

Denver Botanic Gardens
Sam Mitchel Herbarium of Fungi (DBG)

Field Collecting Protocol for Macrofungi



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i. Introduction

The Sam Mitchel Herbarium of Fungi documents the diversity of “macrofungi” in the Southern Rocky Mountain region. For the use of this collecting protocol, we use the definition of Mueller et al. (2007, *Biodiversity Conservation* 16:37-48) to define macrofungi as, “...distinguished by having spore-bearing structures visible to the naked eye (mushrooms, brackets, puffballs, false-truffles, cup fungi, etc.)” Macrofungal surveys and collections represent baseline data critical to understanding species diversity and distributions. To make macrofungal collections valuable for current and future research, both the physical specimen and all associated data must be of the highest quality.

If we use flowering plants as an analogy, the macrofungal spore bearing structure (AKA basidiocarp or “mushroom”) is analogous to the fruit of a plant. Consequently, we tend to refer to the mushroom as the “fruit” of the fungus. The ‘business end’ of the fungus (ie. the leaves, stems, and roots) are in the form of **mycelium** which form underground or within the substrate the fungus is deriving nutrition from (Figure 1).

Please note that this protocol focuses on the field collection process. In Appendix see **Resources 1-2** for information about processing museum-quality specimens for fungal herbaria.

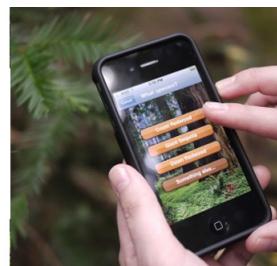
The directions outlined in this protocol are designed to research quality mushroom collections. This includes...



Make a quality collection.



Take and record necessary metadata in the field.



Properly record macroscopic notes and process specimens.

The six steps necessary to collect museum-quality macrofungal specimens:

- 1) Planning and scope of the collecting trip.
- 2) Preparing for the field.
- 3) Describing the survey location so that its physical location and habitat characteristics can be well understood by others.
- 4) Collecting a macrofungal specimen with all the necessary physical structures, associated descriptors, and pictures/tissue collections.
- 5) Take notes in the data and features described in steps 3 and 4 above.
- 6) Drying to achieve a high quality and lasting specimen.

Of these six steps, 1-4 and 6 are outlined in detail in this protocol. Step 5: Taking notes of fungal features, requires more detail than provided here. See **Resources 2 and 3** for further information on this topic.

1. Planning and Scope of the Collecting Trip

Field collecting of macrofungi is not necessarily restricted to specific research goals or questions. The purpose of collecting is often limited to surveying the fungi fruiting at a given place and time. Any desire to frame collecting around a specific question requires evaluation of environmental conditions in effort to take advantage of collecting opportunities when they present themselves. The availability of macrofungal fruits adequate for research specimens depends highly on conditions of the habitat and the availability of soil and atmospheric moisture that will trigger fruiting in fungi. In addition to these conditions, one must gain appropriate access to these habitats prior to collecting.

Some questions you should be able to answer before heading into the field:

- **Is the availability of fruiting bodies likely?** What has been the recent history of temperature and precipitation in the area? Depending on the fungi one seeks, the collector must do some background on the temporal and habitat specific growth patterns of the fungi.
- **What is the survey area of interest?** Does it have pre-defined political boundaries (e.g., a wildlife refuge) or ecological boundaries that you must define (e.g., if you are sampling a wetland, will you include the surrounding habitats as well)?

2. Preparing for the Field

A. Collecting permits and access to private land

- Obtain the necessary collecting permits from county, state or federal agencies.
- Secure permission from private landowners.
- Carry permits and landowner contact information with you at all times.
- Follow all terms and conditions of any granted permits.

B. Reconnaissance of the survey area

- Google maps satellite.
- Check recent precipitation records in the area. (<https://water.weather.gov/precip/>).
- Generate a species list for the survey area before going into the field.
- Use the online database Mycology Collections Portal (AKA MyCoPortal, <http://mycoportal.org/>) to evaluate the taxa that are available at a given location and date.



Figure 2. MyCoPortal aggregates fungal collection information from around the country. Referencing dates and locations of fungal fruitings will help to ensure successful collecting in the field.

Further preparatory reading should include **background on the plant communities and soil substrates** (or wetland characteristics) known to occur in the desired collecting sites. See **Resource 4** for additional info on Colorado plant communities and soils. **Resource 4** has guides for plant (predominantly tree and shrub) identification which is important for associating with fungal specimens.

C. Essential collection materials

Preparing supplies for the field is important. Lacking important equipment for your trip can result in a loss of time and money. Make a checklist of the following field items before each trip (Figure 2):

- **GPS units** for marking waypoints of collection locations. Be sure to include extra batteries. Also, physical maps can always be used as a backup.
- **Collecting permits** and contact information for landowners
- **Collecting basket:** Or some vessel that is easy to carry in order to contain your collecting gear (outlined below) and specimens.
- **Sampling/digging Tool:** Generally this is in the form of a collecting knife. A good knife can be used for digging up the specimen from the ground, or cutting it from whatever substrate it is growing from. Additional options include:
 - A trowl or hori hori.
 - A hard tined rake can be used to scrape for hypogeous fungi.
 - Clippers to collect branches with fungi on them, to ID plants later.
- **Wax paper bags** for collecting and storing individual specimens. In a pinch, you can substitute foil, or small paper bags. Plastic bags are not desired and only acceptable when there are no other options.
- **Tackle box** is useful for collecting and keeping separate smaller specimens.
- **Field notebook** for writing each plant collection number and associated data
- **Camera and camera book** for taking pictures and logging associated data for each specimen
- **Tubes of silica** for collecting tissue samples.
- **Hand lens and keys to species** for making identifications in the field.

D. Essential specimen processing materials

For field collecting where you do not anticipate returning home at the end of the day you will need to bring additional items in order to effectively process specimens as research collections (Figure 3). This is necessary because macrofungal specimens do not maintain their integrity for very long after being collected. As a result, we need to describe the characteristics of the fresh specimen, before going through the process of preserving it as a voucher:

- **Specimen cards** for transcribing macrofungal notes of fresh specimens.

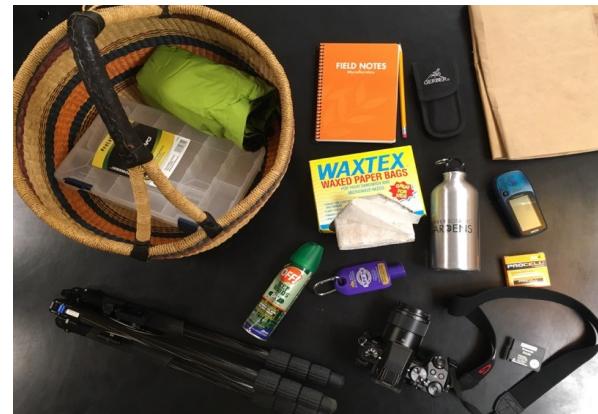


Figure 2. Gather essential materials and use a checklist to make sure you have everything for the field.

- **Specimen labels** to provide collection numbers and associated collection data with specimens.
- **Field guides** to assist in putting a name on your collection.
- **Metric ruler** to record the range of sizes of the collection in mm or cm.
- **Color guide** to describe the colors of the specimen prior to preserving.
- **Sealable containers** to protect dried specimens from moisture. Basically zip-lock bags, but tupperware containers can work in a pinch.

3. Foray Details

A. Describing the Foray

Every foray has important details that must be noted prior to collecting. Notes on the date of the foray, location, habitat type, and participants should be written down in your Field Notebook so that this information can be attributed to the collections. It is also important to assemble all participants and discuss the plan for collecting, a timeframe for the collecting, and a place to assemble at the end in order to make sure everyone is accounted for (Figure 4). It is also fun to do a show-and-tell afterwards.

B. Physical location

Another person should be able to navigate to any given collection location using the site name and associated information you provide. The information should include municipal/government/political boundaries, recognizable landmarks, and precise location information using a GPS.

Physical location descriptors

- Country
- 1st political division, i.e. state.
- 2nd political division, i.e. county.
- Nearest population center, town, village, and/or well recognized (and immutable) landmark (eg. Mountain pass, river crossing, etc.).
- Directions and distance to the collection site from that town/landmark.
- Any landscape features that would help locate the specimen.
- GPS coordinates (preferably in decimal degrees), elevation, datum and, if possible, uncertainty.



Figure 3. If you are collecting in a remote location, you should bring along equipment and materials necessary to process and preserve the specimens.



Figure 4. All foray participants should assemble before and after collecting to share information and understanding of the collecting goals.

Example: USA, Colorado, Phillips County, Frenchman Creek SWA, 5 miles W of Highway 6 and 0.5 miles S of County Road 29. Frenchman Creek SWA is approximately 71 acres and ranges from 1159 m to 1173 m in elevation. 40.59248, -102.39977. WGS84. ~1164 m elevation.

C. Habitat type

Upon arrival to a study site, take time to familiarize yourself with the fungal habitat (vegetation and substrate) in which fungi are growing.

- Make note of dominant plants (trees) that make up the habitat. Record to at least the genus level of the dominant plants that make up the habitat to better establish the likely fungal hosts.
- Make a physiographic description of the site. This includes **elevation, aspect and slope**.
- As you move around the study site, you might enter a new plant community meaning the substrates on which the fungi are growing might change.
- Be aware of substrate on which the mushroom is growing. Saprobic fungi tend to grow on woody debris while ectomycorrhizal fungi will grow directly from the ground. However, keen observation will let you know that this isn't a strict rule. Ectomycorrhizal fungi can grow through and fruit on severely decomposed logs, and wood-decay fungi can fruit from the ground directly above buried wood such as a large root mass.

Example description: Quaking fen dominated by several species of *Carex* grading into upland of shrubby cinquefoil-*Salix* spp. and Quaking aspen-Douglas-Fir-Engelmann Spruce overstory with a mixed forb and grass understory. Low-lying wetland has a thick peat layer that is flooded year-round. Upland soils are of very gravelly sandy loam with 15-30% slopes. Elevation 2750 m.

Summary of habitat type and physiographic descriptors

- Plant community type (see **Resource 4** for plant habitat categories)
- Soil texture (see **Resource 4** for soil texture categories)
- Elevation (from GPS)
- Aspect (from GPS)
- Slope (using a clinometer or visualized along a continuum from flat to a 90-degree angle)

Additionally the substrate on which it is growing should be mentioned. If it is on the ground, material's composition (organic: mulch, mossy, manure, etc; or mineral: rocky, sand, clay, etc.) should be noted. If it is growing from plant material, it should be mentioned if it is from woody substrates. If the woody debris is merely branches and twigs. Otherwise, the plant material may be straw or hay or leafy. Lastly, you should try to identify the host, and whether or not the plant substrate is dead or alive.

D. Habit

It is important to not only understand what and where the fungus fruits, but how it is fruiting. Figure 5 a-e provides examples of different types of fungal habits with the underlined terms being the terms used.



Figure 5. Examples of fruiting habits: a) a solitary, *Russula* species fruiting on mossy ground; b) scattered *Cortinarius violaceus*; c) clustered or gregarious *Hypholoma fasciculare* growing on a mossy log; d) caespitose *Mycena overholtzii* where many stems are arising from a single base; e) a below-ground (hypogeous) *Rhizopogon*.

Almost all of the examples in Figure 5 depict “hypergeous” or above-ground fruiting structures, which are comparatively easier to see than “hypogeous” fungi that produce fruiting structures below-ground (Figure 5e). These are your truffle-like fungi, and it’s important to make this distinction.

How it’s growing includes morphological growth habits. Is it sessile? These fungi typically lack a stem and produce a structure directly on the substrate they are growing on. If this structure is essentially a pileus without a stipe then you could refer to it as astipitate (Figure 6a). Otherwise you would say the fungus is stipitate if the cap is attached to a stipe/stem. Stipitate fungi can have various stipe connections to the pileus (cap), such as: lateral (off to the side), eccentric (off center) or central (Figure 6). On occasion, you will be required to distinguish between stipitate, sessile, and species that are considered astipitate BUT with a pseudostem. Many of these distinctions require a discerning eye and knowledge that comes with experience. Regardless, the notes you take will help future researchers in determining the species you collect, regardless of whatever terminology you use as long as you attempt to be as accurate as possible.



Figure 6. Examples of a sessile (astipitate) *Crepidotus* sp. (a) and other stipitate (b-d) attachments: b) lateral attachment in another *Crepidotus* sp.; c) eccentric (=off center) attachment in *Cortinarius violaceus*; d) central attachment in *Hypholoma fasciculare*; e) and an example of *Phylotopsis nidulans* that can ambiguously span the distinctions between sessile and laterally stipitate species.

Many aspects of collecting fungi in the field are similar to collecting plant specimens. Others differ for practical reasons. Because part of the day must be reserved for writing up the specimen while it is still in good condition the process of collecting tends to occur in the first part of the day. Because of the limited time in the field, the ability to take good *mental* notes is important. This or the ability to capture important environmental features in photos is also

good so that these features can be written down for every specimen when the opportunity becomes available. For this reason, be observant when collecting. If you are not confident about your ability to retain important info, then write it down in your field notebook then. Otherwise take a photo, make a mental note, and move on to make more collections.

As long as the above information is gathered appropriately, then each specimen should have the following associated information:

- Exact collection date
- Geographic location as described above
- Habitat description as described above
- Names of primary collector *and* associated collectors in the party
- Collector number (tied to collector, preferable sequential numbering for the life of the collector)
- Associated taxa
 - Include the names of plants growing nearby the collected individual; this information is in addition to the plant community description you make upon arrival to the collection location

4. Collecting a Macrofungal Specimen

A. Specimen assessment

A macrofungal specimen should represent variation you see among individuals within the population.

Specimens worthy of collecting have the following characteristics:

- Specimens are fresh and whole.
- There are >2 basidiomes >2cm in stature to collect.
- The fruit bodies represent a variety of shapes, colors and features for the species.
- The fruit bodies represent a range of maturity from a simple button mushroom to fully formed and sporulating basidiomes.

Specimens *unworthy* of collecting:

- Specimen is too ripe, buggy, decomposed, or mangled in some way.
- If there are <2 basidiomes then the quality and value must be scrutinized, otherwise the collection can be passed over
 - Some groups that often function as exceptions are: *Lepiota*, many different kinds of “polypores”, etc.
- Specimens are too young and immature (not sporulating) to yield good morphological characteristics.

This is also where you are making an assessment of habitat and habit in which the mushroom is growing. How to make these assessments are detailed in the previous sections 3C and 3D respectively.

B. Collecting the specimen – “Dig, don’t pick”

How the fruit body is collected will depend largely on the aforementioned substrate on which the fungus is growing. For most specimens it is VERY important that in the process of collecting your work to keep the specimen whole in order to protect and maintain important features that are necessary for the identification of the specimen. (Figure 7) Here are some key points to keep in mind when collecting:

1. Handle gently – This pertains to some specimens more than others. Features on the stipe, pileus, or other tissues may be damaged simply by handling them.
2. Collect substrate – It is important to know what the fungus is growing on, so collecting some of it is important. HOWEVER, only a little is necessary. You want to keep the collection clean. Unless you have another reason to do so, don’t collect dirt because it will make the collection messy.

C. Keep specimens separate

As you collect multiple specimens of a species, you will want to keep them isolated in their own container, separate from other collections. (Figure 8) This is where your paper or wax paper bags and tackle boxes come in handy (see section 2C). For smaller and fragile collections, gently place them into individual compartments in your tackle box. Use some of the surrounding vegetation, (leaves, moss, etc.) to like the compartment to keep the specimen from being jostled about. This may also provide important substrate for later identification. If the specimen you are collecting is more robust, then place them in a wax paper bag and store somewhere in your basket. The more robust material can rest at the bottom of your basket, with the more delicate collections at the top.

If you are collecting at more than one location that day, then having an additional regular sized grocery bag or other receptacle handy can be good to keep the specimens from different foray locations separate. Use a scrap paper from your note pad to write down which specimens are from which location and keep it with the foray’s specimens.



Figure 7. Dig, don’t pick. It’s important to collect the entire, and undamaged specimen to obtain all the important identification features. This can require digging out partially buried specimens.



Figure 8. Keep specimens separate using a tackle box or bags.

D. Taking pictures and recording picture data

Pictures help capture habitat and attributes of the fungus. Photos and their associated data should be taken in the field, or within 24 hours of returning from the field. See **Resource 6** guidance in using iNaturalist on your phone to document specimens.

- Upon arriving to each site, take several landscape-level pictures that document the habitat type. (Figure 9)
- Capture variation in plant community types
- Capture variation in topographic and physiographic features
- For each fungal collection, display the collection appropriately with some specimens having been dug up and others undisturbed. (Figure 10) The display should help to feature:
 - Habitat that the fungus is growing in.
 - Growth habit.
 - Colors of all the different tissues.
 - Shape of the pileus, hymenium, and stipe.
 - Features occurring on the pileus and stipe surface.



Figure 9. Taking landscape-level pictures provides additional information on habitat type that researchers can use to better understand collections.



Figure 10. Arrange individual fruit bodies in the collection to display important features. It's often best to do this in the field so habitat, habit, and metadata from the image can be captured.

5. Writing Up the Collection and Drying the Specimen

A. Initial specimen processing.

Upon returning from the foray, specimens should be written up and dried as soon as possible. If they cannot be processed right away, then they should be stored in a cool dry space until they are ready to be processed.

B. Field Notebook and Specimen Label.

For each specimen important collection information should be recorded in the **Field Notebook**. (Figure 11a) This includes:

- The unique **collector number** assigned to the specimen. A Field Notebook (Figure 11a) is critical for keeping a record of your collector numbers and what species and associated metadata that number is assigned to.
- Date.
- Associated collectors.

- Physical location data as described in chapter/section 3B.
- Genus species. The precise identification isn't necessary, but some taxonomic identifier should be assigned. This can be refined and updated at a later time.

Once all this information is assembled and added to the Field Notebook, it should then be replicated on to a specimen label (Figure 11b) this will be kept with the collection at all times.

C. Specimen Writeup.

The Sam Mitchel Herbarium of Fungi used Specimen Cards to write up specimen details. (Figure 11c and d) These details include everything written down in the Field Notebook and includes:

- Habitat and habit information discussed in sections 3C and 3D above.
- Morphological Features. This protocol will not go into detailed instruction on how to properly describe macrofungal specimens. Please refer to **Resources 1-3** for directions on this activity. Regardless, some descriptive features the following:
 - Basidiocarp structure: stipitate-piliate, coralloid, gasteroid, hypogeous, etc.
 - Ascocarp structure: apothecia, perithecia, or hypogeous.
- Data from the Field Notebook as well as habitat and habit information for each specimen must be recorded in “**TEMPLATE_DataFields_Vouchers_Fungi**” spreadsheet, as mentioned in the in the specimen accessioning workflow.

A Specimen Card *is not* required in order to write down all of the above information. Important specimen meta and morphological data can be just as easily written down on a blank piece of paper, a personalized template, or composed directly onto a computer.

D. Drying the Specimen.

After filling out the details of the specimen in the Field Notebook, Specimen Label, and Specimen Card the specimen should be prepared for drying. (Figure 12) **Large fruit bodies** (examples: Boletales species, *Agaricus* sp., *Amanita* sp., etc.) should be sectioned longitudinally to increase the surface area of the mushroom and facilitate drying. **Medium sized** basidiomes (examples: *Hebeloma* sp., *Laccaria* sp.,

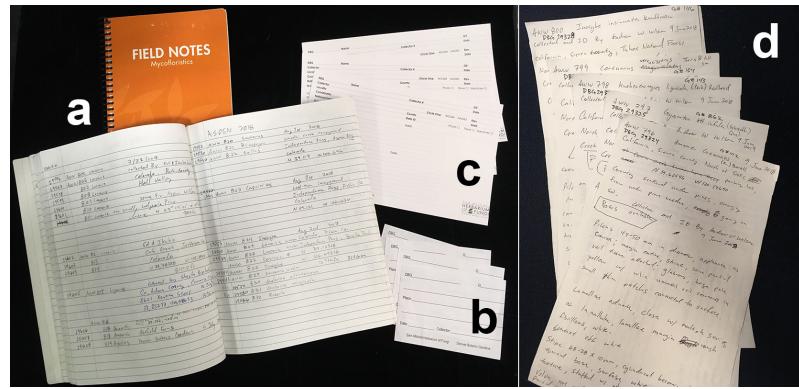


Figure 11. For processing specimens you will need: a) a Field Notebook; b) Specimen Labels; and c) Specimen Cards to properly record all the required specimen information. Formal specimen cards are not entirely necessary. One half of and 8.5"X11" piece of paper (d) to take specimen notes on will do in a pinch.



Figure 12. In drying your specimens, large collections should be sectioned to facilitate drying. Tiny specimens should be kept protected in a boat. All specimens should be kept with their specimen labels.

etc.) can be kept whole. **Small specimens** (examples: *Marasmius* sp., *Mycena* sp.) should be placed in some sort of boat (made out of paper, foil, or top/bottom of a petri dish, etc.) to keep the specimen from falling through the narrow holes that are in the drier's trays.

The Specimen Label should be kept with the specimen on the dryer (Figure 12). Specimens should be dried at a medium low heat (> 100°F/38°C and <120°F/49°C) with sufficient airflow. Small to medium sized specimens should be dried for a minimum of 5-6 hours. Large specimens should be dried for a period of >12 hours until you can be reasonably confident that all moisture has been removed from the specimen.

CAUTION!: If all the moisture has not been completely removed from the specimen mold will set in. Mold will effectively ruin the specimen. Although it still fungal, it will be very difficult to distinguish between the specimen and the mold under the microscope and DNA will be contaminated with >1 fungal species.

Upon removing from the drier, the specimen should be placed in a sealable ziplock bag or similar airtight container, along with the specimen label, until it is ready to be processed through specimen accessioning workflow. This is to prevent the specimens from hygroscopically re-absorbing moisture from the air immediately after drying. This process is not nearly as great a concern in the arid regions of Colorado, as it is in the humid climates of Midwestern USA or in Tropical regions.

Polypores and similar “persistent” fruiting bodies need not be dried immediately due to their excessive skeletal hyphae, which do not readily attract mold. These can be dried last, but will likely involve much longer drying times than most fleshy fungi.

Additional Resources

Resource 1: Relevant chapters in: *Biodiversity of Fungi: Inventory and Monitoring Methods.* (2004) Mueller, G. M., Bills, G. F., and Foster, M. S. eds.

Chapter 2 *Preparation, Preservation and use of Fungal Specimens in Herbaria*

Chapter 8 *Terrestrial and Lignicolous Macrofungi*

Resource: 2 MyCoPortal Guides for Collecting and Specimen Processing

The “Halling Guide” for collecting fungi, by Roy Halling:

http://sweetgum.nybg.org/science/projects/boletineae/wp-content/uploads/sites/6/2016/08/collecting_illustrated.pdf

Preparing and Maintaining a Collection Fieldbook by Barbara Theirs:

http://mycoportal.org/portal/documents/Preparing_and_Maintaining_a_Collection_Fieldbook.pdf

Techniques for Preparing Macrofungal Specimens as Scientific Vouchers by Barbara Theirs:

http://mycoportal.org/portal/documents/Techniques_for_Preparing_Macrofungi_Specimens_as_Scientific_Vouchers.pdf

Resource 3: Terminology for macroscopic and microscopic features of macrofungi

[Else Vellinga's 1998 Glossary of Macroscopic and Microscopic features in Macrofungi. From Flora Agaricina Neerlandica 1.](http://ElseVellinga's%201998%20Glossary%20of%20Macroscopic%20and%20Microscopic%20features%20in%20Macrofungi.%20From%20Flora%20Agaricina%20Neerlandica%201.pdf)

Largent, David L. "How to identify Mushrooms to genus I: Macroscopic Features." *Eureka, California: Mad River Press* (1977).

Johnson, D., and R. Watling. "How to identify mushrooms to genus. III. Microscopic features." *Eureka, California: Mad River Press* (1977).

Resource 4: Colorado Plants and Soils

The Colorado Natural Heritage Program has put together an accessible overview of Colorado's vegetation types: Our plant community types borrow from this publication.

<https://cpw.state.co.us/Documents/CNAP/RevegetationGuide.pdf>.

See the US National Vegetation Classification website at <http://usnvc.org/>. From here you can explore different vegetation types using their hierarchical classification and drop-down menu.

A map of the different soil orders in Colorado is here:

https://www.nrcs.usda.gov/Internet/FSE_MEDIA/stelprdb1237749.pdf

An overview and pictures of each soil order is here:

https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/class/?cid=nrcs142p2_053589

Explore soil types at different locations using the USDA's Web Soil Survey:

<https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>

Soil survey publications for various Colorado locations are here:

<https://www.nrcs.usda.gov/wps/portal/nrcs/surveylst/soils/survey/state/?stateId=CO>

Resource 5: Plant Identification

A good reference for newer botanists is:

"*Field Identification of the 50 most common plant families in temperate regions*" located at
http://www.sci.sdsu.edu/plants/plantsystematics/Identifying_50_major_plant_families.pdf.

Colorado State University Forest Service's Website: [Colorado's Major Tree Species](#)

Website: [The Native Trees of Colorado.](#)

Resource 6: Taking Pictures

[Using iNaturalist](#)