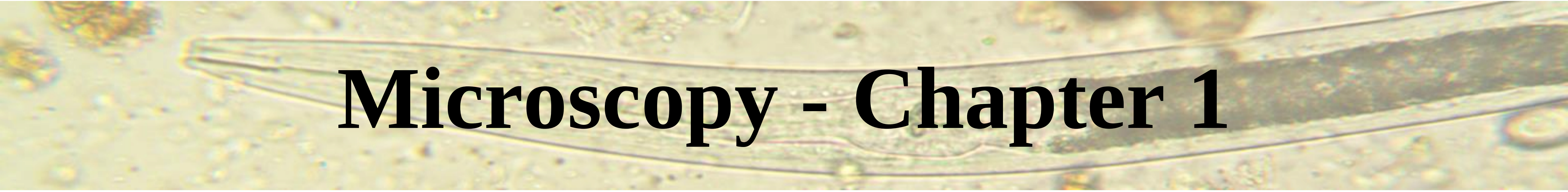


Microscopy - Chapter 1

Lecture 7

Fungal Morphology (Part 3)

How to compare fungi and bacteria

Counting the number of strands of fungi makes no sense, as each strand may be very different in length and in diameter.

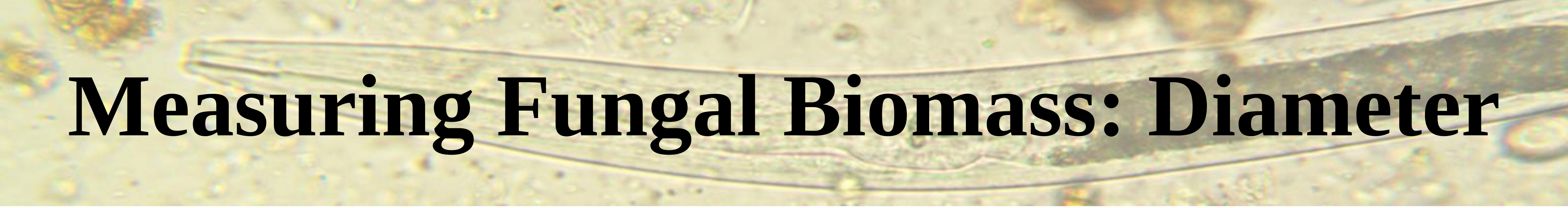
Comparing numbers of bacteria with the number of hyphal strands doesn’t make sense. The largest organisms on this planet are fungi, whose bodies can be 20 miles across and 25 or more feet deep into the soil, while in individual bacterium is a couple micrometers long and only 1 or 2 micrometers wide.

Thus, we need to compare BIOMASS of bacteria and fungi, not the number of individuals.

Measuring Fungal Biomass: Units

All measurements of fungal hyphae are done using 400X total magnification. The diameter of a field of view at 400X magnification is usually 450 µm although 550 diameter objectives can be bought. Put the correct diameter into your calculations.

Split the diameter into 10 equal parts. Use the 0.1 units to determine the length of each fungal strand in the field of view. A hypha that is half the width of a field of view would be recorded as 0.5 units long in the spreadsheet, for example.



Measuring Fungal Biomass: Diameter

The second measurement need for any fungal strand is the diameter of that strand.

Find the smallest bacterium and take that diameter as 1 µm. Determine how many of those 1 µm bacteria would fit in the width of the strand. Record diameter for each strand in the field of view.

Measuring Fungal Biomass: Example

The next two pictures show fungal strands. Estimate the length of the hyphae in these two pictures, as well as the diameter using what we just discussed.

After making your estimates, compare them with mine below and see how well we agree. If your estimates are very different from mine, you might want to re-determine your 0.1 unit size.

Example measurement #1

Example measurement #2

Practice Measuring Fungal Length

My estimates for the length and width of of the fungal strands in the next two pictures (example #1 and #2) are given here.

Example #1: One unit long (1.0 units; full width of the field of view), 2.5 – 3 µm diameter. Brown color, uniform distance septa. Good fungus

Example #2: There are two very different diameter strands of hyphae in this field. A long, looped strand 2.4 units long, 2.5 – 3 µm wide, and a second strand that starts center-field, but lies on top of the first strand. Length 0.5 units, width 2.0 µm. Brown, uniform septa. Probably a good fungus.

Calculate Fungal Biomass per Field

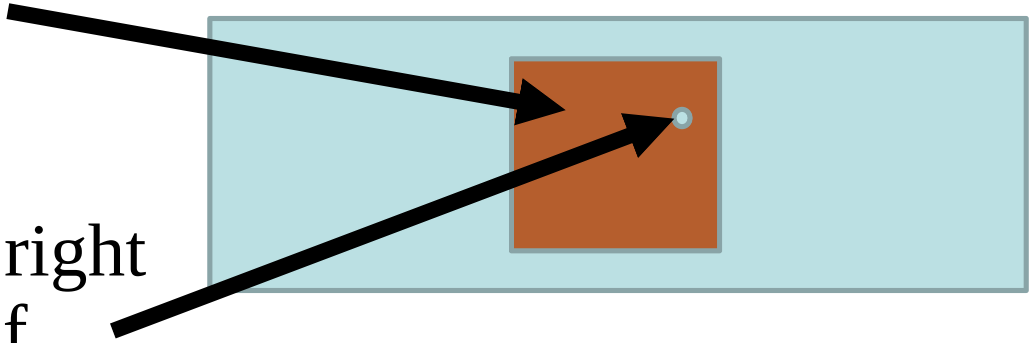
All the equations needed are in the spreadsheets, so you don't have to remember all these steps each time you want to write a report.

If you want to go through these equations and make sure you understand these conversion steps, please contact one of our mentors (info@soilfoodweb.com).

Fungal Biomass per field

But we need to now convert from per field to per gram of the sample (see previous discussion about this).

Determine the volume of sample in 1 field of view. How many fields of that volume are in 1 drop of sample (brown) under the coverslip on this slide?



The first field of view is the tiny dot in the upper right hand side of the coverslip. How many of fields of view must be looked at obtain a representative value for biomass?

Fungal Biomass per diluted sample in the test tube

The **area of a single field** is:

(Pi * Radius of the field^2)

--- or ---

(3.14 * (0.0225 cm)^2)

The **area under the coverslip** is 1.8 X 1.8 cm.

There are 2,036 fields of view in that area. Convert biomass per FoV to biomass in the one drop under the coverslip by multiplying by 2.036.

Two factors left to go: Multiply by the number of drops to reach 1 ml and then… (next slide)

Fungal Biomass per gram of the original sample

To correct for the dilution you used to observe your sample, multiply by the dilution factor.

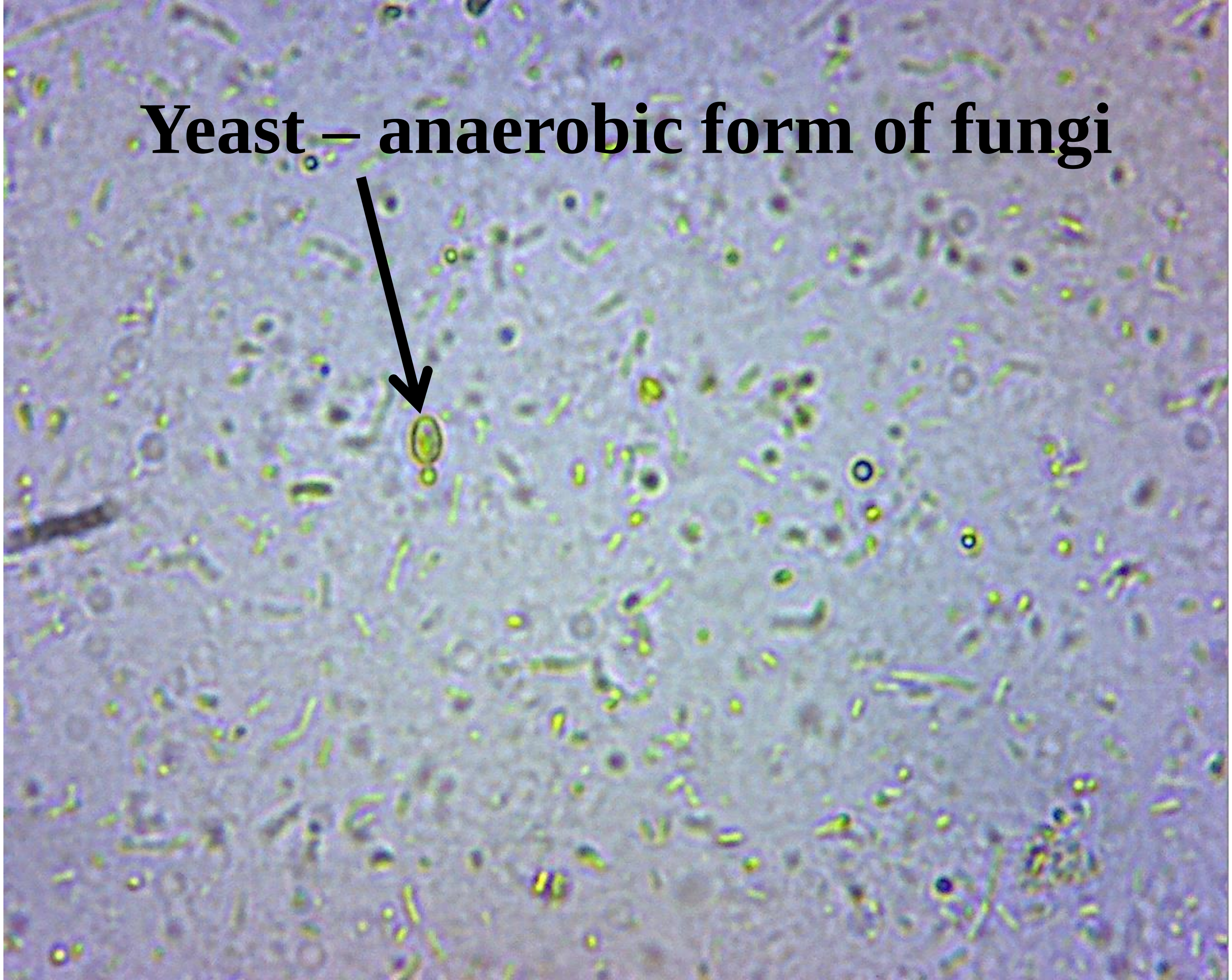
This will result in biomass per 1 cm^3 of the soil.

Please note that there is a difference in whether we measure per volume of soil, rather than per gram dry weight of soil. The amount of free water is more important than total amount of water present in the sample.

These equations are in your microscope spreadsheets, so you don’t have to remember all these conversions!

Fungi in Brewers

- Fungi grow at the tips. If there is lots of food present, the new biomass will branch often, look soft and puffy-looking, because the cell wall is only one cell layer thick.
- Older fungal hyphae have thicker cell walls, easy to measure that thickness. Thus, a great deal more carbon, harder to chow down on the older hyphae.
- When conditions become anaerobic, anaerobic bacteria attack those young hyphae, completely decompose the fungus within a few minutes. An indicator of low oxygen conditions. The next three pictures show examples.


Yeast – anaerobic form of fungi

Mycorrhizal Fungi

- **Form a symbiosis** with plants to exchange soluble plants nutrients collected by the fungus for energy provided by the plant
- **Suppress** disease
- **Retain** nutrients and water
- **Build** soil structure: macroaggregates
- **Decompose** toxins

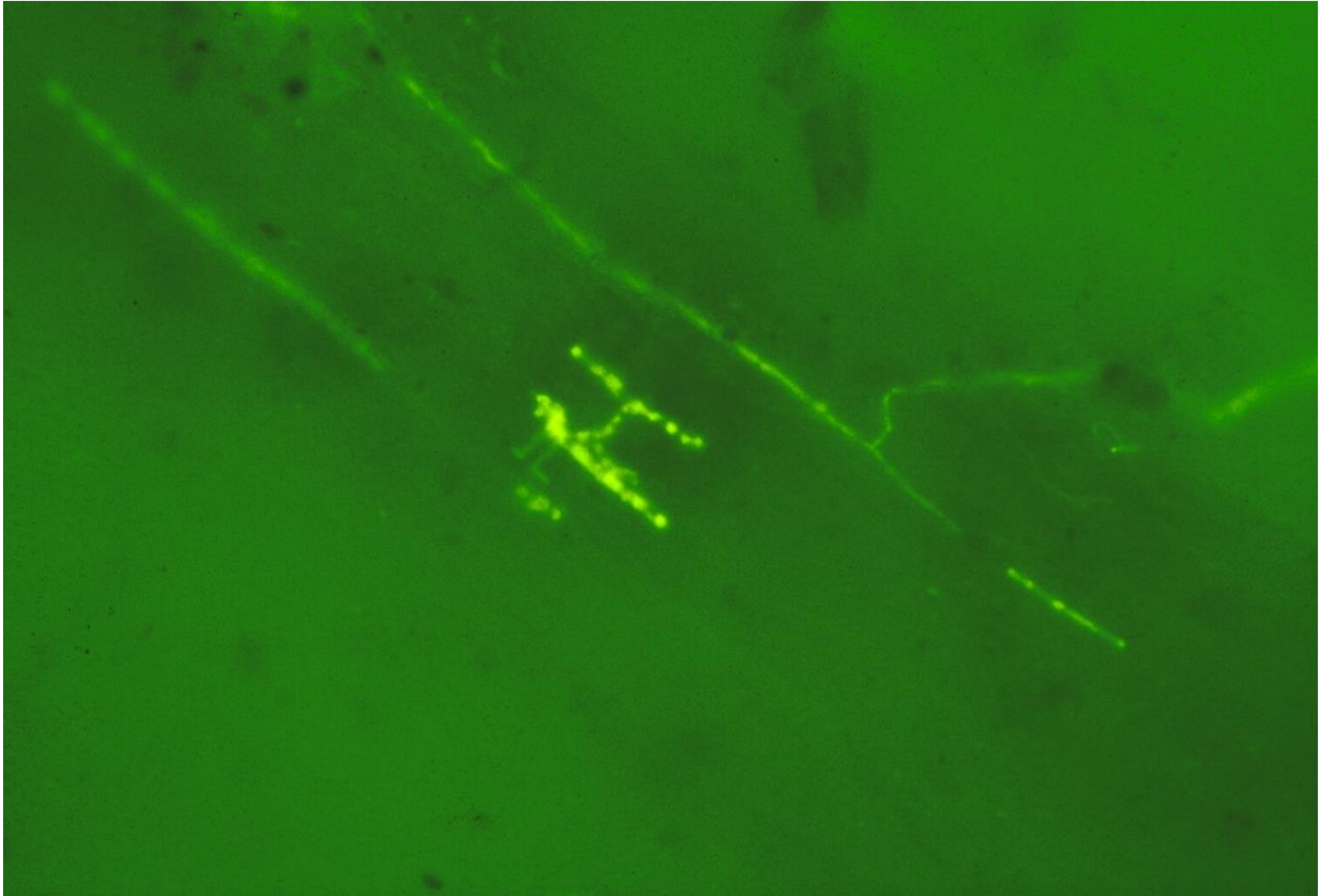
Mycorrhizal Fungi

The plant releases specific exudates to wake up the right species of mycorrhizal fungus. The spore germinates and grows toward and into the root of the plant.

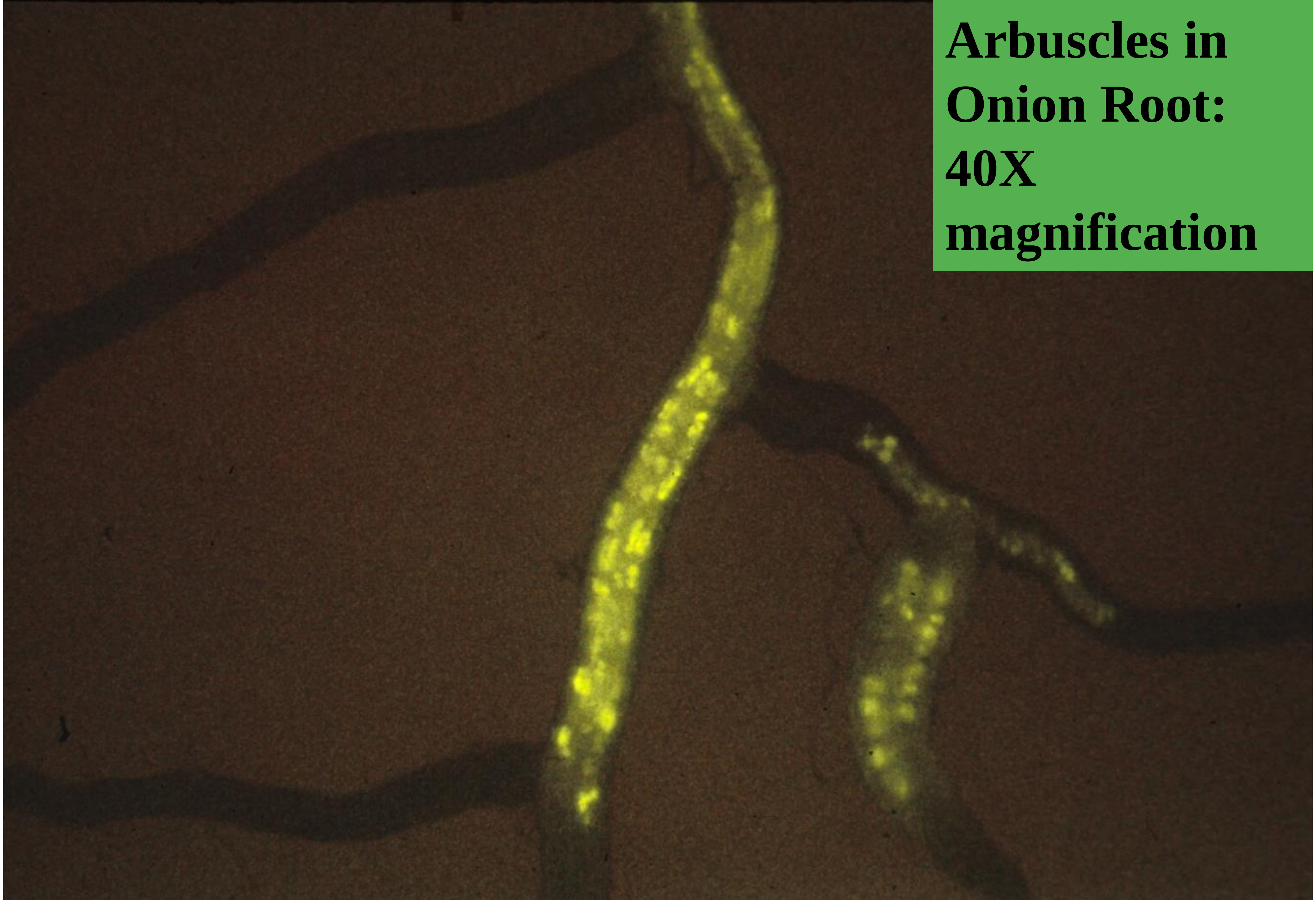
The fungus finds an “infection site”, the fungal enzymes chew through the cell wall, the fungus grows between the cell wall and cell membrane.

The “arbuscle” is where sugar from the plant and nutrients from the fungus are exchanged.

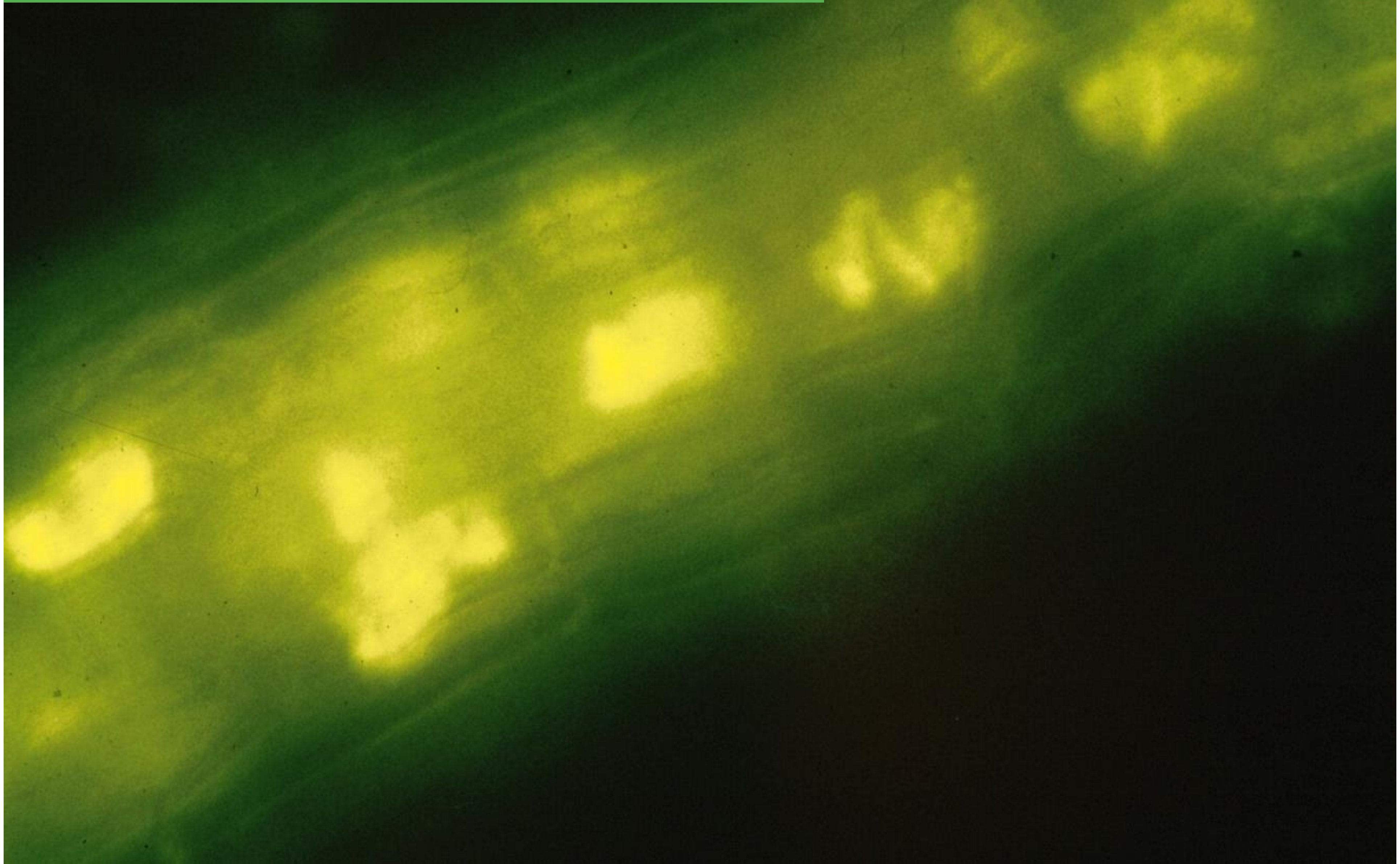

See any fungi?
No shadowing…..



Arbuscles in Onion Root: 40X magnification



Up close on arbuscles; 400X magnification



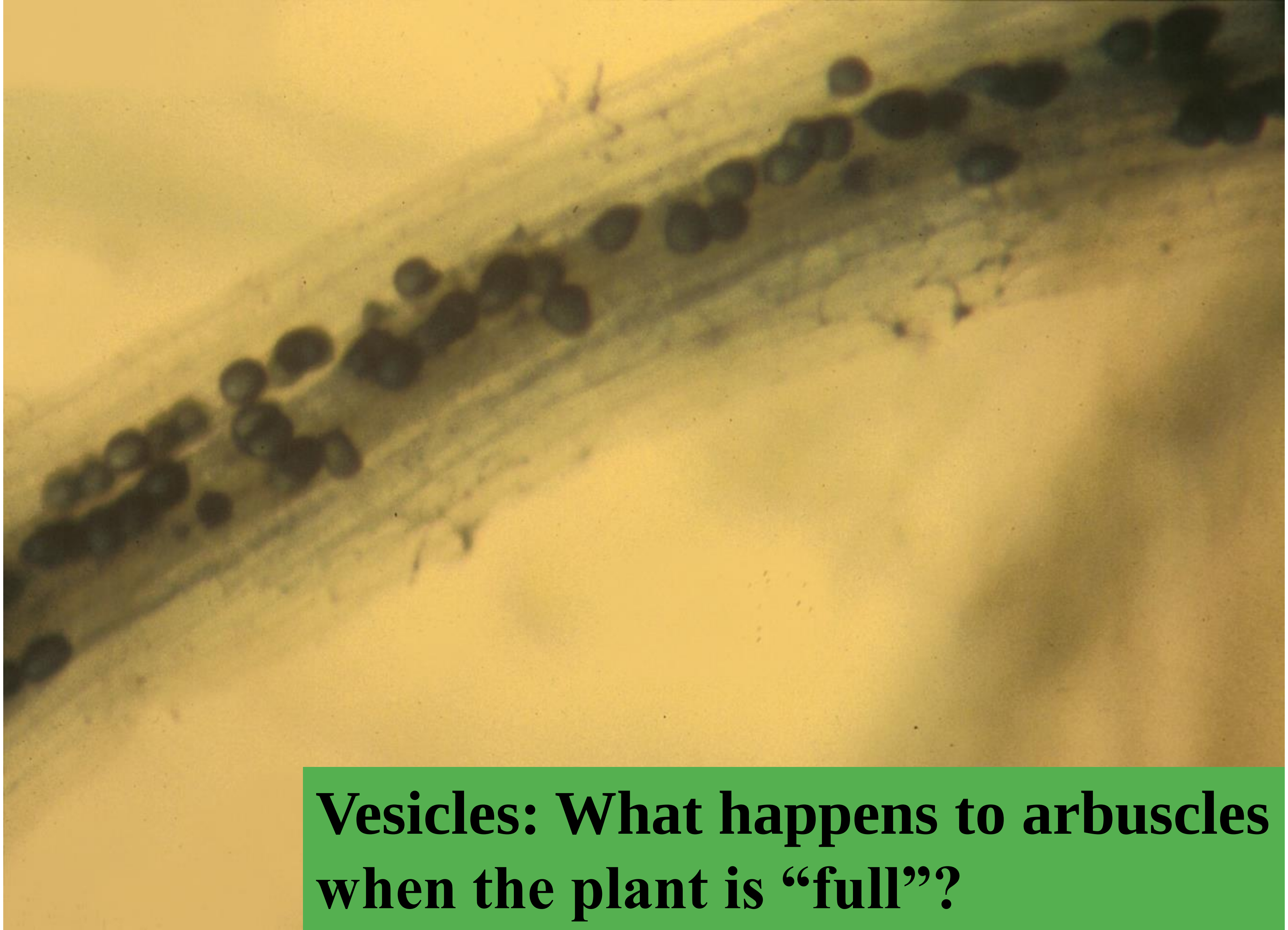
Measuring Mycorrhizal Colonization

Measure total root length in the field of view, measure the length of the root occupied by active colonization or auto-fluorescing arbuscles.

Length colonized by mycorrhizal arbuscles divided by total root length examined X 100 = Percent root system colonized by mycorrhizal fungi.

Do at least 3 separate roots so variability can be assessed.

A classic tree-like VAM arbuscle



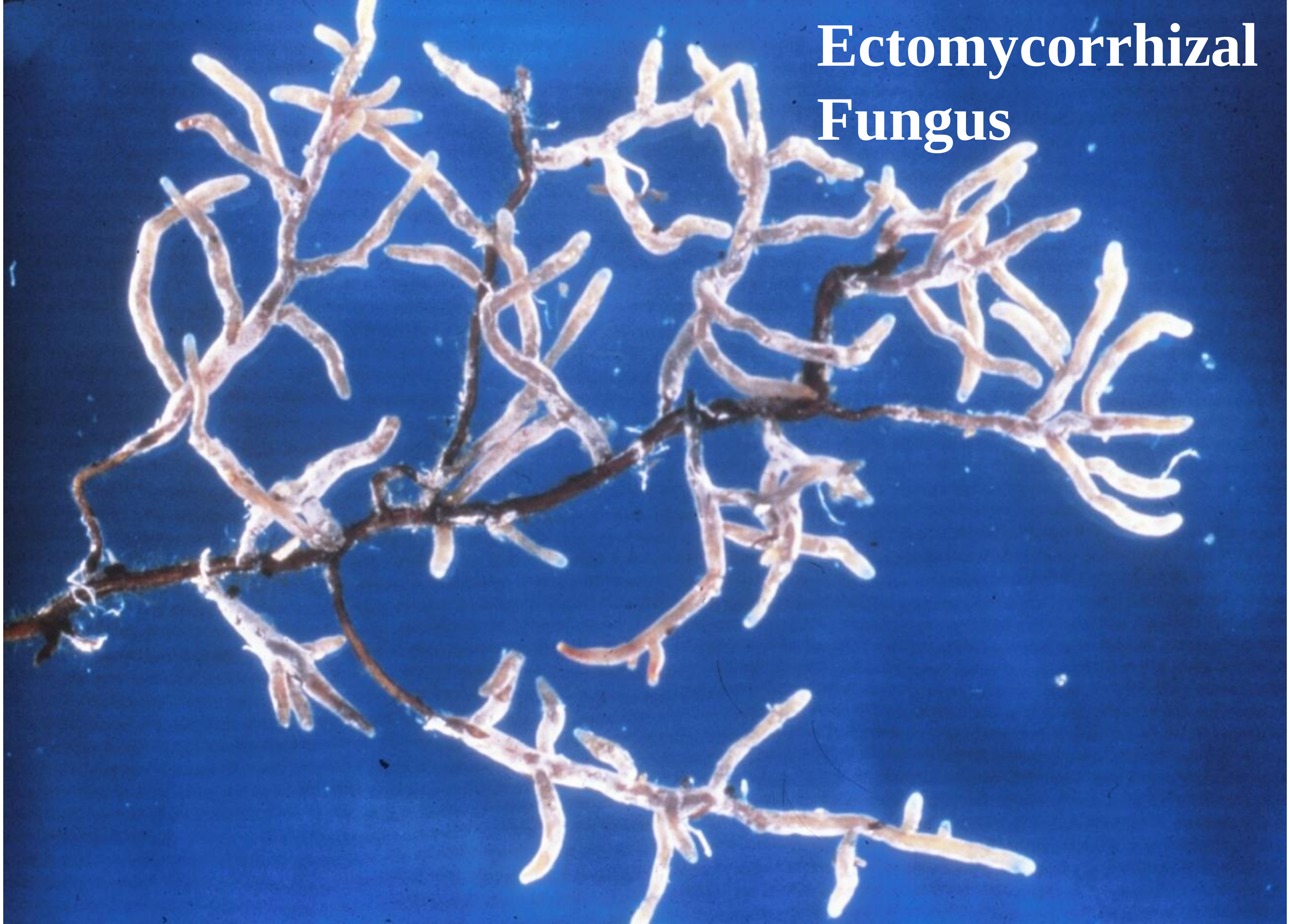
Vesicles: What happens to arbuscles when the plant is “full”?

Extramatrical Spores

VA Mycorrhizal Spore

Not mycorrhizal spores

Fungal pathogen in root (probably Fusarium)


Ectomycorrhizal
Fungus

A Different Ectomycorrhizal Fungus