Using Chemical Reagents to Help ID Mushrooms in the Field

Western Pennsylvania Mushroom Club
Fungi, fun and friends!

By Dawn Wehman - Mushroom Education Day 2025

Who Am I?

- Joined the Club in 2021
- Membership Chairperson since Summer 2023
- Lincoff Foray Chairperson 2024, 2025
- Became a Club Identifier this time last year
- Member of this club as well as MAWDC, WV Mushroom Club, Central PA Mushroom Club and the Ohio Mushroom Society because I love to go to Forays

- Lifelong volunteeraholic
- 2 grown children, husband of 35 years
- B.S. in Elementary Education
- Day job as an Order Entry Specialist for a Commercial Industrial Fan Manufacturer in Zelienople PA

https://www.inaturalist.org/observations/180766165

What brought me here today?

FYI - I did not know this was Chicken of the Woods *Laetiporus sulphureus* in 2018
https://www.inaturalist.org/observations/89341590

I came to MED in 2022 as a participant & was handed a kit

I had no idea what to do with it but I **kept it with me**

Every time I ran into Chemical Reagents in books, dichotomous keys, online descriptions, when hiking in the field and it was brought up by someone **I TRIED THEM**

OMG they worked!

How did we first start identifying mushrooms?

Morphological Characteristics

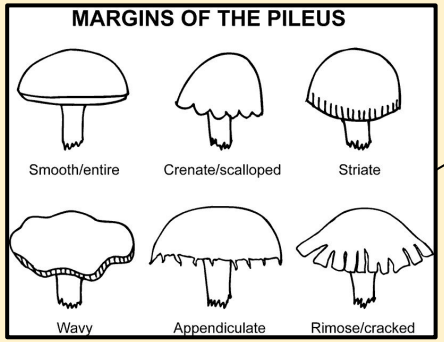
(what does it look like, smell like, taste like, etc.)

March 2025 Chaga at Walk at Gallitzin State Forest

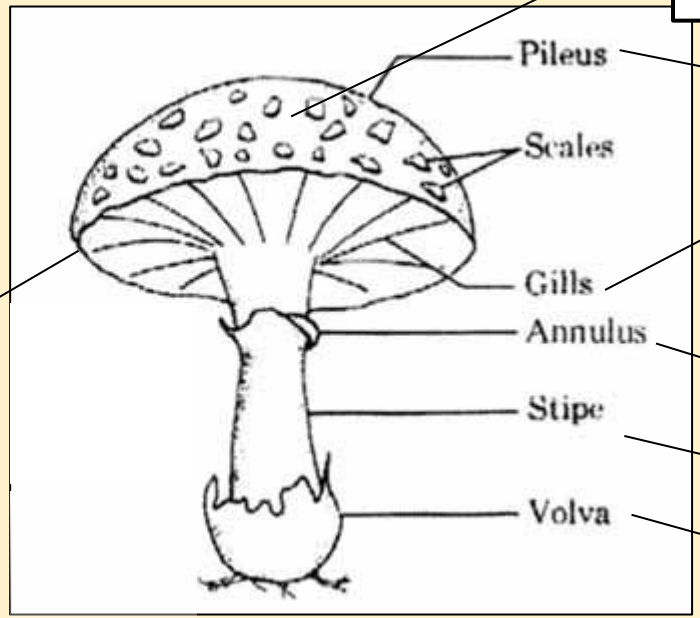
What are Morphological Characteristics

- See
- What happens when it bruises?
- Observe its Environmental Surroundings
- Smell
- Taste
- Feel

What We See



[6]



[5]

Hymenium
The fleshy spore producing layer under the Pileus

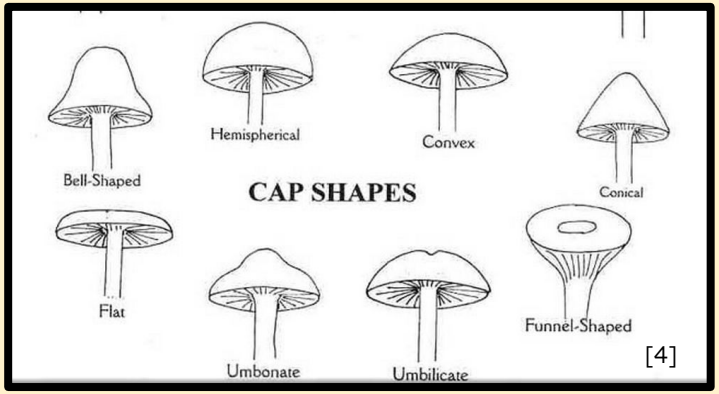
aka Cap or Cuticle

Fertile, Sporing Surface
Lamellae - Gills
Tubes or Pores
or Teeth

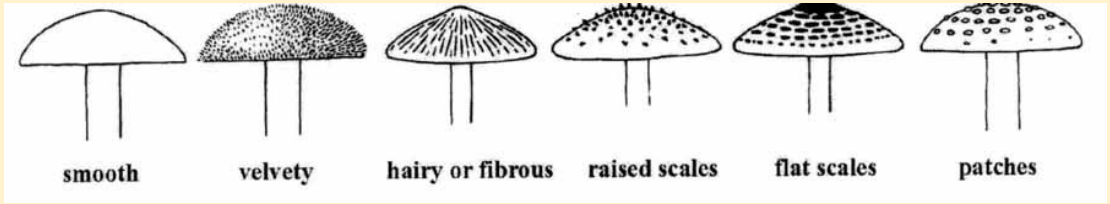
aka Ring

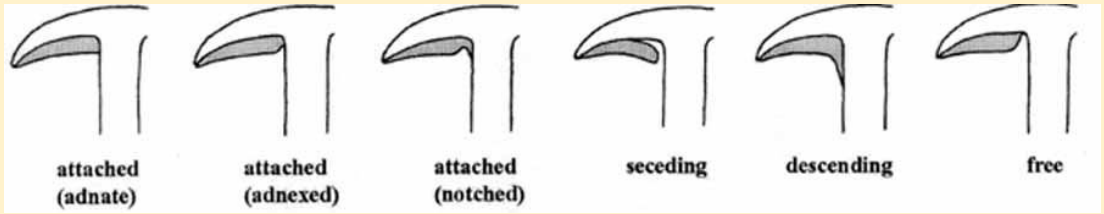
aka Stem

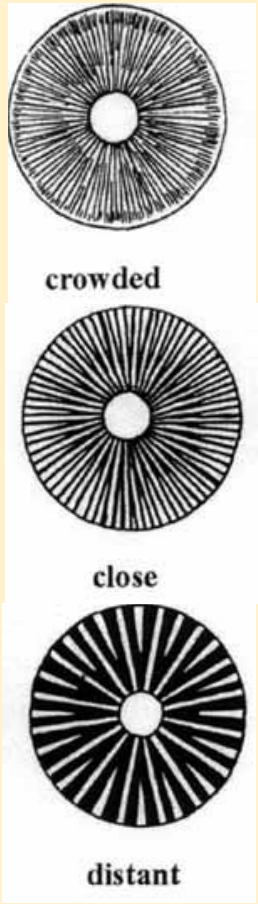
aka Basal Cup


CAP SHAPES
Bell-Shaped
Hemispherical
Convex
Conical
Flat
Umbonate
Umbilicate
Funnel-Shaped

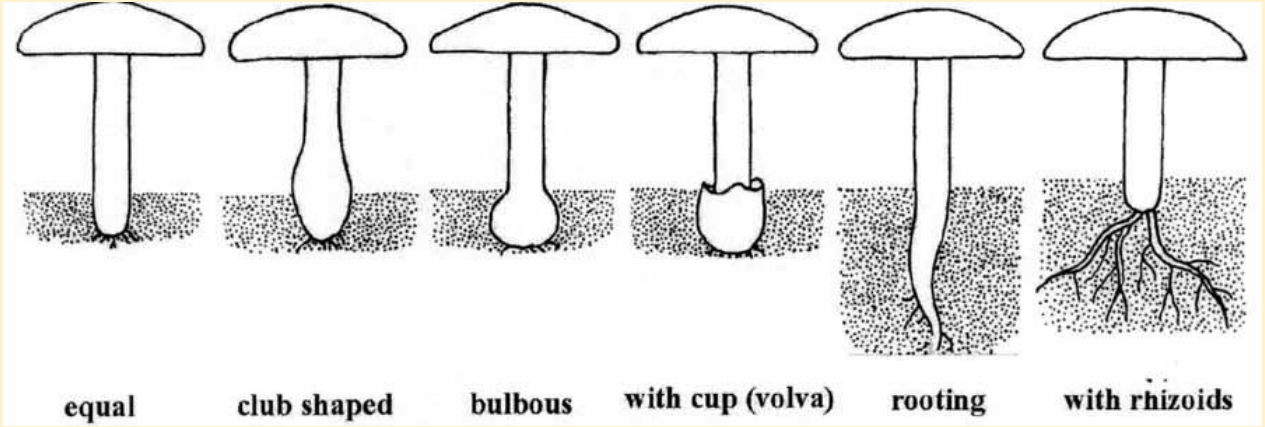
[4]

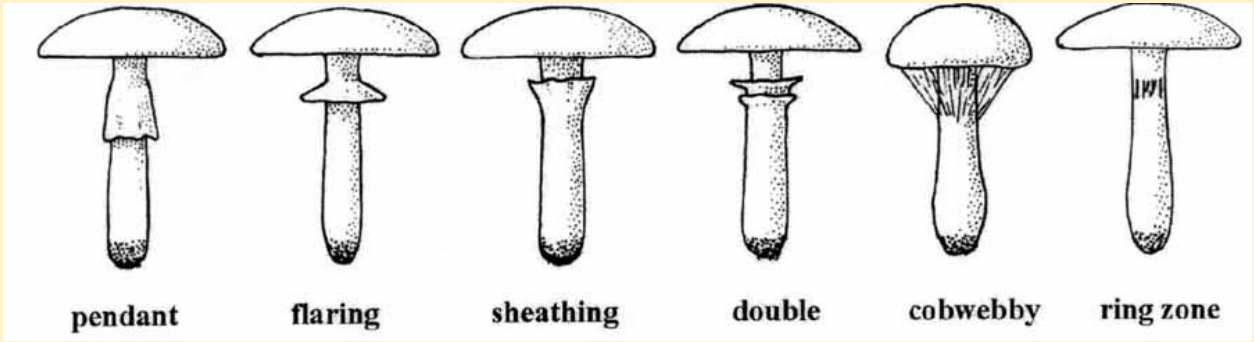

smooth
velvety
hairy or fibrous
raised scales
flat scales
patches


attached (adnate)
attached (adnexed)
attached (notched)
seceding
descending
free


crowded
close
distant

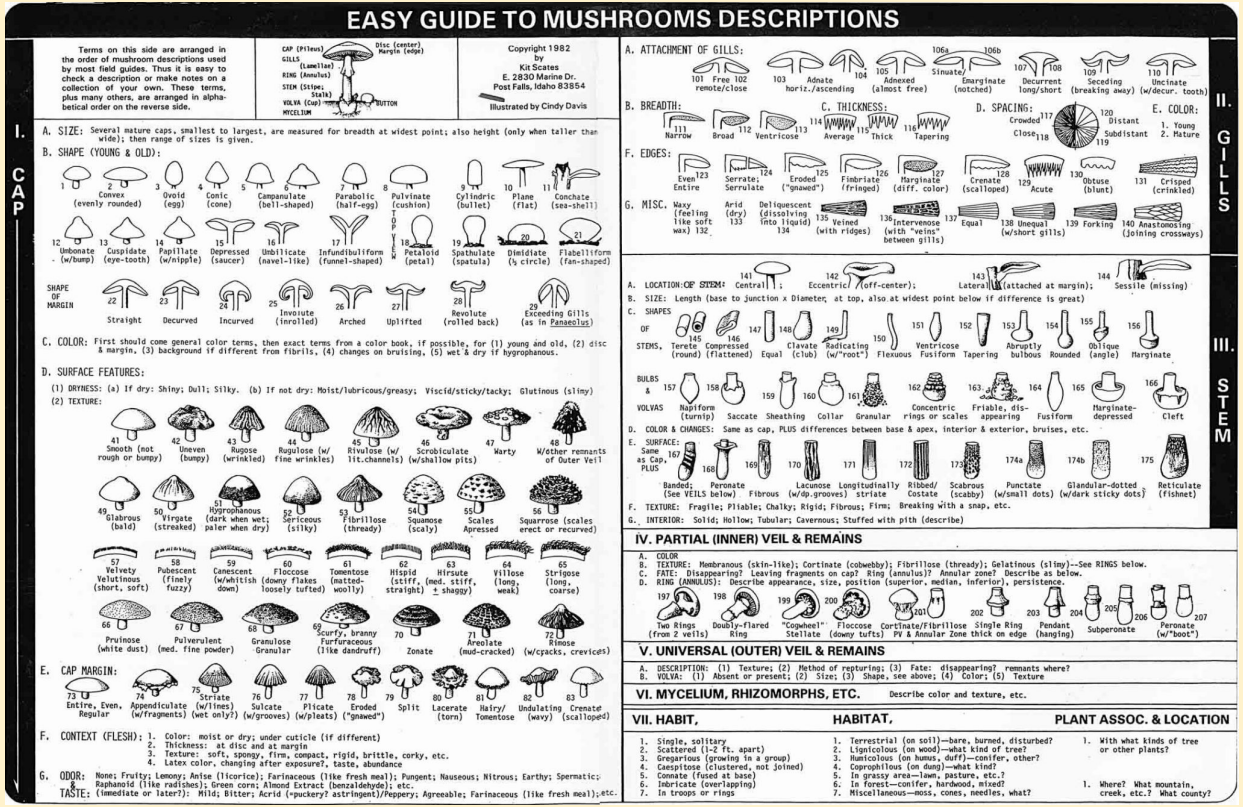
[5]


equal
club shaped
bulbous
with cup (volva)
rooting
with rhizoids


pendant
flaring
sheathing
double
cobwebby
ring zone

[5]

OMG all on one page!



[7]

What We Observe in its Environmental Surroundings

Is it growing on

- Wood?
- Dung?
- Dirt?
- Leaves?
- Another Mushroom?
- An insect?

If so can we tell what? Everything counts!

What We Smell

Just a few:

- Like fish or shrimp
- Like almonds
- Like apricots
- Like bleach
- Like cucumbers
- Like sperm
- Like anise
- Foul - dung or a corpse
- Etc.

This is HARD!

“Like a Mushroom!” is NOT a smell characteristic

What We Taste

Chew and spit test is typically very safe

- If your mushroom has a mealy or bleach like odor, do not waste your time (or your taste buds) testing its taste. It will undoubtedly taste more or less like it smells—and assessing the odor is already enough for identification purposes.
- If you have tasted *Lactarius piperatus*, *Tylopilus felleus*, or another excruciatingly acrid or bitter mushroom, be prepared to regret the experience. [11]

Just a few:

- Spicy
- Non-Distinctive
- Hot
- Chemically
- Mildly sweet

This too is HARD!

What We Feel

- Dry
- Slimy
- Powdery
- etc.

Age

How old is the specimen?

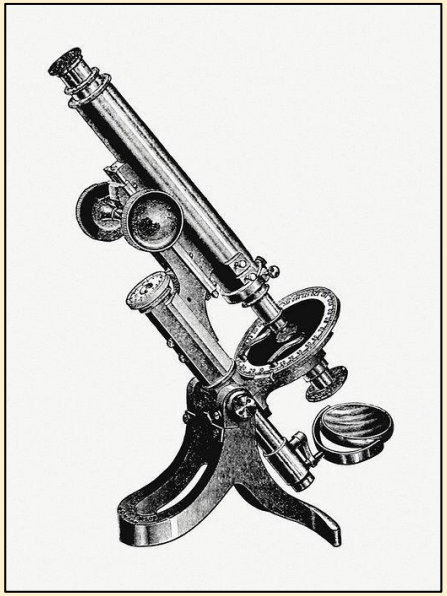
- Fresh
- Dried
- Decaying

This will be key when you get to using reagents.

Microscopy

“**Pier Andrea Saccardo** (1845-1920) was an Italian botanist and mycologist. His multi-volume *Sylloge Fungorum* was one of the first attempts to produce a comprehensive list of identified fungi, using their **spore-bearing structures** for classification.[1]”

This was only 150 years ago!



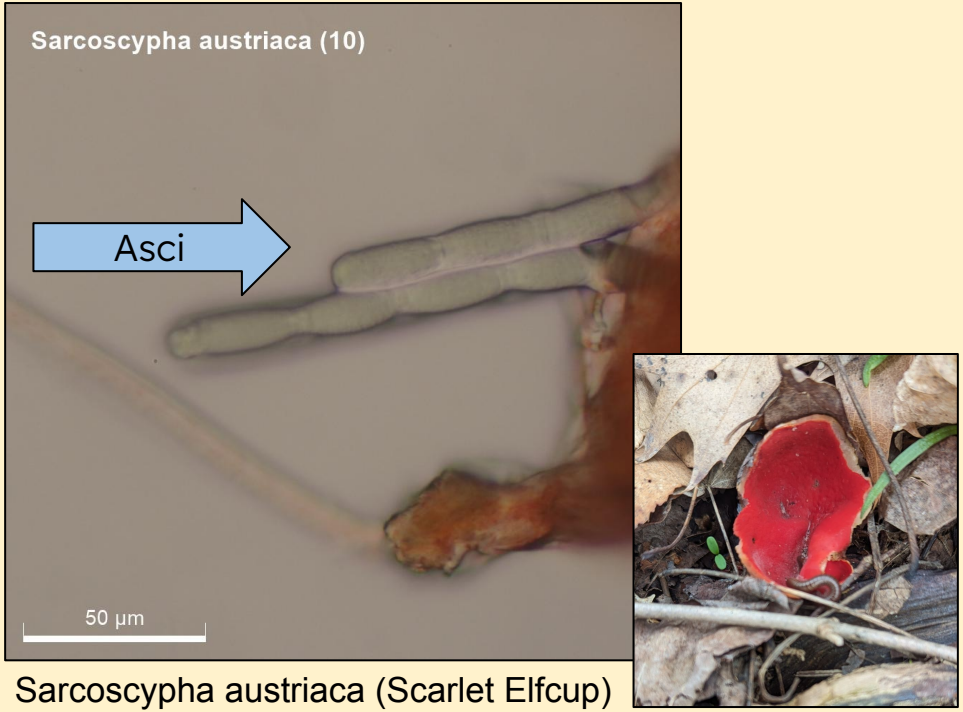
Spore-Bearing Structures can only be seen via a microscope

Ascomycetes vs Basidiomycetes

Briefly:

Macro fungi can be divided into two broad groups, called ascomycetes and basidiomycetes, depending on how their sexual spores are formed.

“In **ascomycetes** the spores are produced within microscopic structures called asci. The asci vary in shape from cylindrical to spherical. Commonly, each ascus holds 1 to hundreds of spores, with the most common being 8.” [2]



Sarcoscypha austriaca (Scarlet Elfcup)
https://www.inaturalist.org/observations/202902123

“In **basidiomycetes** the spores develop on projections that grow out of structures called basidia. Commonly, each basidium has four projections and four spores - but some species may have just one projection others up to eight. The projections from the basidia are called sterigmata.” [2]

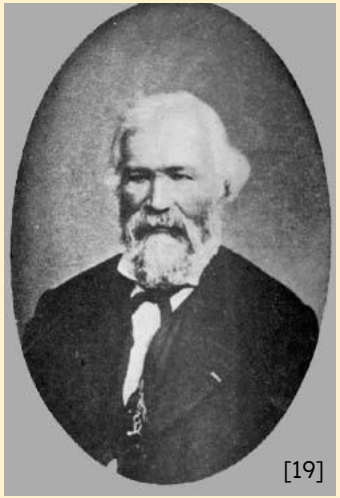
Galerina marginata (Funeral Bell)
https://www.inaturalist.org/observations/264726355

Chemical Reagents

(likely mid 1800's)

"According to (Lawrence) Leonard, the earliest reference to the usage of iodine to identify fungi was by Currey in 1858 on the ascomycete *Amylocarpus encephaloides* and by the Tulasne brothers in 1861 with lichens …"[12]


[18]


[19]

Charles and Edmond Tulasne

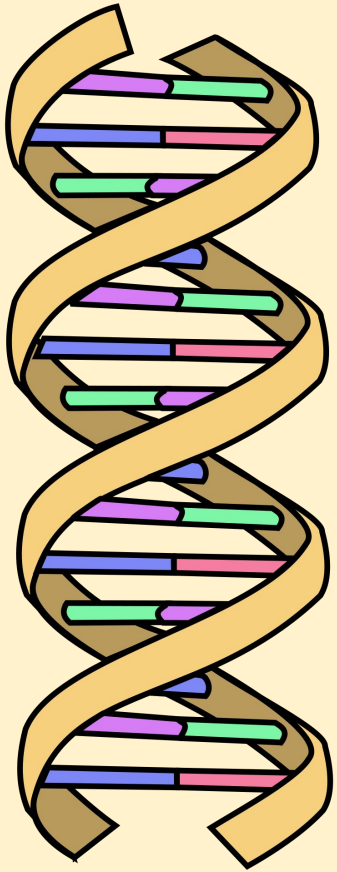
DNA

Sanger Sequencing - developed by Frederick Sanger & his colleagues in 1977. It's expensive and the machine takes lots of maintenance.

PCR, a polymerase chain reaction, invented in 1983 by American biochemist Kary Mullis at Cetus Corporation. Mullis and biochemist Michael Smith, who had developed other essential ways of manipulating DNA. [9]

PCR targets a certain gene, doubles it, copies it billions of times.
It's cheaper and easier, but doesn't work on every type of mushroom. This is typically what the club sends out to Stephen Russell at Mycota during the Continental MycoBlitz' each year.

Fun fact:
The whole genome of the mushroom is approximately 40 million base pairs long.



Why go through with all of these previous slides if we have DNA?

We amplify a small region of DNA for barcoding analysis (if PCR is used)

Some mushrooms are NOT easy to sample

Samples fail

Regarding Fungarium samples, the sample may be too old or preserved with something that degraded the DNA

DNA is only one part of the “story”

For the everyday person, it can take up to a year to get your results back

Science is evolving - who knows what we’ll use next!

Important notes at this stage

“Many mycophiles keep a few simple chemicals close at hand that, when applied to collected mushrooms, can elicit a color change and assist identification.” [12]

- Field guides don’t always agree
- Field guides get out of date
- Lots of individual people observe the same exact thing in different ways
- Guides don’t always approach identification in the same way
- Dichotomous keys differ from one another due to authors. Another author can approach the key differently from one version of a book to another based on things they picked up from listening to their audience and new things they have learned themselves

Chemical reagents are only a small part of this deciphering process,
but can be key in differentiating between similar looking species
that might otherwise require microscopy or DNA work

A reaction alone will not make an ID (but they certainly help)

What are Chemical Reagents?

Chemicals that are used to initiate, carry out, speed up or monitor a reaction but are not themselves used up.

Chemical Reactants vs Reagents?

Term	Reactant	Reagent
Definition	Chemicals that are consumed in a reaction in order to form products	Chemicals that are used to initiate, carry out, speed up or monitor a reaction but are not themselves used up.
Example	$2H_2 + O_2 = 2H_2O$	Initiators Solvents Catalysts **Indicators**

[3]

Safety First!

Most of the chemicals are safe if used as described.

Obviously you should not drink the liquids or put them in your eyes or eat anything that you have put the chemical onto.

If you get them in your eyes accidentally, it is heavily advised to seek prompt medical attention.

Gloves aren't a bad idea, but not necessary because the chemicals have been diluted. If you get them on your skin, just rinse it off with water as promptly as permitted.

We also recommend protecting the surface where you work and wearing old clothes.

Take care to inspect the bottles in your kits for damage. The club may have some reserves or you can purchase bottles on Amazon.

Safety Data Sheets have been provided in your kits.

What we could possibly use

The Club put together a kit in the past that included some items that are not included in today's kit:

Sulfo-Vanillin
Sulfuric acid 1N
Hydrochloric acid
Lugol's Iodine

What are the most popular, useful and safe

Ammonia (NH3)
Ferrous sulphate (FeSO4) 10%
Potassium hydroxide (KOH) 10%

Why we don't use some of the chemicals we first mentioned?

Depending on the concentration of the hydrochloric acid you are working with, significant injury can occur as it comes into contact with skin, eyes, ingestion or inhalation of acidic vapors.

Exposure to sulfuric acid can occur as skin/bodily contact, ingestion, or inhalation of vapors. Each type of exposure can pose serious hazards to your health and should be managed immediately and appropriately by a medical professional to minimize damage and health risks.

Lugol's Iodine 5% - is really worth the long read but is about $30 for a 1 oz. bottle. The 2% is available on Amazon.com but is more suitable for medical uses.

Melzer's reagent - we'll talk about in a slide or two

Some additional test include:

The Meixner test (also known as the Wieland test)
This test uses concentrated **hydrochloric acid** and **newspaper** to test for the deadly amatoxins found in some species of Amanita, Lepiota, and Galerina. The test yields false positives for some compounds, such as psilocin. [10]

Let's see if this embedded video works
https://www.facebook.com/watch/?v=720245335123987

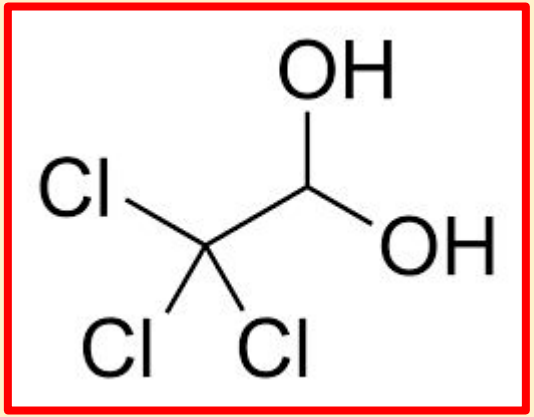
Ehrlich's reagent
A very common Ehrlich test is used to identify psychoactive compounds such as tryptamines, lysergamides (LSD), and psilocybin (the active substance in psychedelic mushrooms). This test will also give a positive reaction to opium because of the tryptophan. "Psilocybes are very difficult to discern from other little brown mushroom look alikes, including Deconicas, Stropharias, Conocybes, Panaeolus, Galerinas and others." [12] This reagent is expensive - $20 for up to 100 tests. [14]

“Far and away, Melzer’s reagent and KOH are the most important and most used.” [12]

Melzer's reagent used by mycologists is an aqueous solution of chloral hydrate, potassium iodide, and iodine. Due to the legal status of chloral hydrate, Melzer's reagent is difficult to obtain in the United States, [13] but you can order Melzer’s and other reagents from https://www.myko-service.de/

The chloral hydrate in Melzer’s reagent clears the cell contents so that the color reaction is more obvious [12] which is what makes it superior to all of the other iodine based reagents.

Melzer's reagent ... is normally performed on white spored mushrooms. If the spores are not light colored, a change will not be readily apparent. It is easiest to see the color change under a microscope, but it is possible to see it with the naked eye with a good spore print. [10]



The color change described above is either **amyloid** (means “starch like”) where the spore walls turn blue/black, or **dextrinoid** where they turn red brown. If no change happens, the correct term is **inamyloid**.

Getting Started

It is important to test for chemical reactions on *fresh* mushrooms, preferably within an hour of picking them … try to test mushrooms as soon as possible when you get home, remembering that the longer you wait, the less reliable your results may be. [11]

Good habits in the field

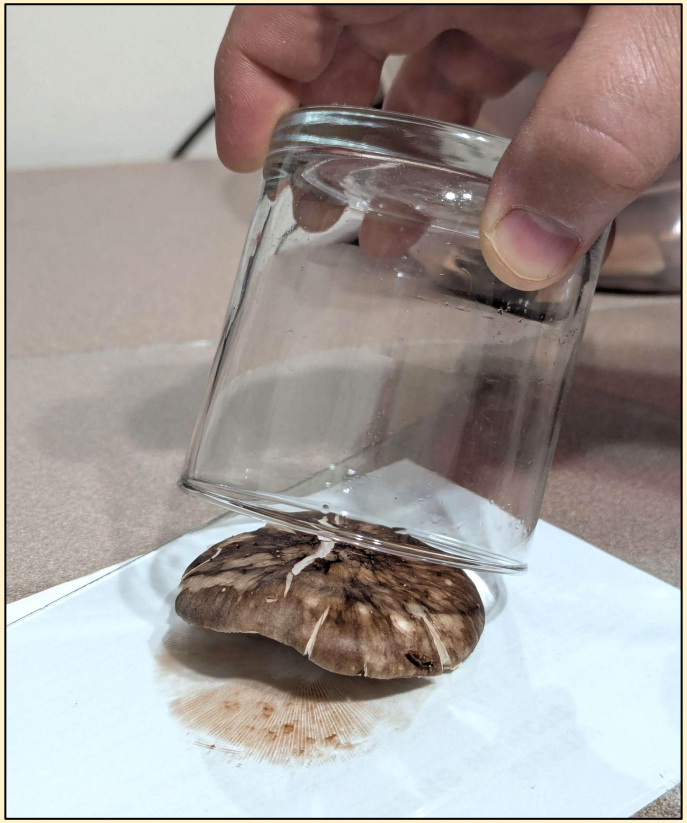
- Protect your specimens as you travel (fishing tackle box, mesh bag, NOT your pocket, lol)
- Get pictures for iNaturalist, in situ w substrate or environment, top, gills, vulva, stipe and any distinguishing features.

Once you get in the door:

- Clean glass ready w cups ready to do a spore print (typically takes an hour to several hours)
- Have your field guides nearby
- What color are the spores - really helps when looking at dichotomous keys
- Look hard at your specimen for those distinguishing features and WRITE IT DOWN
  (notes field in iNaturalist)

Advanced users (those w/ a microscope):

- Have your slides and chemicals ready and don't over harvest (just a little experience talking here!)



NOTE:
There are separate chemical concentrations that are used for under the microscope (KOH, iodine based solutions like Melzer's, Lubol's, Hoyer's medium, ammonium hydroxide and several other that are key that we won't discuss today) - The concentrations are NOT what is in your field kit today.

My home set up

Ammonia (NH_3)

Standard household cleaning ammonia, undiluted

Don’t get the orange citrus scented

Do not inhale.
Can cause skin irritation
Can cause serious issues if gets in the eyes

Ferrous Sulfate (FeSO4)

FeSO4: a blue-green powder, is mixed with water before application to the surface of a mushroom. The liquid will turn orange within several days as ferrous sulfate begins oxidizing to liquid rust. The bottles provided in our kit are premixed for you and in plastic squeezable liquid “eye” dropper bottles with a tamper resistant cap.

If the contents crystallize, this is normal

Your bottles are marked with an expiration date of approximately 18 months from when they were mixed.

Green vitriol test (the common name for ferrous sulfate) on boletes and russulas [8]

Potassium Hydroxide (KOH)

KOH: The bottles provided in our kit are premixed for you and in plastic squeezable liquid eyedropper bottles with a tamper resistant cap. KOH slowly eats glass.

At full concentration KOH causes severe chemical burns. Gloves are recommended but not necessary due to the eyedropper tip & dilution.

Rinse off the skin, surfaces and clothing with soapy water.

Which families of mushrooms have regular reactions

- Russulas
- Polypores
- Amanitas
- Boletes
- Gilled Mushrooms (Cortinarius, Lactarius & Agaricus)

MushroomExpert.com

I use this site almost daily during mushroom season and Michael Kuo is my first go to for microscope chemicals as well as chemical reagents to try **BECAUSE THIS SITE IS RIGHT AT MY FINGERTIPS** and doesn't have the inherent limitations of a textbook

Russulas

Russula grata aka laurocerasi
(almond or maraschino cherries scented russula)

Photo by Barbora Batokova
https://www.inaturalist.org/observations/242995546

KOH on cap surface pinkish to orangish.
FeSO4 on stem surface negative to pinkish or orangish [20]

Photo by Robert Bosiljevac
https://www.inaturalist.org/observations/228684382

FeSO4
For Russulas, place a drop on the stem surface. [11] Three results are expected with the iron salts tests: no change indicates a negative reaction; a color change to olive, green or blackish green; or a color change to reddish-pink. [10]

KOH
“Red, yellow, green, purple or black color reactions can be expected. Some Russula and Lactarius species may give a strong olive green color.” [12]

Russula aeruginea
(Green brittlegill)
Photo by Fluff Berger
https://www.inaturalist.org/observations/93658633

Mushroom Expert has been my favorite “go to” for what chemical to start with as well as Microscopic Features.
Russulas are particularly difficult to identify, but according to Michael Kuo,
“KOH on the cap surface turns orange. Iron salts the on flesh and stem surface slowly turn pink.” [22]

Russula xerampelina
(Shellfish-scented Russula)
Photo by Fluff Berger
https://www.inaturalist.org/observations/56659632

“Stem surface and flesh turn green to gray-green or olive with iron salts.” [23]

If you have a particular interest in Russulas, check out these additional links:

Field Mycology Volume 5(3), July 2004 on the Use of Guaiac in the Identification of Russula by Mario Tortelli

Sorting Out Russula by Tavis Lynch recorded Spring 2025 by the North American Mycological Association

Polypores

Photos by Jared White, KOH provided by Barbora Batokova

https://www.inaturalist.org/observations/176894629

Ammonia or KOH on the cap of

Hapalopilus rutilans aka nidulans

will result in a vibrant purple (or vibrant red in other parts of the world) [15]

Little Latin Lesson:
As of April 2021, both MycoBank and Species Fungorum treat H. nidulans as a synonym of H. rutilans. This species of polypore is commonly known as the "purple dye polypore", "cinnamon bracket", or the "tender nesting polypore". Rutilans is Latin for "orange-red",whereas nidulans means "nesting".[17]

KOH can be applied to the to the flesh and the cap surface of polypore mushrooms. Black reactions among polypores are crucial separators on dichotomous keys. [11]

Picipes badius
(Bay Polypore)

https://www.inaturalist.org/observations/62851797
aka Polyporus badius,
aka Polyporus picipes,
aka Royoporus badius

KOH negative (inamyloid reaction) on cap surface, or grayish on darker, redder caps; negative on flesh

Cerioporus varius
(Elegant Polypore)

https://www.inaturalist.org/observations/121173276
aka Polyporus varius,
aka Polyporus elegans,
aka Polyporus leptocephalus

KOH brownish orange on flesh and cap surface.

Amanita Family

KOH on the cap of
Amanita bisporigera
(Eastern Destroying Angel)
will result in a bright yellow

BTW, almost all white Amanitas
will give you a yellow reaction

KOH on the cap of
Section Vaginatae
Negative to bright yellow [25]

Amanita fulva (Tawny Grisette)
Photo by Dana Driscoll
https://www.inaturalist.org/observations/245157112

KOH negative (inamaloyd) on the cap [26]

Amanita jacksonii
(Jackson's Slender Caesar)

https://www.inaturalist.org/observations/173135310

KOH on cap surface causes "erasing" of red pigment, causing it to turn yellow [27]

Boletes

Ammonia is primarily used in the identification of boletes. Place a drop of ammonia on a fresh bolete's cap, stem, sliced flesh, and pore surface. Note any color changes that take place. Some species, like Xerocomus illudens, will demonstrate a quick flash of one color (for example, blue-green), then settle into another, more permanent color change (for example, grayish). Other species, like Boletus separans, may demonstrate a single color change. [11]

Xerocomus illudens
Photo by Robert Bosiljevac
https://www.inaturalist.org/observations/127908802

Boletus separans
(Lilac Bolete)
https://www.inaturalist.org/observations/180751169

FeSO4

- For boletes, place a drop of Iron Sulfate on the cap, stem, sliced flesh, and pore surface [11]
- Three results are expected with the iron salts tests: no change indicates a negative reaction; a color change to olive, green or blackish green; or a color change to reddish-pink. [10]

KOH

- For boletes, place a drop on the cap, stem, sliced flesh, and pore surface.
- Various colors are produced with boletes [11]

Rusty Bolete (Xerocomus ferrugineus) vs. Suede Bolete (Xerocomus subtomentosus)

These two macroscopically similar species can easily be distinguished by adding a drop of ammonia to their cap surfaces. X. ferrugineus “displays a vivid blue or blue green reaction that turns reddish brown, whereas the cap surface of” X. subtomentosus “immediately stains reddish brown” [16]

Photo by John Plischke III
https://www.inaturalist.org/observations/106378360

Photo by Dirk Cappo
https://www.inaturalist.org/observations/124185101

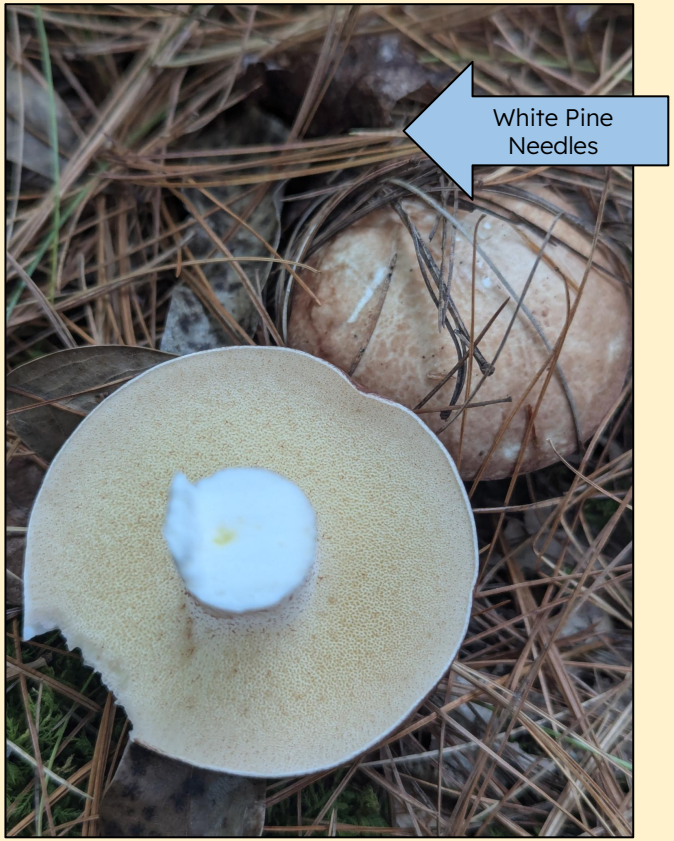
Suillus weaverae (Butterball)

Because we want to “play” with these chemicals and this mushroom flushes in quantity, Suillus weaverae is an excellent mushroom to try FeSO4 on.

Be sure to safely dispose of your “test” mushroom and don’t consume it.

Bucket List:

The cap surface turns bluish gray to olive with Ferrous Sulfate [16]



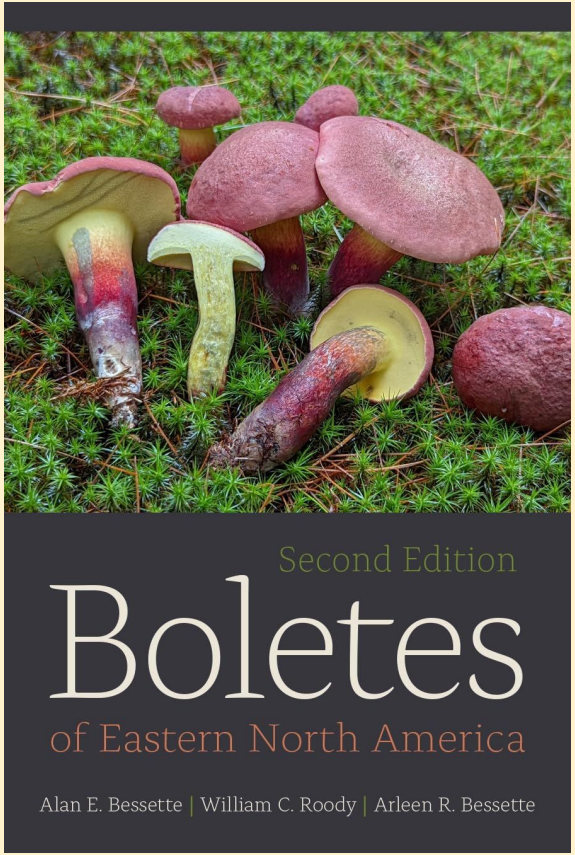
https://www.inaturalist.org/observations/246988365

Boletes of Eastern North America, 2nd Edition

Appendix A has a long passage that details temperature of the reagent, temperature of the mushroom, reagent quantity and application (they recommend using a cotton tipped swab) and reaction times that I won't just site here. If you are interested in Bolete identification this book is fantastic and from some of the best experts in the field.

A final statement that the authors made as part of this appendix worth mentioning is the inconsistencies in the literature and variable results. [16]

This book references chemical reagents on many many mushrooms!

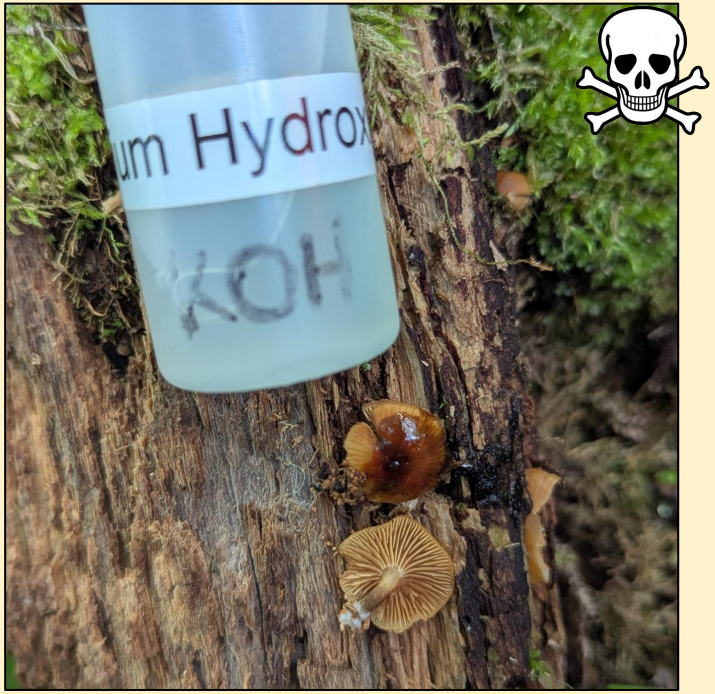


Gilled Mushrooms

Potentially Pholiota granulosa

https://www.inaturalist.org/observations/278048195

If you look at this observation online, you will note all of the other details provided to debate if this could be granulosa. This was a small very young specimen and has also been retained for eventual DNA testing.



Galerina marginata

https://www.inaturalist.org/observations/279347352

- KOH can be placed a drop at a time on on the cap surface
- Yellow is sometimes found in species of Agaricus
- Magenta or Olive is sometimes found in Lactarius
- Deep Red or Black reactions can help sort out many gilled mushrooms [11]

Cortinarius distans

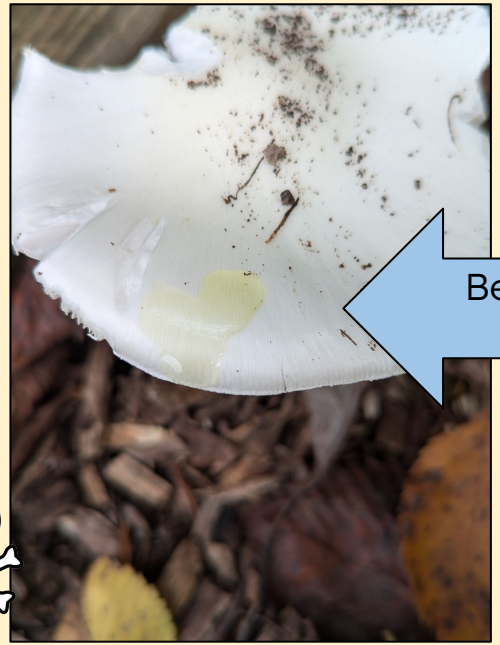
KOH on cap surface slowly turns dark reddish brown. [28]

https://www.inaturalist.org/observations/228547795

Documenting what you've observed

If you try chemical reagents but your key doesn't provide test results, check MushroomExpert.com

How to document reagent reactions or non-reactions within iNaturalist

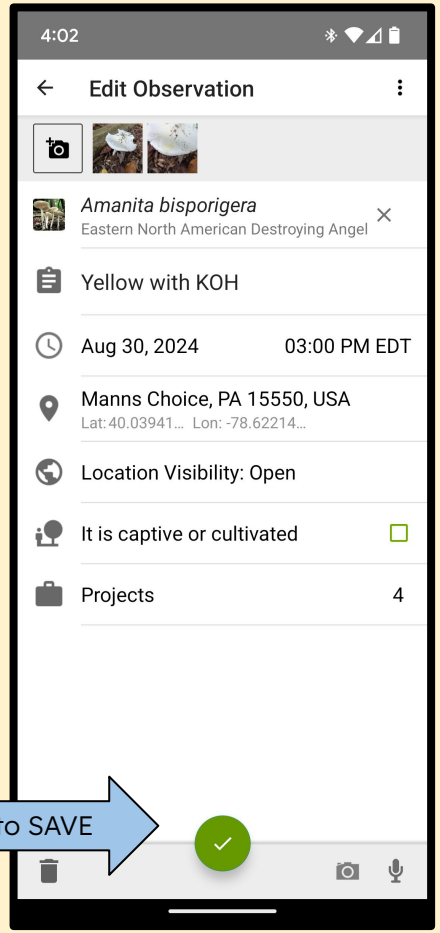


Be ready. Get a picture!

Eastern North American Destroying Angel (Amanita bisporigera)
https://www.inaturalist.org/observations/238865221/

iNaturalist mobile for Android or iPhone

Make a comment in the “notes” field



Don’t forget to SAVE

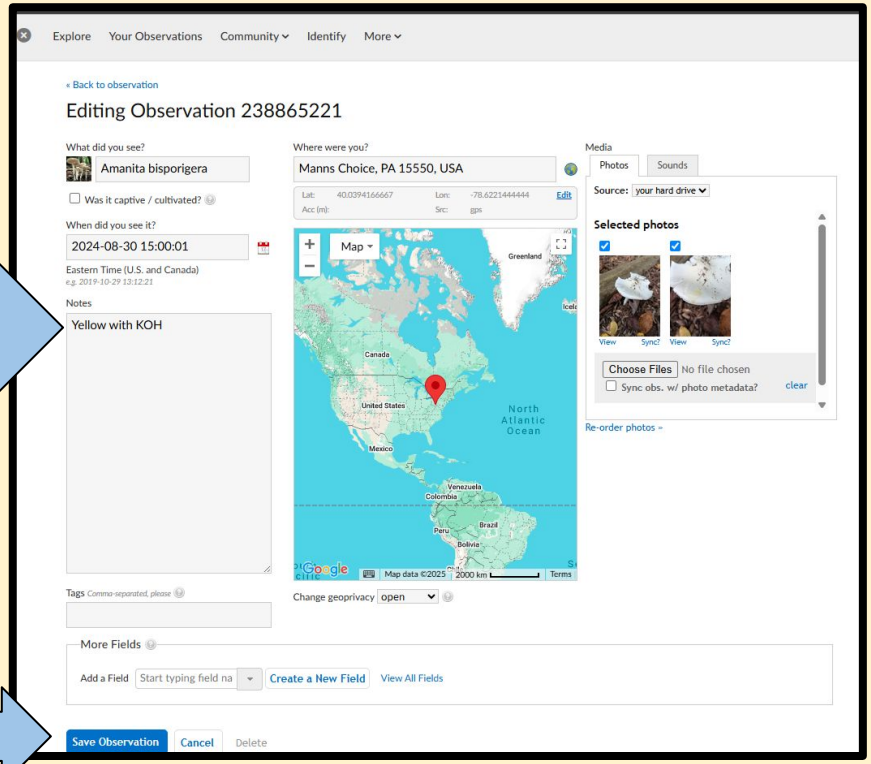
EASY Peasy

iNaturalist for desktop

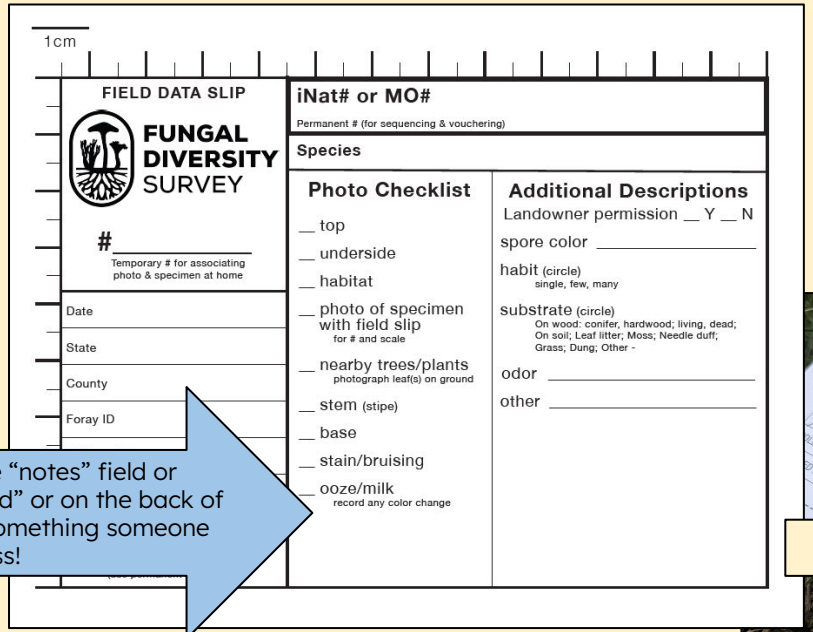
(highly recommended for advanced users!)

Make a comment in the “notes” field

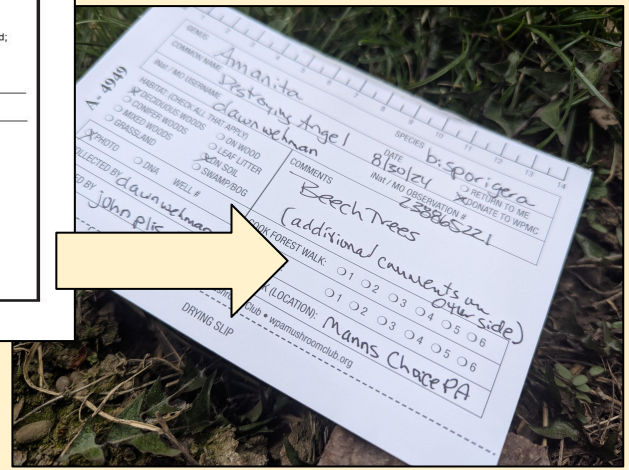
Don’t forget to SAVE



How to document reagent reactions or non-reactions on common foray or walk ID slips



Make a comment in the “notes” field or “additional descriptions field” or on the back of the slip noting there is a something someone might miss!

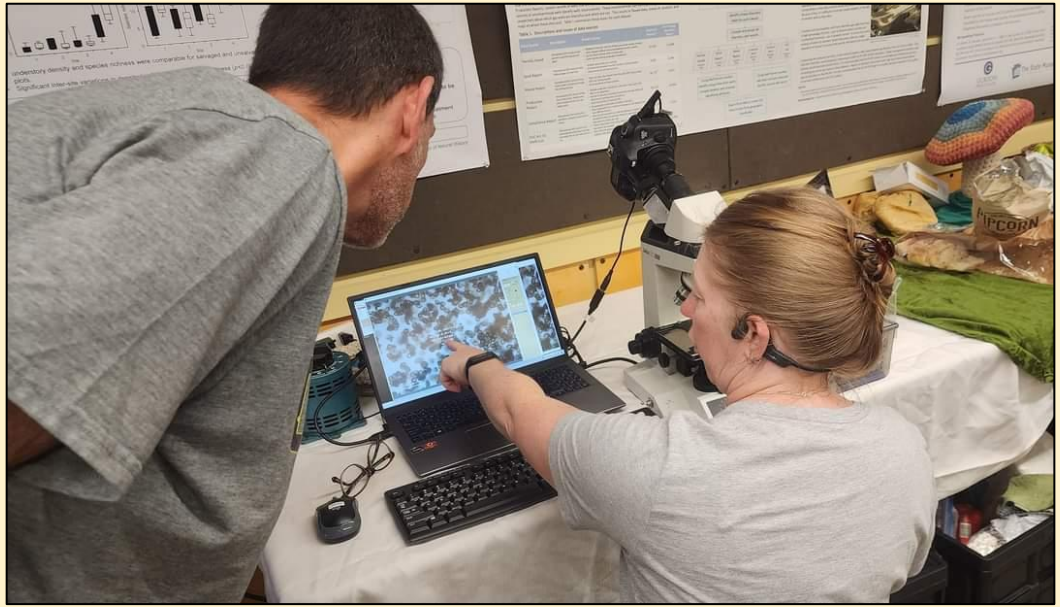


What's in your kit?

Where's the QR Code take me to?

The QR Code will take you to our website where you will find the flyer and safety data sheets that are also in your kits, just in case you lose them and/or need them in an emergency.

Questions?



Contact me at
dawnwehman@gmail.com or
membership@wpamushroomclub.org

Thank You

The Western Pennsylvania Mushroom Club
is a 501(c)(3) non-profit organization.

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[24] https://www.mushroomexpert.com/polyporus_varius.html
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A huge

goes out to all of my “Mushroom Mentors”