abundance in the dietaries of the animals captured in the paired control stands showed a similar decline in spring 1993 (rank in control stands spring pre-thin 2, post-thin: 0, 2, 3). In the 2 pre-treatment fall GLSA samples *Melanogaster* ranked 3, and 4, (Table 4.2) and in the three falls post-thinning it ranked 5,3,2. Again the paired control stands showed a similar trend, with pre-thin ranks of 2, and 2 and post thin 4,2,2 (Table 4.3)

Table 4.3. Rank order of dominant (≥5% RF) dietary hypogeous fungi for Townsend's chipmunks (TATO) and northern flying squirrels (GLSA) captured in the FES study stands. Rank order of fungal genera collected by mycologists (biomass and frequency on sample plots) in thinned stands and paired control stands by season.

	Spring 1993								Spring 1994								Spring 1995							
	GLSA		TATO		Mycologists				GLSA		TATO		Mycologists				GLSA		TATO		Mycologists			
	T	C	T	C	T		C		T	C	T	C	T		C		T	C	T	C	T		C	
Genus					B	F	B	F					B	F	B	F					B	F	B	F
*Rhizopogon**	1	1	1	1	1	1	2	2	1	1	1	1	2	2	4	2	1	1	1	1	3	1	1	1
Hysterangium	2	3	3	+	2	2	1	1	6	3	3	4	9	6	1	3	3	2	3	3	4	5	4	1
Melanogaster	6		+	+	4	9.5	9	8	2	2	7	2	1	3	3	7.5	2	3		+	1	2	3	6.5
Leucophleps	5		2	2	3	6	5	8	3	6	2	3	6	6	6	6		4	2	2	7	7	5.5	4
Elaphomyces	+	5							+	+	+	+	7	9.5				+						
Endogone			+	+	7	3	8	5				+	4	1	7	5					8	4	8	6.5
Gautieria				+	5	6	3	3.5	5	5			5	9.5	2	4	5	5			6	7	2	6.5
Genia	+	+	4	3			4	8			5		8	9.5					+	+				
Genabea													10	9.5				+					7	6.5
Glomus			+	+					4		6	+	11	6	8	7.5			+	+				
Hydnotrya	+	+	+	+	9	4												+	4	4				
Hymenogaster	3	6	5	+	6	9.5	7	6		4	+						4	6	+	+				
Leucogaster	4	4	+																	+				

Table 4.3 (Continued)

	Spring 1993								Spring 1994								Spring 1995							
	GLSA		TATO		Mycologists				GLSA		TATO		Mycologists				GLSA		TATO		Mycologists			
	T	C	T	C	T		C		T	C	T	C	T		C		T	C	T	C	T		C	
Genus					B	F	B	F					B	F	B	F					B	F	B	F
Pachyphleous			+								4	5					+	+	+					
Radiigera	7	2																			2	7		
Tuber					8	6	6	3.5			+		3	4	5	1					5	3	5.5	3
Genus	Fall 1993								Fall 1994								Fall 1995							
*Rhizopogon**	1	1	1	1	3	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
Melanogaster	5	4	2	+	1	3	1	4.5	3	2	+	2					2	2	3	+	5	5		
Leucogaster		+		+	5	6	4	2	4	5	+	4					4	4	2	2				
Tuber		+	+	+			5	4.5		+	+	+							+		1	3		
Elaphomyces	3.5	3			2	6				+	.	+	3	2	2	2	.		+		3	5		
Glomus			+	+	7	4	6	4.5			+	+	2	3					+	+				
Gautieria		6		+			2	4.5	2	4	+	+					3	3						
Hysterangium		7	+	+					5	3	2	3					5	+	+					
Pachyphleous	3.5	2	+	+	6	6						+												

Table 4.2 (Continued)

Genus	Fall 1993								Fall 1994								Fall 1995							
	GLSA		TATO		Mycologists				GLSA		TATO		Mycologists				GLSA		TATO		Mycologists			
	T	C	T	C	T		C		T	C	T	C	T		C		T	C	T	C	T		C	
Genus					B	F	B	F					B	F	B	F					B	F	B	F
Endogone					4	2						+									4	2		
Leucophleps			+								+									3	6	5		
Radiigera	2	5																						

* May include related taxa not distinguishable by spores alone; T=VDT stand; C= Control stand; B= rank order based on g/ha estimates; F= rank based on frequency of occurrence on $4m^2$ sample plots; += genus present in dietary in trace amounts (<5%RF) (not all trace items are shown).

It would appear that 1993 was a bad year for *Melanogaster* consumption and based on the spring biomass estimates collected by the truffle survey, the rank abundance was rather low in the spring. Fall of 1993, *Melanogaster* was the largest biomass producer and relatively frequently encountered (Table 4.3).

Melanogaster in TATO dietaries was absent in the fall pre-thin sample, while it ranked 2 in the paired control stands. Post thinning *Melanogaster* ranked 2, trace, 3, in the three falls post thinning; in the paired controls, it ranked trace, 2, trace. Based on frequency, mycologists ranking ranked *Melanogaster* 1 in both thinned and control stands the first fall after thinning, found none in fall 1994, and ranked only a 5 in thinned stands in 1995. If the TATO in thinned stands were using the flush of *Melanogaster* brought on by the thinning, TATO in the control stands were not using the *Melanogaster* available to them. Animals in the control stands in spring 1994 were finding *Melanogaster* when the mycologists were not.

Rank order correlation between GLSA dietaries and hypogeous sporocarps collected by mycologists was poor. The dominant hypogeous fungal component of both TATO and GLSA dietaries had a more positive correlation with rank order of frequency of truffle taxa than with rank order of estimated biomass. Spearman's Roh for correlation of GLSA dietary items averaged 0.109 (SD=0.75) for truffle biomass and 0.391 (SD=0.66) for truffle frequency. The means were not significantly different (t=-1.68, DF=18, P= 0.11). Spearman's Roh for correlation of TATO dietary items averaged 0.349 (SD=0.63) for truffle biomass and 0.514 (SD=0.53) for truffle frequency. Again the means were not significantly different (t=-1.92, DF=35, P= 0.063).

Discussion

Dietaries of the small mammals captured during this portion of the FES were similar to those recorded by previous researchers. *Rhizopogon, Melanogaster,* and *Hysterangium* have been shown to be dominant food items for GLSA and TATO by many other researchers, (Maser *et al.,* 1978; Maser *et al.,* 1985; Maser *et al.,* 1986; Carey, 1995; Carey *et al.,* 1997b). North *et al.* (1997) found these to be some of the most highly consumed truffle genera in forest stands in western Washington. Most other researchers have focused on dead trapped animals or fecal pellets collected over a very narrow time frame. This study is the first to examine the same populations of mammals in a relatively small forest over an extended period of time.

Small mammals are more efficient than mycologists at locating fungal sporocarps. This fact is not surprising, however the magnitude of the difference was greater than expected. The concurrent truffle study (Chapter 2) was one of the most comprehensive ever, yet small mammals found as many fungal genera during one 2 week sampling period as we found in our 3 year effort. Many factors likely contributed to these differences. The animals were not limited to randomly assigned plots $4m^2$, 10 per control stand, 30 per VDT stand. Squirrels are dependent on these fungi as food and have coevolved with these fungi for mutual exploitation. The animals are continuously present in these forests and have located fruitful foraging areas. Flying squirrels are at high risk from predators while on the ground foraging for truffles. The ability to quickly locate and extract truffles is necessary to avoid excessive expenditures of energy, as most of the truffle species studied

have been shown to be of low to moderate nutritive value (Cork and Kenagy 1989, A. Claridge, unpubl. data) This inherent ability makes mycophagists very powerful tools to study hypogeous fungal dynamics in these forests.

Johnson (1994) found that Bettongs (*Bettongia gaimardi.*) foraging in a *Eucalyptus* forest used fungal sporocarps essentially in proportion to their abundance (available biomass). This study suggests that sporocarp frequency is more indicative of TATO and GLSA use of this resource. Other researchers (Waters and Zabel, 1995) found flying squirrel densities correlated with sporocarp frequency in *Abies* spp. forests in northeastern California at 17 and 20 years post harvest. In our study, GLSA foraging activity was reduced in the VDT stands, however den sites occupied prior to thinning were still used (Carey unpbl. data). TATO densities increased in VDT stands after thinning (Carey unpubl. data). These observations suggest that the apparent drop in truffle frequency (Chapter 2), as well as other changes induced by thinning (canopy structure, microclimate, etc.) recorded in the thinned stands affected GLSA to a greater degree than TATO. The increase of herbaceous plants and woody shrubs in VDT stands may also have increased the suitability of these stands for TATO.

I hypothesize that animals foraging by smell will detect the odor "nearest" to them (probability says that that will be the truffle with the most homogeneous distribution across the landscape, i.e. the most frequently encountered on the randomly placed $4m^2$ plots). This may not be the largest truffle biomass producer, or even the most nutritious. I would expect these to be consumed first, then other less frequently encountered taxa would be sought after to balance out the nutritional needs, and or preference. This hypothesis is

supported by the Spearman rank order correlation data. While the difference between rank order in TATO and GLSA dietaries and rank order of truffles collected by human based on biomass and on frequency were not definitive, they are strongly suggestive that frequency is a better reflects animal use of truffles.

Rhizopogon was the most frequently encountered truffle, as well as the most abundant in biomass. However taxa such as *Glomus* and *Endogone* are relatively frequent in Douglas-fir forests, (Luoma *et al.*, 1991; Chapter 2 this volume) but contribute to only a small percentage of the available (standing crop) biomass. Each animal can only consume a finite amount of food in a single meal, one truffle could easily fill the stomach of one of these small mammals; yet other researchers consistently found numerous hypogeous taxa in the stomach contents of small mammals (Maser *et al.*, 1985; Maser *et al.* 1978). These observations are also supported by this paper as well as chapter 3. No single animal sampled consumed only one hypogeous genus, 3-4 was the norm. Feeding trials with these two mammals species (A. Claridge unpbl. data.) strongly suggest that a diversity of truffle taxa are required to maintain the health of GLSA and TATO.

TATO Home ranges are smaller and animals are less likely to forage away from the stand in which they den (Carey *et al.,* 1997b; Carey, 1995). Therefore small scale disturbance would be (such as the VDT) should have a greater impact on dietaries TATO (compared to GLSA). The fact that rank order did not change significantly raised many questions. *Gautieria* and *Hysterangium* showed the greatest decline in the VDT stands compared to control (Chapter 2). Other studies have shown that *Gautieria* apparently is not a very "palatable" truffle for TATO (Carey *et al.,* 1997b; Maser and Maser, 1988),

although it is commonly consumed by GLSA. *Gautieria* occurred only in trace amounts in TATO dietaries at Fort Lewis. *Hysterangium* was found in greatly reduced numbers in thinned stands (Chapter 2) but occurred in relatively large quantities in the TATO dietaries in these stands (over 25% RF the first spring post thinning). TATO are apparently finding *Hysterangium* sporocarps much efficiently than mycologists, or are foraging at the stand edges where mycological survey did not sample. Nutritional value of the fungi vary from species to species (A.W. Claridge pers. comm.) and perhaps *Hysterangium* is particularly nutritious (or just palatable) and is sought after by TATO. Part of the perceived decline in the *Hysterangium* species in VDT stands could have been caused by TATO consumption of the fruit bodies of fungal colonies remaining after thinning.

GLSA often have home ranges in excess of the 2.8 ha treatment stands (Witt, 1992; Carey, 1995), and have been shown to move more than 1 kilometer in a single evening (Carey unpbl. data) These fact helps to explain the presence of *Gautieria* (often in relatively large amounts) in the feces of animals trapped while foraging in thinned stands. Fecal pellets reflect a previous meal, and therefore animal could be consuming these fungi from adjacent control stands or buffer zones between stands where the mycological survey did not sample. Flying squirrels could be preferentially seeking out *Gautieria* sporocarps. Zabel and Watters (1997) found *Gautieria* sporocarps to be highly palatable to GLSA in a feeding trial. Again sustained consumption of truffles produced by retained *Gautieria* colonies could account for a portion of the measured drop in abundance.

The extreme efficiency of small mammals in locating and consuming hypogeous fungi make them excellent tools for studying fungal dynamics in forest ecosystems. As

more sophisticated tools (such as PCR DNA amplification and others) from fecal material (Hoss *et al.* 1992) become available, tracking the contribution of a single fungal individual (or population) may become practical. Further research is needed on the interactions of fungi, mammals and forest ecosystems.

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Chapter 5

Tuber anniae sp. nov. (Ascomycotina)

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MYCOTAXON

(in press)

Abstract

A new species of truffle associated with *Pseudotsuga menziesii* on the Fort Lewis Military Reservation near Olympia, Washington, is described as *Tuber anniae*. It differs from related species, *T. monticola* and *T. shearii* in peridial color and spore shape.

Introduction

Integrated research on the northern spotted owl in forests of 55 to 65-year-old Pseudotsuga menziesii at Fort Lewis Military Reservation near Olympia, Washington includes studies of the small mammals that constitute the owls' food base. As hypogeous fungi are a major food base for the small mammals, standing crops of those fungi have been monitored at 6-week intervals for nearly 3 years (Colgan *et al.*, 1996). During this time, the genus Tuber was represented by three species: *T. monticola* Harkness was relatively common, *T. gibbosum* Harkness was found once, and an undescribed *Tuber sp.* was represented by 4 collections. We propose the name *Tuber anniae* for this new taxon.

The resemblance of the new species to *Tuber shearii* Harkness prompted us to re-examine some other collections in our herbarium tentatively assigned to that species but not comfortably fitting its characters. As a consequence, the range of *T. anniae* was extended to Oregon and Idaho.

Methods

At each collecting time, 10 plots of 4 m2 each were raked into mineral soil along each of 16 transects. All specimens found in plots were collected, dried and identified Specimens were then weighed to estimate standing crops (Colgan *et al.* 1996). Colors of fresh specimens are in general terms of the authors. Specimens were dried the day of collection with a forced air dehydrator set at 49°C (120°F). Microscopic characters were determined from hand sections mounted in 5% KOH, Melzer's reagent, or cotton blue, as indicated. Spore dimensions are based on at least 50 randomly selected spores and do not include ornamentation. The light photomicrograph is from material mounted in 5% KOH. For scanning electron microscopy, dried spores were mounted on pegs with double sided tape, coated with gold and examined with an Amray 3000 scanning electron microscope. The holotype and paratypes have been accessioned into the Mycological Herbarium of Oregon State University (OSC).

Taxonomy

Tuber anniae sp. nov

Ascomata subglobose to irregular, 6-18 mm in diam, glabrous, in youth pale yellow, by maturity brown to dark olive brown with grayish white furrows and patches, crisp, drying hard. Gleba at maturity with dark brown fertile tissue marbled with narrow, white

to ivory veins alternating with brownish gray veins. Odor mild to slightly paint-like; taste not recorded.

Peridium 100-200 µm thick, with two layers. Ectal excipulum 75-120 µm thick, a compact tissue of isodiametric to elongate, rounded or subangular, hyaline to light yellow cells 3-20 µm in diam, the walls 0.2-1 µm thick. Ental excipulum 25-80 µm thick, hyaline, of tightly periclinal-interwoven, hyaline hyphae 2-4 µm broad at the septa, the cells often slightly inflated, the walls thin to somewhat thickened. Medullary excipulum (gleba) of hyaline, interwoven, thin-walled, hyphae 2-5 µm broad with scattered cells inflated up to 10 µm. Asci 1-4 spored, crowded within the medullary excipulum, globose to ellipsoid, 75-90 x 50-80 µm, hyaline, the walls ±1 (-2) µm thick, lacking a stem or occasionally with a simple stem 8-20 µm long. Spores globose to subglobose or infrequently broadly ellipsoid, brown, the walls up to 5 µm thick at maturity; in 1-spored asci 43-45 µm in diam excluding ornamentation when globose, 45-48 (-50) x 40-44 µm when ellipsoid; in 2-spored asci mostly globose and 28-45 µm in diam; in 3-spored asci mostly globose and 25-38 µm in diam; in 4-spored asci mostly globose and 20-25 µm in diam. Ornamentation alveolate, the alveolae 6-10 along the spore and with 5-6 sides, the alveolar walls 3-5 µm tall and yellow brown to dark brown in KOH and Melzer's reagent. Lens effect prominent on larger spores (in transmitted light the alveolae on the underside of the spore are projected to the upper spore surface).

Etymology: "anniae" in honor of Annie Kiel, who facilitated the Fort Lewis Ecosystem Study in many useful ways.

Collection examined: Holotype - Washington: Thurston Co., Fort Lewis Military Reservation, Star Forest Block, light thinning treatment area 03, plot 25, 3 Nov. 1993, D. Thysell (OSC 58992). Paratypes - Idaho: Nez Perce Co., Craig Mt., 20 Sept. 1983, L. Terwilliger 83-177 (OSC 59330). Oregon: Polk Co., Black Rock Rd., 5 Oct. 1996, A. Beyerle (OSC 59329). Washington: Lewis Co., 1 mi. N. of Vader, 10 July 1969, J. Trappe (OSC 59328); Thurston Co., Fort Lewis Military Reservation, Farley Forest Block, non-thinned stand 04, plot 18, 16 Aug. 1993, W. Colgan III (OSC 58991); Star Forest Block, non-thinned stand 02, plot 100, 31 Aug. 1995, W. Colgan III (OSC 59000); Star Forest Block, light thinning treatment area 01, plot 115, 24 Oct. 1993, J. Trappe (OSC 59001).

Habit, Habitat and Season: Hypogeous at 400 ft elevation in 55 to 65-yr.-old stands of *Pseudotsuga menziesii*, with which it probably forms ectomycorrhizae. August through November. The August and October collections are not fully mature, so we infer that ascomata begin to form in late summer and mature in late autumn.

Discussion

Tuber anniae is intermediate between *T. monticola* Harkness and *Tuber shearii* Harkness. *T. anniae* has a brown to dark olive brown peridium and mostly globose spores with 6-10 alveolae across the spore surface. *T. monticola* has a dingy white to gray peridium and mostly globose spores with (8) 10-16 alveolae across the spore surface. *T. shearii* has a

light yellowish brown peridium and mostly subglobose to broadly ellipsoid spores with 2-8 alveolae lengthwise along the spore surface. The anatomy of the peridium and gleba is similar among all three species, although *T. shearii* has areas of obtuse to tapered, often granulated hyphae emergent from the surface. At present, *T. anniae* and *T. monticola* are reported only from the Pacific Coast, whereas *T. shearii* has been reported only from east of the Mississippi (Gilkey, 1954). *Tuber sphaerosporum* Gilkey and *Tuber californicum* Harkness differ from *T. anniae* in having uniformly globose spores.

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Chapter 6

Pachyphloeus thysellii sp. nov. (Ascomycotina)

Wesley Colgan III

Abstract

An undescribed truffle found on the Fort Lewis Military Reservation near Olympia, Washington, is described as *Pachyphloeus thysellii*. This new species of truffle associated with *Pseudotsuga menziesii*. It differs from related species, *P. melanoxantha* and *P. virescens* in peridial color and ascus shape.

Introduction

Integrated research on the ecosystem function in forests of 55 to 65-yr-old *Pseudotsuga menziesii* at Fort Lewis Military Reservation near Olympia, Washington includes studies of the small mammals that feed on fungi. As hypogeous fungi are a major food base for the small mammals, standing crops of those fungi have been monitored at 6-week intervals for nearly 3 years (Colgan *et al,*. 1996). During this time, the genus *Pachyphleous* was represented by one undescribed species. I propose the name *Pachyphloeus thysellii* for this new taxon.

Methods

At each collecting time, 10 plots of $4m^2$ each were raked into mineral soil along each of 16 transects. All specimens found in plots were collected, identified and dried for obtaining dry weights of standing crops and for herbarium deposit (Colgan *et al.,* 1996). Colors of fresh specimens are in general terms of the authors. Specimens were field-dried

with a forced air dehydrator at 49°C (120°F). Microscopic characters were determined from hand sections mounted in 5% KOH, Melzer's reagent, or cotton blue, as indicated. Spore dimensions are based on at least 50 randomly selected spores and do not include ornamentation. Light photomicrograph from section mounted in 5% KOH unless otherwise indicated. For electron microscopy, dried spores were mounted on pegs with double sided tape, coated with gold and examined with an Amray 3000 scanning electron microscope. The holotype and paratypes have been accessioned into the Mycological Herbarium of Oregon State University (OSC).

Taxonomy

Pachyphloeus thysellii nom. prov.

Ascomata subglobose to irregular, 0.8-2 cm in diameter, warty reticulate, brown to tawny with yellow veins and patches, texture rubbery, odor mild to slightly onion like, taste not recorded, hard when dried. Gleba off-white to yellowish translucent, white and dark alternating veins emanating from a central cavity containing cottony hyphae.

Ectal excipulum (peridium) 200-300 µm thick, comprised of a two layers: Ectal excipulum 150-250µ thick, brown to pale yellow, compactly arranged, of inflated polygonal hyphal cells, 35-45 µm in diam, cell walls ca. 1µ thick. Ental excipulum 40-60µ thick, hyaline, comprised of tightly arranged hyphae 6-8µ in diam. with inflated, rounded cells up to 10µ diam., cell walls ca. 1µ thick

Medullary excipulum (gleba) of hyaline, interwoven, branched hyphae 6-10μm in diam. Asci 8-spored, elliptical to subglobose, 40-55 x 60-80 μm, hyaline, wall 1μ thick, not amyloid, mildly cyanophilic in cotton blue.

Spores predominantly globose, 12-17μ in diameter excluding ornamentation, walls hyaline in KOH and Melzer's reagent, walls strongly cyanophillic in cotton blue. Ornamentation of symmetrical rods ≥2 μm tall, also cyanophilic; spore wall ±1 μm thick. Episporium hyaline, inconspicuous, adhering closely to spore wall and in between the ornaments.

Etymology: "*thysellii*" in honor of the collector of the type specimen, David Thysell, a research botanist with the USDA Forest Service and cooperator on the Forest Ecosystem Project at Fort Lewis

Collection examined: Holotype, Washington, Thurston Co. Fort Lewis Military Reservation, Hill block, stand 3, in root-rot treatment area, below an excavated wasp nest, 8/24/1994, D. Thysell. Isotype, Washington, Thurston Co. Fort Lewis Military Reservation, Stellar block, stand 1, heavily thin treatment, 8/18/1993, Wes Colgan III

Habit, Habitat: Hypogeous; found at 400 ft elevation in a 55-65-yr.-old thinned stands of *Pseudotsuga menziesii* (Mirb.) Franco.

Discussion

P. thysellii is closest to *P virescens* Gilkey but differs in peridial coloration and structure. *P thysellii* possesses darkly pigmented angular warts with yellow streaks in underlying tissues similar to *P. melanoxanthus* (Tul. ex Berk.) Tul. Gior. The asci of *P. thysellii* are elliptical to subglobose as found in *P. virescens*, not unisereate as in *P. melanoxanthus*, *P. thysellii* is an intermediate between these two species.

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Chapter 7

Conclusions

"If the things described so far were all that the fungi had to offer, it would appear that the harm done by fungi just about balances the good. But when you add the most important function of these tiny organisms, you will see that the good far out weighs the bad. Fungi are essential to the life cycle of every living thing on earth."

From: Mushrooms, Molds and Miracles

The Strange Realm of Fungi,

Lucy Kaveler, 1965

Human manipulations of forest systems are an inevitable aspect of our existence on this earth. If such manipulations can be done in a manner that enhances and diversifies the landscape, we will better be able to sustain life on home. This study shows that while production of hypogeous sporocarps may be reduced by thinning, diversity can increase. Small mammals that forage in these areas were much more efficient than mycologists at finding hypogeous fungal genera in these forests. The use of hypogeous fungi by small mammals was highly variable and largely unaffected by the thinning of these forests.

This study encompassed three years of field sampling for forest fungi and five years worth of small mammal dietary collection. Few previous studies in these areas have had this duration. The idea put forth by Amaranthus and Perry (1994) "Disturbing the

forest to remove commodities does not necessarily conflict with maintaining spatial and temporal linkages between plants via ectomycorrhizal fungi" is largely supported by this study. The VDT installed shows every indication that structural complexity in young forests can be achieved without long term deleterious effects on hypogeous fungi and the animals that depend on them for food.

This work in conjunction with the planned 20 year Forest Ecosystem Study should be continued and these data considered as a benchmark. Future researchers must continue to examine the long term effects of human manipulations to our forest ecosystems.

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