Survey & Manage Category B Fungi Equivalent-Effort Survey Protocol

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Cover photo: Old-growth Forest. 2008. Eugene District BLM. Photo author unknown, USDI BLM Photo Gallery [Accessed May 2010, http://www.blm.gov/wo/st/en/bpd.html].

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Background

The Survey and Manage standards and guidelines (USDA/USDI, 2001) require equivalent-effort surveys for Category B fungal species (rare, pre-disturbance surveys not practical) when National Environmental Policy Act (NEPA) decisions or decision documents are to be signed for habitat-disturbing activities in old-growth forest in fiscal year 2011 and beyond if strategic surveys are not considered completed. The survey protocol described herein will be used to conduct equivalent-effort fungal surveys for Category B Survey and Manage fungal species. It is a modified version of a macrofungal survey protocol (Van Norman et al., 2008) developed for the R6 Forest Service and Oregon/ Washington BLM Interagency Special Status/Sensitive Species Program (ISSSSP) with an emphasis on required survey effort to assure that the standards and guidelines' intention of "equivalent-effort" surveys are met.

Equivalent-effort surveys are defined in the Survey and Manage standards and guidelines (page 25) as:

> pre-disturbance surveys conducted similarly to practical surveys (to the same intensity and effort—usually one field season and no more than two), according to written Survey Protocols, and during the times when the likelihood of detecting the species is highest.

The standards and guidelines point out there are only two differences between equivalent-effort surveys and practical surveys. One difference is that equivalent-effort surveys are not expected to meet the description of "likely to determine the presence" of a species because the characteristics of these species make finding sites less certain. The other difference is that equivalent-effort surveys may need to accommodate one or more of the practicality factors. Regarding these practicality factors, the standards and guidelines say the survey is an "equivalent effort" to practical surveys, with protocol adjusted to deal with the one or more of the factors...that make determining presence of the species unlikely. For the Category B macrofungi, these factors include:

1. The taxon does not appear annually or predictably, producing identifying structures that are visible for a predictable and reasonably long time;
2. The taxon cannot be authoritatively identified by more than a few experts;
3. The taxon cannot be readily distinguished in the field and needs more than simple laboratory or office examination to confirm its identification.

Hence, survey protocol adjustments to address each of these factors are included herein.

Equivalent-effort surveys for Category B Survey and Manage fungal species are only required for habitat-disturbing activities in old-growth forests. Old-growth is defined in the standards and guidelines glossary (page 79) as:

> an ecosystem distinguished by old trees and related structural attributes. Old growth encompasses the later stages of stand development that typically differ from earlier stages in a variety of characteristics which may include tree size, accumulations of large dead woody material, number of canopy layers, species, composition, and ecosystem function. More

specific parameters applicable to various species are available in the USFS, Region 6, 1993 Interim Old Growth Definitions (USDA Forest Service Region 6, 1993 [for this document see http://www.blm.gov/or/plans/surveyandmanage/sp.htm]). The Northwest Forest Plan SEIS and FEMAT describe old-growth as a forest stand usually at least 180 to 220 years old with moderate-to-high canopy closure; a multi-layered, multi-species canopy dominated by large overstory trees; high incidence of large trees, some with broken tops and other indications of old and decaying wood (decadence); numerous large snags; and heavy accumulations of wood, including large logs on the ground (USDA, USDI 1994).

This survey protocol is generally the same as the original 2008 version with the following exceptions:

1. A requirement for survey frequency has been added;
2. Language specific to the Survey and Manage standards & guidelines has been added; and
3. Language specific to the Interagency Special Status/Sensitive Species Program has been removed.

Introduction

The vegetative body (thallus) of a fungus usually grows within a substrate such as wood or soil with filamentous growth known as a hypha individually and mycelium collectively. A thallus may be present within the substrate at one location for many years, yet the mycelium cannot be easily detected or identified to species. Instead, surveyors typically rely upon finding the fruiting bodies (sporocarps) of macrofungi, those fungal species whose fruit can be seen by the unaided eye, to locate and identify species.

Most sporocarps are ephemeral. Sporocarp production by many species can be irregular and infrequent, often fruiting every several years. For this reason, single-visit surveys have a relatively high probability for missed detections (a false negative conclusion: a survey result of the target species being ‘not present’ when they actually are present) (Brown, 2002; Molina, 2008).

Multi-year surveys spanning a greater range of climatic conditions are recommended to increase the probability of detecting resident fungi (North et al., 1997; Brown, 2002; Castellano et al., 2004; Molina, 2008). Sporocarp production is a function of substrate, host availability and climate; namely seasonal rainfall and temperature (Lodge et al., 2004). In years with drought or atypical weather, fewer species produce sporocarps.

Because species produce sporocarps at different times throughout the year and in response to specific weather conditions, multi-season and multiple surveys per season are recommended to increase both the probability of detection and the proportion of fungal diversity sampled. The greater the number of survey visits, the less chance of a false negative survey result and the greater chance of sampling maximum species richness (Smith et al., 2002; Lodge et al., 2004).

One objective of this survey protocol is to meet the intent of the Survey and Manage standards and guidelines for equivalent-effort surveys in which usually one and no more than two field seasons are required. This protocol emphasizes a 2-year, 4-visit per year survey effort for most habitat-disturbing projects in old-growth. Exceptions to this 2-year survey effort are for projects that restore and maintain medium- and large- diameter shade-intolerant tree species within dry forests or when project disturbances are of limited spatial extent. For these exceptions, a 1-year, 4-visit survey effort is applicable based on the reduced risk to species persistence from these activities.

Another objective of this survey protocol is to establish written standards for: 1) conducting sporocarp presence surveys for multiple target species; 2) documenting survey effort, sporocarp collection, and site data; and 3) characterizing habitat and ecological conditions for occurrences of Category B Survey and Manage macrofungal species. If an occurrence is found, the site will require management as directed under the Survey and Manage standards and guidelines for Category B species (page 9). The presence of a Category B Survey and Manage fungal species sporocarp does not delineate the population, but rather its presence in a particular habitat is meant to extend our knowledge of the species. The exact probability of a missed detection using these methods cannot be determined at this time.

This survey protocol is written to target more than one fungal species, which maximizes field visits and efficiency. These methods can also be used for site revisits that are occasionally needed to monitor continued species presence. The protocol was developed by agency botanists and biologists based upon experience, journal articles, consultation with fungal survey contractors and researchers, and other survey protocols and guidelines for macrofungi (Castellano et al., 1999; O'Dell, 1999). We do recognize that alternative survey methods may differ depending on project objectives, but offer these methods as a minimum requirement for Category B Survey and Manage equivalent-effort fungal surveys for habitat-disturbing projects in old-growth forests.

Preliminary Field Planning

To effectively meet the objectives of this protocol, planning and preparation is necessary prior to conducting field surveys. The following steps are recommended:

1. Determine if the project is within old-growth forest. Old-growth forest (as defined in the standards and guidelines, page 79) can be determined by using agency GIS records, timber stand records, aerial photos, USGS maps, predictive habitat models, and individuals knowledgeable with the project area. ***If the project is not in old-growth, no equivalent-effort fungal surveys are required.***

2. Determine if the project is exempt from surveys. Activities associated with routine maintenance of structures or improvements are exempt from surveys (see the standards and guidelines, page 22). Additional activities are exempt under the 2006

Northwest Ecosystem Alliance et al. v. Rey et al. known as the "Pechman exemptions" and the 2011 Conservation Northwest v. Sherman case known as the "2011 Settlement Agreement" (see table of exemptions at http://www.blm.gov/or/plans/surveyandmanage/gg.htm). ***If a project meets any of the exemptions, no equivalent-effort fungal surveys are required.***

3. Determine if the project is considered a "habitat-disturbing activity." ***For management actions that are not considered habitat-disturbing, no equivalent-effort fungal surveys are required.***

   Habitat-disturbing activities are defined in the standards and guidelines (page 22) as:

   … those disturbances likely to have a significant negative impact on the species' habitat, its life cycle, microclimate, or life support requirements. The evaluation of the scale, scope, and intensity of the anticipated negative impact of the project on habitat or life requirements should include an assessment of the type, timing, and intensity of the disturbing activity. "Habitat-disturbing" is not necessarily the same as "ground-disturbing."

   Consider the type, intensity, and extent of disturbance (as defined in the standards and guidelines, pages 22 and 76) expected as a result of project implementation. Consider the degree that the old-growth components may change as a result of the activities. Habitat characteristics upon which old-growth fungi depend include, but are not restricted to (Cushman and Huff, 2007):

   - Presence of coarse woody debris and forest floor organic matter in a variety of decay classes,
   - Forest floor canopy shade provided by a canopy and sub-canopy overstory with crown-to-crown contact,
   - Retention of old-growth or legacy trees in the stand,
   - Retention of tree species diversity that is present in the stand.

   Because of the variability in ecoregions, soils, and plant communities across the Northwest Forest Plan area and the variability associated with specific activities, consider and determine thresholds or measurements that define fungal habitat-disturbing activities in old-growth for that particular area. Document the rationale for your determination in the NEPA analysis for the project.

   Examples of management activities that are not be considered habitat-disturbing include activities that are enacted to maintain old-growth stand characteristics, do not pose a direct or indirect impact to the fungal organism, and do not substantially change the microclimate. For more information about potentially beneficial versus negative impact activities, see the Conservation Assessment for fungi (Cushman and Huff, 2007).

4. Determine the list of target species for the project area based on species range and habitat requirements. Documenting known sites of potential target species on all

land ownerships within the vicinity of the project area will help narrow the target species list. Agency and Natural Heritage Program occurrence databases are the primary sources for target species locations though other sources, such as university herbaria, field guides, and knowledgeable individuals may be useful. Gather known information about the habitat and ecology for each targeted species. Known habitat information for FS and BLM special status and Category B Survey & Manage fungi has been documented in Appendices 1 and 2 of the Conservation Assessment for fungi and in the Region 6/BLM Oregon and Washington Habitat Summary for Sensitive Fungal Species spreadsheet (http://www.fs.fed.us/r6/sfpnw/issssp/planning-documents/assessments.shtml).

5. Determine the ecological association of fruiting for target species. In particular, consider what is known about habitat and the seasonality or timing of when the target species produce sporocarps (see Timing of Surveys section below).

6. Monitor weather conditions and conduct field reconnaissance to determine when to begin field surveys.

Number of Survey Years and Visits

Habitat-disturbing activities in old-growth forest that do not meet one of the survey exemptions require a 1 or 2-year survey, depending upon the scale and scope of the potential impact to fungi and/or their habitat.

One year survey effort
For projects that meet the following criteria, a 1-year survey effort is appropriate:

1. Commercial thinning projects in dry forest Plant Association Groups (PAGs) in the East Cascades Provinces of Oregon and Washington, the California Cascades Province, and the Klamath Provinces of Oregon and California (see table of dry forest PAGs at http://www.blm.gov/or/plans/surveyandmanage/gg.htm) where projects are designed to restore and maintain medium- and large-diameter shade-intolerant tree species through thinning from below, and target the removal of fire-intolerant tree species. These projects should either:
    i. retain 60% canopy closure, or
    ii. maintain > 40% live-tree basal area with dispersed green-tree retention in combination with aggregated retention, which has been shown to maintain sporocarp production (Luoma et al. 2004).

   These stand-scale restoration projects may have short-term negative effects to some late-successional/old-growth (LSOG) associated fungal species, but long-term benefits to these species are expected. A 1-year survey effort suffices as these design criteria retain most old-growth fungal host trees, most down log and snag structures, and moderate levels of canopy closure.

2. Projects in which the old-growth habitat being disturbed is of limited extent and no more than 0.2 acres (Durall et al., 1999) may utilize a 1-year survey effort. These are areas where tree clusters are removed and/or intensive ground disturbance occurs.

For these projects a total of 4 visits to each survey area over 1 year are required with 2 visits per season.

	Spring-Season Fruiting	**Fall-Season Fruiting**
Year 1	2 visits	2 visits

Two year survey effort

For other habitat-disturbing projects including regeneration harvest, commercial thinning not meeting the above criteria, pipeline development, and road construction, a 2-year survey effort with 4 visits each year is required.

	Spring-Season Fruiting	**Fall-Season Fruiting**
Year 1	2 visits	2 visits
Year 2	2 visits	2 visits

Field Survey Methods

Survey Types

Either complete surveys or intuitive-controlled surveys may be conducted, depending on the size of the survey area (O'Dell, 1999).

Complete Surveys

Complete surveys are recommended when the survey area is less than 2.5 acres. These surveys complete a 100% ***visual*** examination of the survey area (distance between transect lines are close enough to permit an overlapping sight distance between transect lines) (Fig. 1). For hypogeous fungi this means looking for and intensively sampling suitable habitat within the old-growth survey area, but not a complete 100% survey of the area (see App. 1).

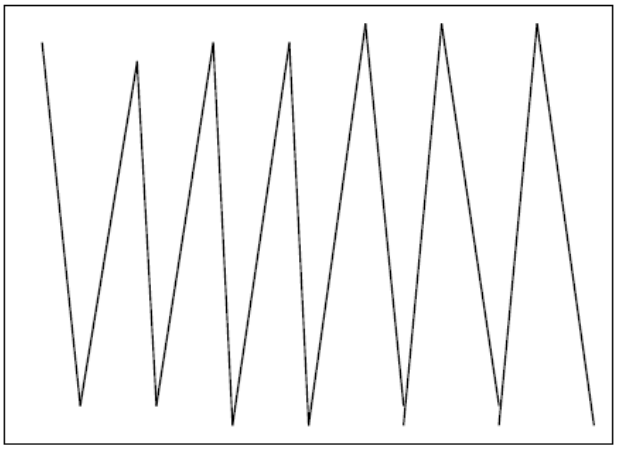


Figure 1. Example of a Complete Survey. Distance between survey lines would depend upon sight distance (from Derr et al., 2003).

Intuitive-Controlled Surveys

Intuitive-controlled surveys are recommended when the survey area is greater than 2.5 acres. These surveys are conducted by traversing through and around the survey area, focusing survey efforts in areas of highly suitable habitat within the old-growth survey area (Fig. 2). Highly suitable habitat delineated on topographic maps during preliminary field planning and observed while traversing the survey area receive complete (100%) visual examination for both epigeous and hypogeous species (see App. 1). It is expected that surveyors will pass through all represented plant associations and significant changes in slope position, aspect and substrate, especially when there are multiple target species. Trained and experienced surveyors looking in terrain that is not excessively steep or brushy should be able to survey, on average, 40 or even 60 acres in a day using this methodology depending on the number of target species found.

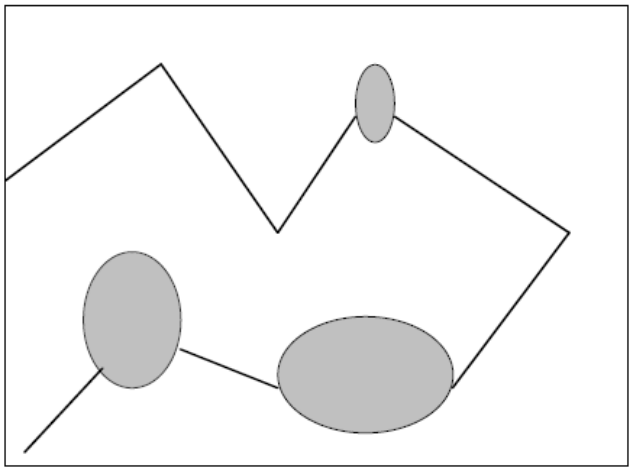


Figure 2. Example of Intuitive-Controlled Surveys. Shaded blocks represent areas of highly suitable habitat (from Derr et al., 2003).

Timing of Surveys

The Northwest Forest Plan area is large with diverse ecosystems and conditions. The guidelines provided below are meant to be informational rather than strict requirements. Conditions that stimulate fruiting of the target fungi within the survey area can differ. Local botanists have the discretion to determine the appropriate survey timing for target species depending on local conditions. Consider your latitude, longitude, elevation, distance from ocean, weather patterns, and other pertinent factors when determining survey timing. Check existing records and with local mycological experts to obtain this information. The Conservation Assessment for fungi, field guides, and guidebooks (Castellano et al., 1999, Castellano et al., 2003) are good references for this information. Monitor weather conditions and conduct on-the-ground field reconnaissance. Check to see which common fungal species are fruiting and use that as a timing indicator for Category B fungal species.

Survey visits 2-3 weeks apart generally accommodate the seasonal variation in weather conditions and variations in the conditions that stimulate fruiting in different species, thereby increasing the chance of detection. However, if a weather change such as the arrival of snow or a hard freeze is imminent, then the interval may be shortened to 10-14 days. If weather conditions are not appropriate (e.g., too dry), an interval greater than 3 weeks is acceptable. A longer interval may also be acceptable if there are multiple target species with widely varying fruiting seasons (e.g., early fall and early winter). When conducting all surveys within 1 year, it may make sense to have a shorter interval, especially at high elevations where the fruiting season may be short. If departing from the 2-3 week interval, write on the survey forms and enter into the survey comments of the database the reason for the departure.

Late Summer/Fall

In general, late summer and fall surveys begin after significant precipitation has moistened the substrate. “Good” fall sporocarp production generally occurs when there are multiple rain events at least every 3 weeks from late August/early September through fall until the first frost and when temperatures have been below 80 degrees F. Consider too that more precipitation may be required to reach the ground thereby stimulating fruiting in areas where dense canopies or shrub layers occur such as the Western Cascade Range. In general, stop conducting fall surveys when there is persistent snow or a persistent hard freeze that impacts the understory. Depending on the target species, freezing conditions may end sporocarp production. Sporocarps often quickly disintegrate when exposed to extreme temperatures. However, some species begin to fruit after the first frost.

The following table outlines general minimum conditions and recommendations by physiographic province for beginning epigeous and hypogeous fungal surveys in the fall. Remember that survey timing may differ depending upon specific geographic location, annual weather conditions, and target species list.

Sporocarp Type	Physiographic Province	Late Summer/Fall Timing
Epigeous (aboveground)	All Physiographic Provinces in the NWFP, Oregon, Washington, and northern California	1-2 weeks after late summer/fall rainfall of ½ inch minimum
Hypogeous (belowground)	Oregon Coast Range, Willamette Valley, Oregon Klamath, Washington Western Lowlands, Washington Olympic Peninsula, California Coast Range	After 2 significant rainfalls of ½ inch minimum within 2 weeks of each other and without 80° F days in between.
	Western Cascades Oregon, Washington, and California and the California Klamath Range	After 2 rainfalls of 1 inch within 2 weeks of each other without 80° F days in between
	High elevations of the Western Cascades and Eastern Cascades Oregon and Washington	After summer or early fall rainfall

Spring

Late winter and spring survey conditions are more variable by locale than autumn survey conditions. For both epigeous and hypogeous fungi, late winter and spring surveys should commence after persistent freezing conditions have ceased. Surveys may continue into early summer. Sampling should occur while the substrate is still moist. Substrate can dry out quickly, although areas such as north slopes and riparian zones retain moisture longer. When planning surveys, elevational gradients following the snow melt line upslope can also be considered.

Surveys may be conducted when 50% or more of the suitable habitat within the target survey area is free of snow and should be scheduled to locate:

- Fungi that fruit just after snow melt
- Fungi that fruit after snow melt, a warming trend and spring rain

Equipment List

- Aluminum foil, wax paper bags (full sandwich size) or brown paper bags and a roll of aluminum or wax paper for larger specimens (fungi can be placed inside and the ends twisted to contain the specimen like a tootsie-roll)
- Trowel or large knife to dig up base of sporocarps
- 4-tine garden cultivator; shorten the handle to 1-meter for a built-in measuring tool

- Sharp knife or razor blade
- Specimen field tags
- Survey data forms and fungal description forms
- GPS unit
- Camera, preferably digital
- Permanent marking pens
- Flagging and permanent tags to mark collection sites
- Basket or bucket to carry collected specimens

Voucher Collection and Data Management

Collection of voucher specimens is ***required*** for documenting most Survey and Manage fungal species and to assure correct species identification. See the Survey and Manage General Guidance web page for current direction (http://www.blm.gov/or/plans/surveyandmanage/gg.htm).

In Region 5 contact the Regional Botanist for current voucher specimen instructions.

In Region 6 and OR/WA BLM, voucher specimens of potential target Survey and Manage species must be collected and sent to the Inventory Coordinator Assistant of the Interagency Special Status/Sensitive Species Program (ISSSSP) who has contracts established for fungal species verification. If a vouchered specimen is verified as a Survey and Manage species, the specimen is then curated and submitted to one of the regional herbaria to allow access to researchers for further work and to provide centralized, permanent, and safe storage.

Follow the vouchering guidelines posted on the ISSSSP Identification Services webpage (http://www.fs.fed.us/r6/sfpnw/issssp/inventories/identification.shtml). The website contains the following:

- R6 FS/OR-WA BLM Vouchering Policy
- Guidelines for Fungal Voucher Collection
- Field Tag instructions and template
- Fungal Description Forms
- ISSSSP Inventory Coordinator Assistant's address to mail voucher specimens to for expert identification and specimen curation

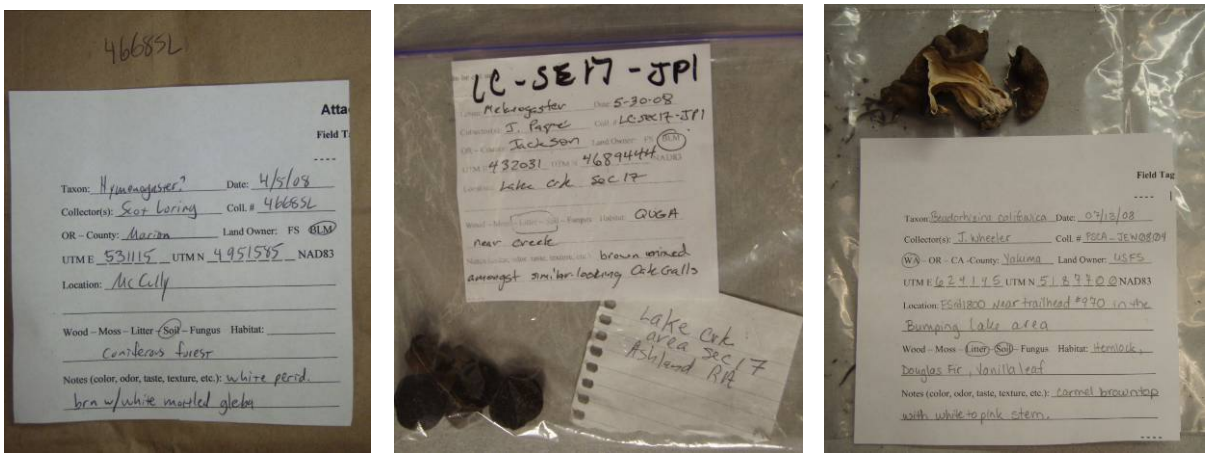
Further information about handling and vouchering fungi such as drying guidelines and collecting spore prints can be found in Castellano et al. (1999; 2003).

In brief, handle fungal specimens as follows. To voucher a fungal collection, first clean specimens of debris. Photograph the fresh specimen, preferably digital and *in situ*. Write the unique collection number legibly on a piece of paper and photograph along with the specimen. For truffles, show a cut truffle in the photo (see appendix for cutting recommendations). Identification of some other genera such as *Ramaria* is also aided

by cutting the specimen. Fill out the entire ***Field Tag*** being sure to include the tentative species identification. Place the specimen and completed field tag in wax paper bags or heavyweight foil and write the unique collection number in permanent marker on the outside of the bag. Never collect in plastic or air-tight containers as sporocarps quickly degrade. Do not mix collections when collecting and storing. Regularly clean your collecting materials (stray spores can hinder identification).

At the location where the potential target species was found, mark the site. Marking sites with labeled metal or hard plastic tags is preferred. Another more ephemeral method of marking a site is to flag 2-3 nearby trees and hang 3 flags above site. Write on one of the flags: collection ID, suspected species, date, and surveyor initials. Keep in mind that flagging has a tendency to break off or be pulled off by animals so write near the knot or top of the flag. Often surveyors initially mark a site with flagging and then return to the site to establish a permanent site marker once a target voucher specimen has been verified by an expert.

The fresh characteristics of specimens such as spore color, exterior traits, and bruising are critical for correct identification. In the office, record characteristics of fresh specimens on the appropriate ***Fungal Description Form*** before drying. Prior to shipping for expert verification, specimens should be thoroughly dried, usually within 1 day of collection. Once a specimen is thoroughly dried, it can be transferred into a recloseable "zip" style plastic bag with the field tag and unique collection number written in permanent marker on the bag (Fig. 3). Fill out the ***Fungal Collection Tracking Spreadsheet*** for the entire fungal shipment being sent to the Interagency Inventory Coordinator Assistant.



Photos by Darci Rivers-Pankratz

Figure 3. Photos of bagged and labeled fungal collections with filled-out field tags.

Data collected during field surveys must be recorded and can be done on paper data forms or electronic data recorders. There are three general categories of information that are collected during BLM/FS species surveys:

- Survey data (information characterizing the survey)

- Detection data (information characterizing site occurrences)
- Supporting data (supporting information including vouchers and accompanying forms)

All of the data collected during surveys must be entered into agency databases (GeoBOB and NRIS TESP) following agency guidance issued for each application. If target species are not encountered during the field survey, list the target species and document their absence within the survey area (negative surveys). If a target species is found, mark the species location on a field map (7.5 minute USGS quad or aerial photo), clearly labelling the species found. UTM coordinates should be taken with a GPS using the North American Datum of 1983 (NAD83). Document the accuracy of how closely GPS reading matches the actual location on the ground.

For surveys and occurrences of Survey and Manage fungal species located on BLM administered lands, complete the ***GeoBOB Flora Survey*** and the ***GeoBOB Observation Forms***. For surveys and occurrences located on national forest system lands, complete the ***NRIS TES Plants Forms***. Both the BLM and Forest Service data field forms are located at:

http://www.fs.fed.us/r6/sfpnw/issssp/inventories/monitoring.shtml

Checklist of Products to be submitted with each dried specimen:

- ☐ Fungal Description Form – hard copy
- ☐ Fungal Field Tags – hard copy
- ☐ Photographs of Fresh Specimens – either on CD or hardcopy
- ☐ BLM GeoBOB Site Form or FS NRIS TESP EO Form – hard copy
  - habitat description
  - relocation directions

Checklist of Products to be submitted with each shipment:

- ☐ Fungal Collection Tracking spreadsheet – shipping checklist (printout of completed tabs) and electronic copy
- ☐ Packaged Specimens

Figure 4. Checklist of items to send to the ISSSSP Inventory Coordinator Assistant with vouchered fungal specimens.

Surveyor Skills and Safety

Personnel with skill in recognition, collection, and identification of fungal species and in the identification of vascular plant species and plant communities for the geographical

area of interest, are required. Personnel need to be experienced in proper collection of fungal voucher specimens and recording appropriate information on fungus features that are needed for later laboratory identification. They also need to be experienced in field techniques, including GPS, aerial photo and map interpretation, route finding, and ecological/floristic field observations to be able to document the location, ecological conditions and habitats surveyed for and occupied by the species.

Follow all agency safety rules and policies for field work. Mushroom hunters may get lost because they are not paying attention to their location as they wander through the forest. Documenting the survey route and paying attention to the survey area map and aerial photos will help surveyors remain aware of their location along with the use of a GPS or compass. Do not eat any mushrooms unless you are absolutely certain of the identification and their edibility.

Acknowledgements

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Glossary

(from Castellano et al., 1999 unless cited differently)

Columella: a sterile central axis within a mature sequestrate sporocarp.

Ephemeral: lasting a short time

Epigeous: growing aboveground

Equivalent-effort Surveys (USDA and USDI, 2001): pre-disturbance surveys for species whose characteristics, such as small size or irregular fruiting, prevent it from being consistently located during site-specific surveys.

Gleba: spore-bearing tissue in sequestrate fungi

Hyphae: one of the filaments of a mycelium

Hypogeous: growing belowground

Macrofungi: fungi with sporocarps large enough to be seen without a hand lens

Mycelium: a mass of hyphae

Old-growth Forest (USDA and USDI, 2001): an ecosystem distinguished by old trees and related structural attributes. Old growth encompasses the later stages of stand development that typically differ from earlier stages in a variety of characteristics which may include tree size, accumulations of large dead woody material, number of canopy layers, species, composition, and ecosystem function. More specific parameters applicable to various species are available in the USFS, Region 6, 1993 Interim Old Growth Definitions (USDA Forest Service Region 6, 1993). The Northwest Forest Plan SEIS and FEMAT describe old-growth as a forest stand usually at least 180 to 220 years old with moderate-to-high canopy closure; a multi-layered, multi-species canopy dominated by large overstory trees; high incidence of large trees, some with broken tops and other indications of old and decaying wood (decadence); numerous large snags; and heavy accumulations of wood, including large logs on the ground (USDA and USDI, 1994)

Peridium: the outer membrane of a sequestrate sporocarp.

Sequestrate: sporocarps that normally retain their spores within until they decay in place or are eaten.

Sporocarp: a general term for a spore-bearing organ

Truffle: sequestrate Ascomycota, Basidiomycota, and Zygomycota

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Appendix 1 – Survey Guidelines for Hypogeous Fungi

The key to locating fungal sporocarps is knowing in general what the target species sporocarps looks like (i.e., size, color), what type of habitat it tends to occur in, and in particular what type of substrate it grows on. Epigeous fungi (mushrooms) fruit aboveground and can be located through visual examination of suitable habitat. Species grow on different substrate such as wood, soil, litter, cones, and dung. In general more people are familiar with looking for and finding epigeous fungi.

Hypogeous fungi (truffles), on the other hand, fruit belowground. They can potentially be found almost anywhere there are trees. Truffles fruit throughout the fall, winter, spring, and summer depending on species and locality. They usually occur at the interface between the organic litter and the mineral soil, about one to six inches deep, but can emerge to the surface or be more than a foot deep. Evidence that small animals have been digging in an area recently is often a good indication that truffles may be about. The information provided herein is derived from a training session by Dr. Michael Castellano (pers. comm., 2007), personal experience, and from various field guides including Trappe et al. (2009), Castellano et al. (1999) and Trappe et al. (2007).

Potential truffle locations that should be sampled in old growth forests include:

- Small humps on the forest floor
- Fresh squirrel digs
- Underside of litter mats or under moss at humus/soil interface
- Depressions and edges of areas where water would naturally collect (start at water margin and work away to the point of the wet/dry soil interface; if conditions are really dry, the surveyor may even need to look around other vegetation)
- Drip-line of big trees on the north side inward to the tree bole
- Around fruiting bodies of epigeous fungi
- Holes around snow banks following snowmelt
- North sides of anything where there may be more moisture, i.e. leaner trees, etc.

Sample by raking roughly ½ meter around potential truffle locations using a four tine rake (mark ½ meter on the rake handle for accurate measurement). Rakes should be used to gently peel back the litter layer (remember to replace the litter when you are done and try to leave the area as you found it). Dig into the soil and work through the tailings (removed soil). Truffles appear like small potatoes, often beige, yellowish, or reddish brown (Fig. 5; for more photos see http://www.natruffling.org/photo.htm). Truffles may appear as small clumps with roots extending out or balls of yarn with loose strands (Castellano, pers. comm.). If you find a truffle, continue to rake in that area. If not, move to the next probable area.

While sampling, use your sense of smell and touch. Truffles are fragrant. *Arcangeliella* smells garlicky or like maple syrup. *Leucogaster* smells fruity-sweet. *Destuntzia rubra* smells like fish, and *Nivatogastrium* smells like bubble gum. DO NOT EAT any truffle sample! Use your hands to touch and feel anything that may appear like a truffle.

Some species are very small, less than the size of a dime and appear like small clumps of dirt. Press these with your fingers. If it is a truffle, it will not break apart. Many truffles are fairly solid, but some are hollow in the center so be gentle.

Other considerations:

- 1-2 weeks after a flush of large, fleshy epigeous fungi (*Boletes, Amanitas*, and late *Russulas*) is a good time to go truffling. If the weather is warm (approx. 70 degree F or warmer), wait 1 week; if cooler (approx. 60 degree F or cooler), wait 2 weeks.
- Mycelia build up, primordia forms and then fruit when conditions are right. Rotting mushrooms may indicate the end of the fruiting period. Watch weather patterns.
- Rake across mycelial fans when they are located.
- Rake small humps as you walk - only takes a few seconds.
- The more sites sampled, the higher likelihood of finding hypogeous fungi.

When cutting open a hypogeous specimen, try to cut it from the base to apex (Fig. 5). This helps to show diagnostic characteristics such a sterile base or columella that may easily be missed if cut in the wrong plane. Cutting fresh specimens is important because it will show features such as lactation, bruising of the gleba, or enhancement of any odor present that could otherwise go undocumented. A photo of both the peridium and gleba is ideal such as a cut specimen with one half facing 'gleba-up' and the other half 'gleba-down'.

Figure 5. Truffles can be quite small, obscure, and difficult to differentiate from dirt clods except by feel and brushing off the dirt. Cut open truffles from the base to the apex and photograph both the peridium and gleba.