RUTGERS
THE STATE UNIVERSITY
OF NEW JERSEY

Soil Regeneration Unlimited Conference

‘Rhizophagy Cycle and Endophytes in Plants’

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What makes a soil healthy?

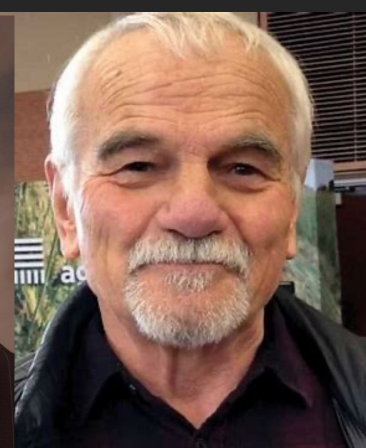
- Soil should be rich in microbes (soil biology)
- Soil should have good structure (aggregation of particles with pore spaces). Plants need oxygen in soil to extract nutrients from bacteria
- Soil should have good drainage to minimize flooding
- Soil should be rich in organic matter (cover crops can build up organic matter in soil). A healthy soil will show increasing organic matter content.
- Soil should show evidence of life (e.g., earth worms)

Elaine Ingham

Christine Jones

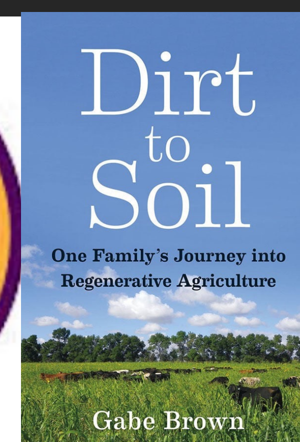
Rick Clark

Kris Nichols



Gary Zimmer

John Kempf

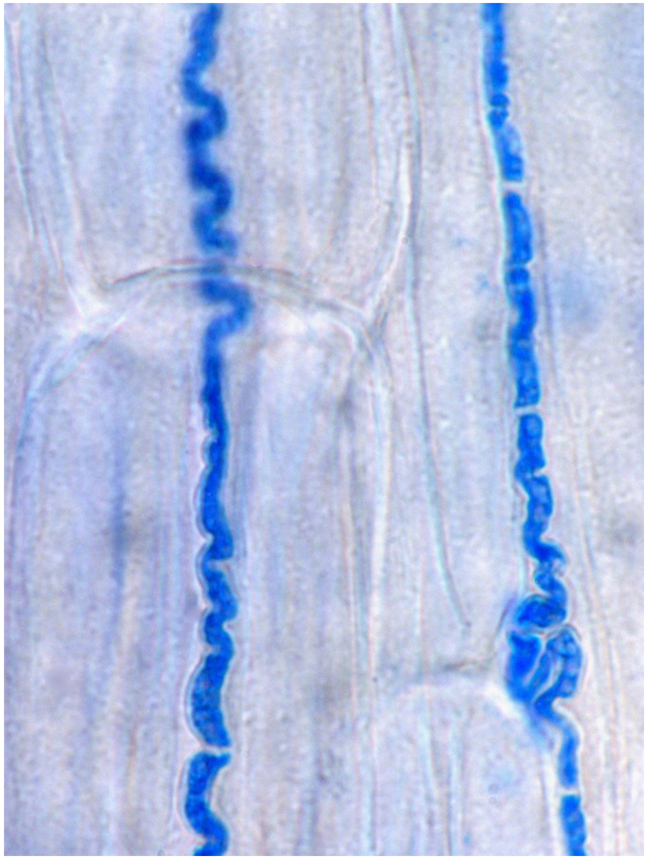


Gabe Brown

Seed plants absorb soil bacteria into growing cells and tissues. These microbes are referred to as endophytes.

What are endophytes?

(Botany): Endophytic/endosymbiotic non-pathogenic microbes (fungi, bacteria or algae) present asymptomatically for all or part of their life cycles in tissues of plants.



(Medical definition): A tumor that grows like a parasite into other tissues.

Fungal hyphae of endophyte in stem tissue of tall fescue grass.

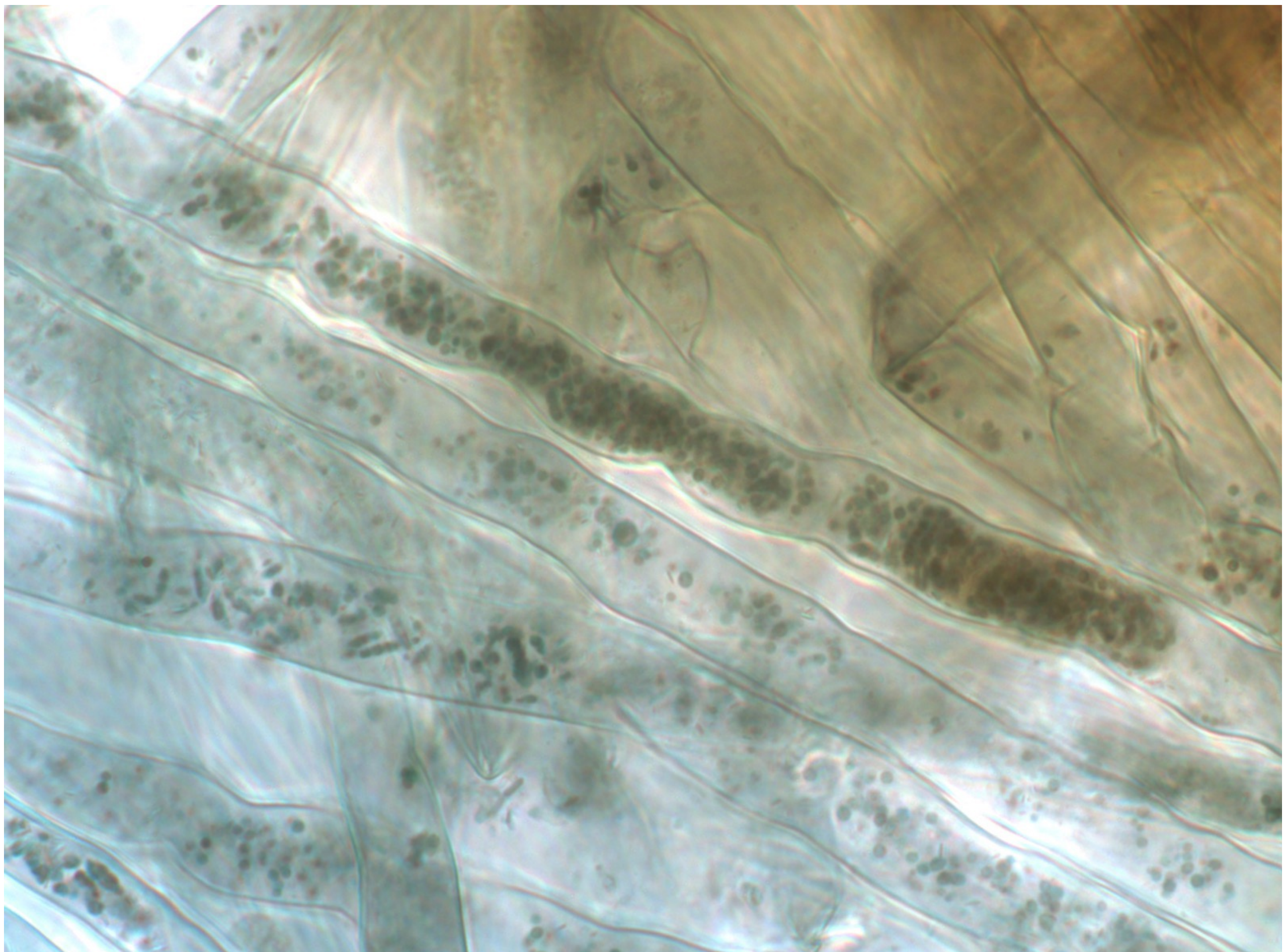
'Cadushy' cactus: *Subpilocereus repandus* in Bonaire

Cadushy fruits

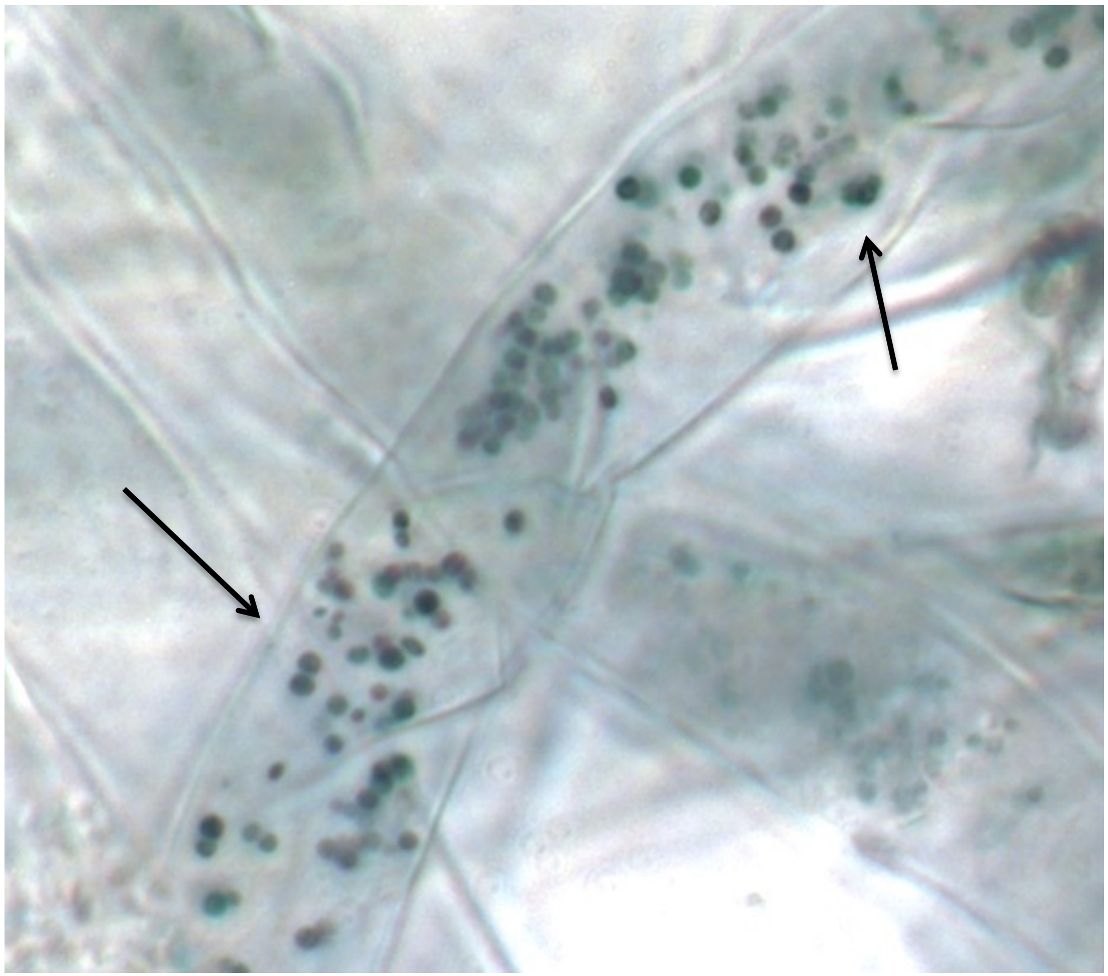
Seeds

Cadushy seedling

Bacteria in root hairs (Stained in DAB followed by aniline blue).

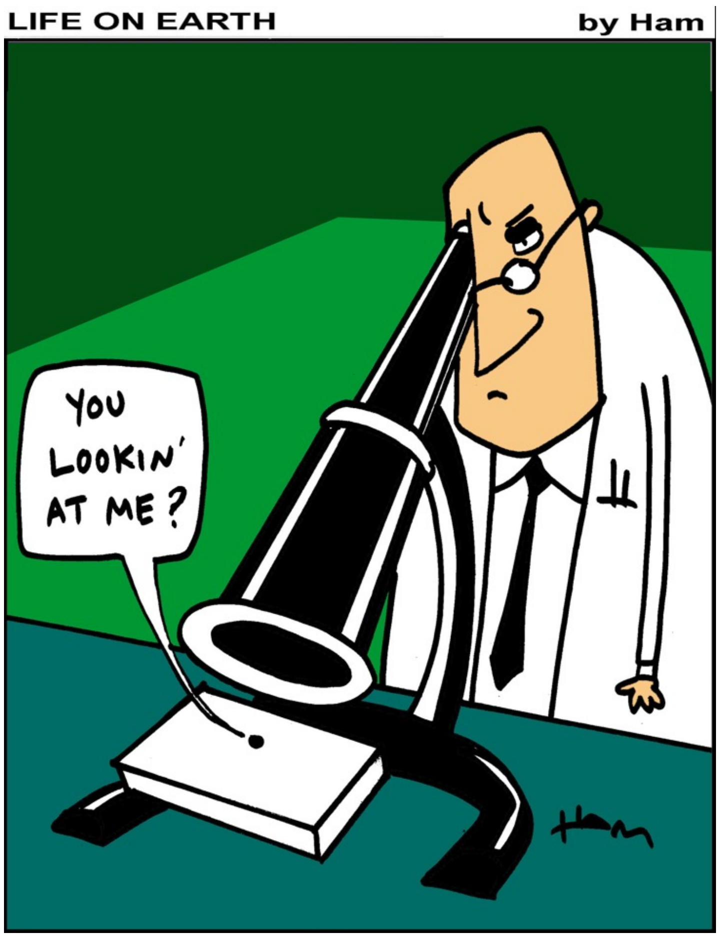


Bacteria in root hairs showing recently divided pairs



All plants naturally host a community of endophytes!

But you need a microscope to see them.



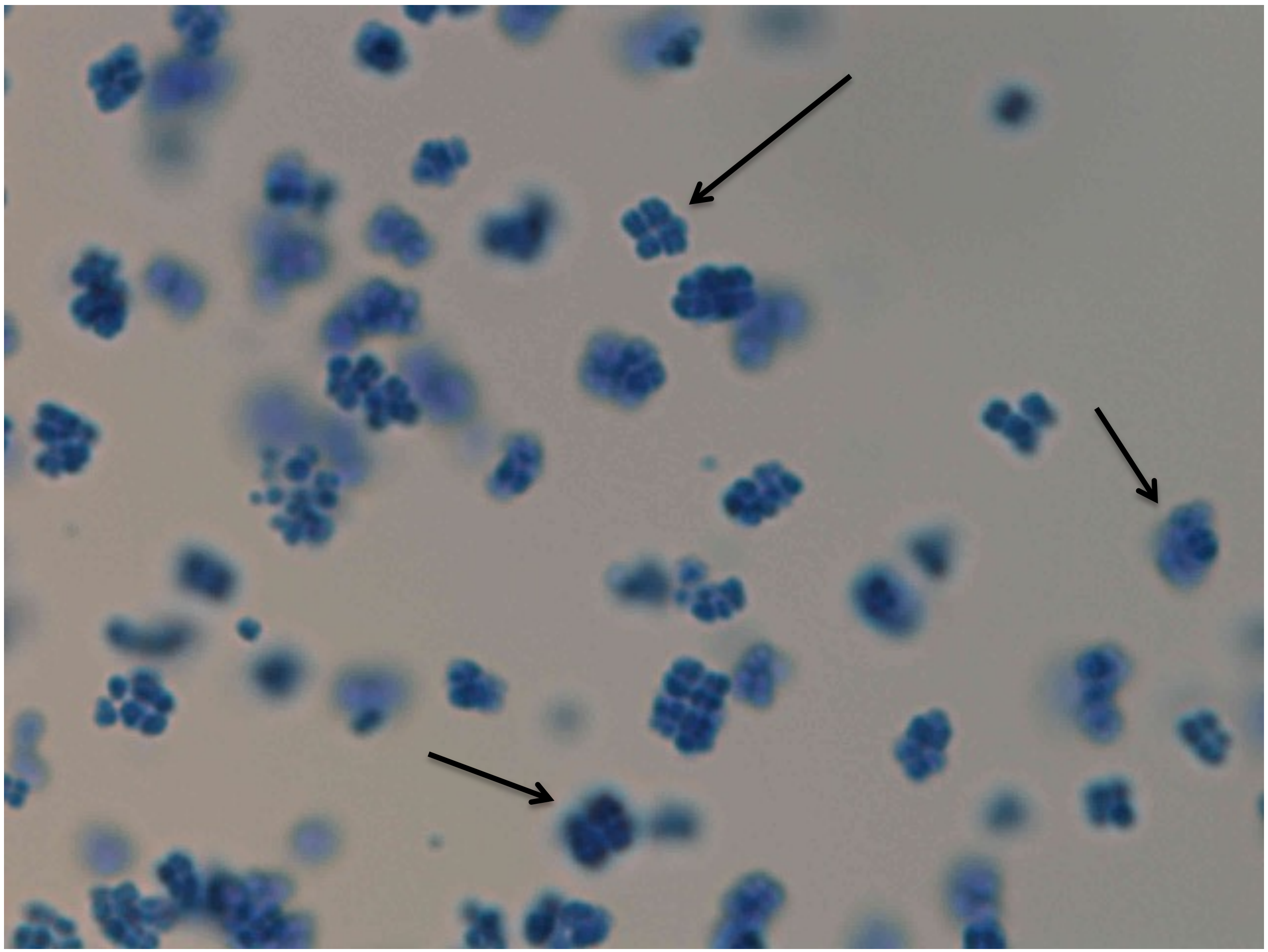
Endophytes are everywhere!

What about crop plants?

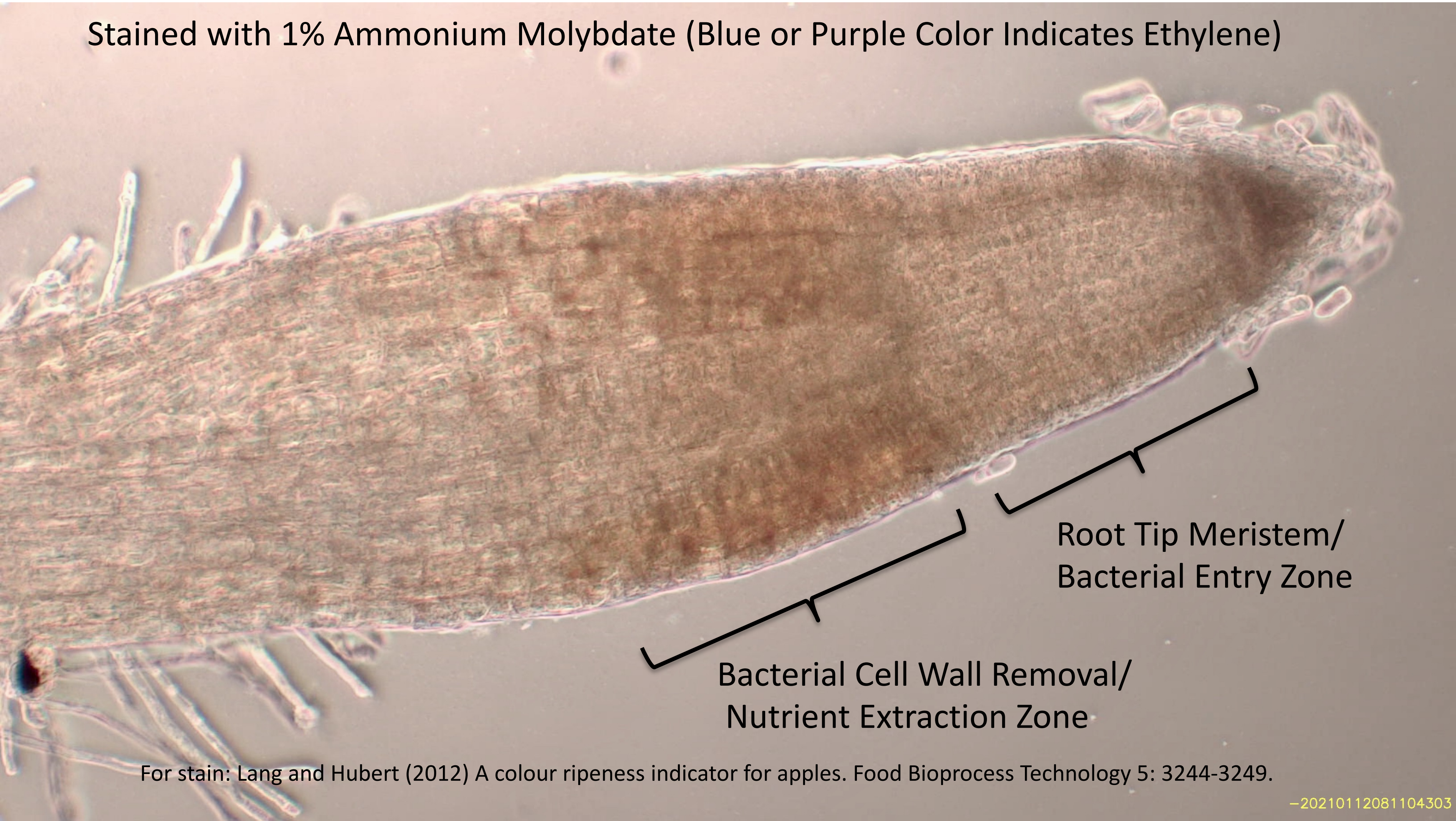
All crops harbor endophytes!

Bacterial endophytes colonize seeds.

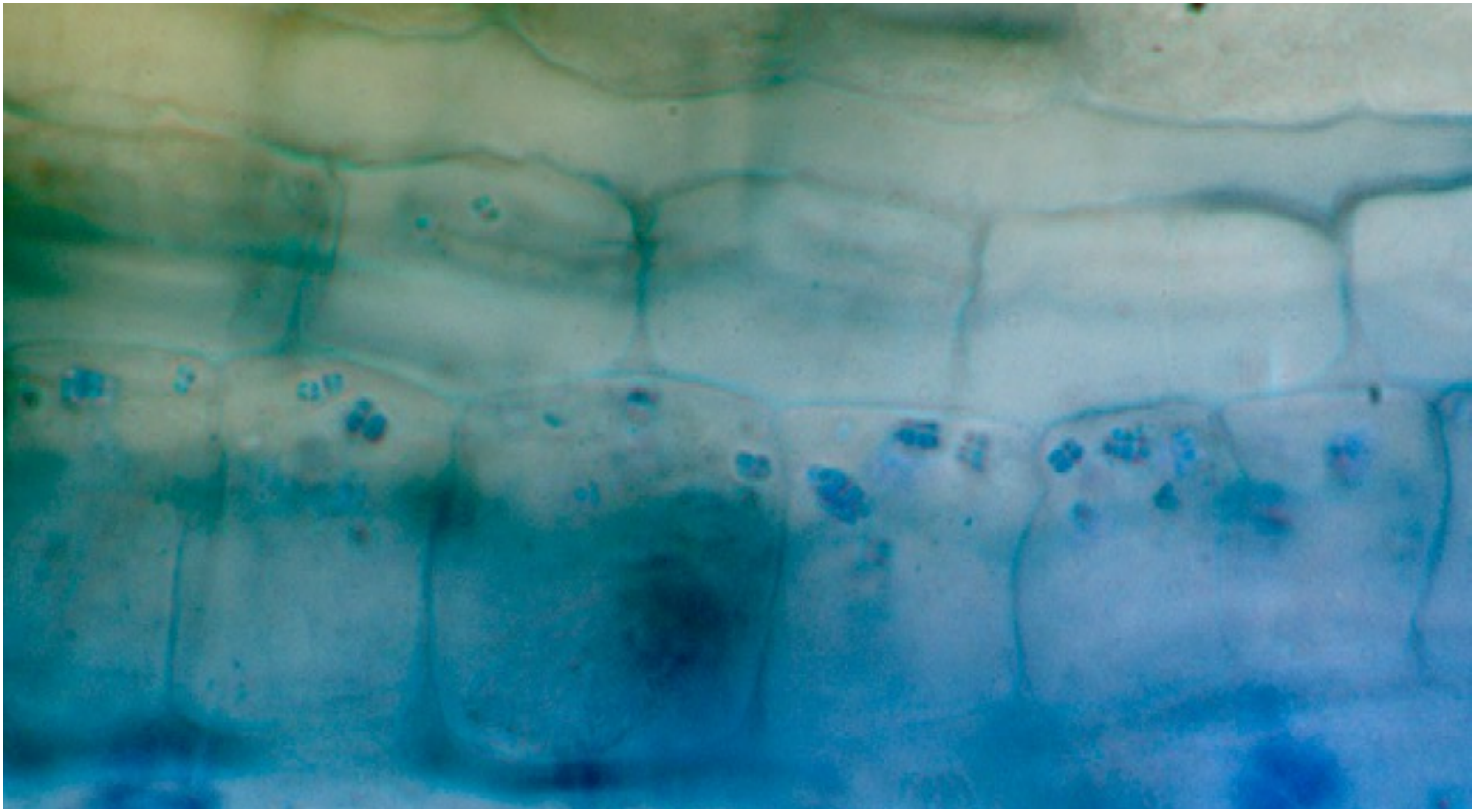
Micrococcus luteus is a bacterial endophyte from tomato seedlings. This photo shows tetrads (clusters of 4 cells) (arrows) of this bacterium.



Tomato seedling root tip showing high ethylene areas to either side of the root tip meristem.



Close-up of *Micrococcus* tetrads in periplasmic space of root meristematic cells.



Plant microbes are:

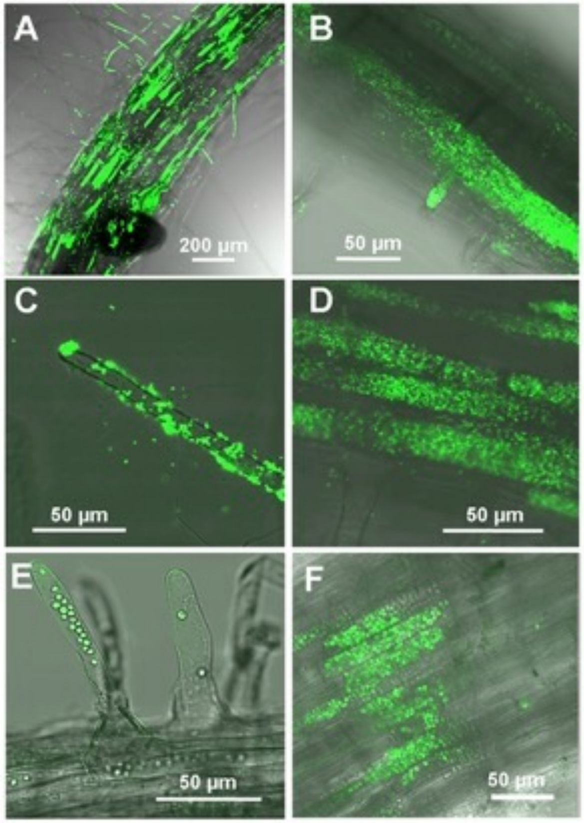
1) Soil microbes

2) Seed vectored

Figure 1. Roots of axenically grown Arabidopsis and tomato were incubated with E coli or yeast expressing green fluorescent protein (GFPE. coli or GFPyeast).

"Rhizophagy"

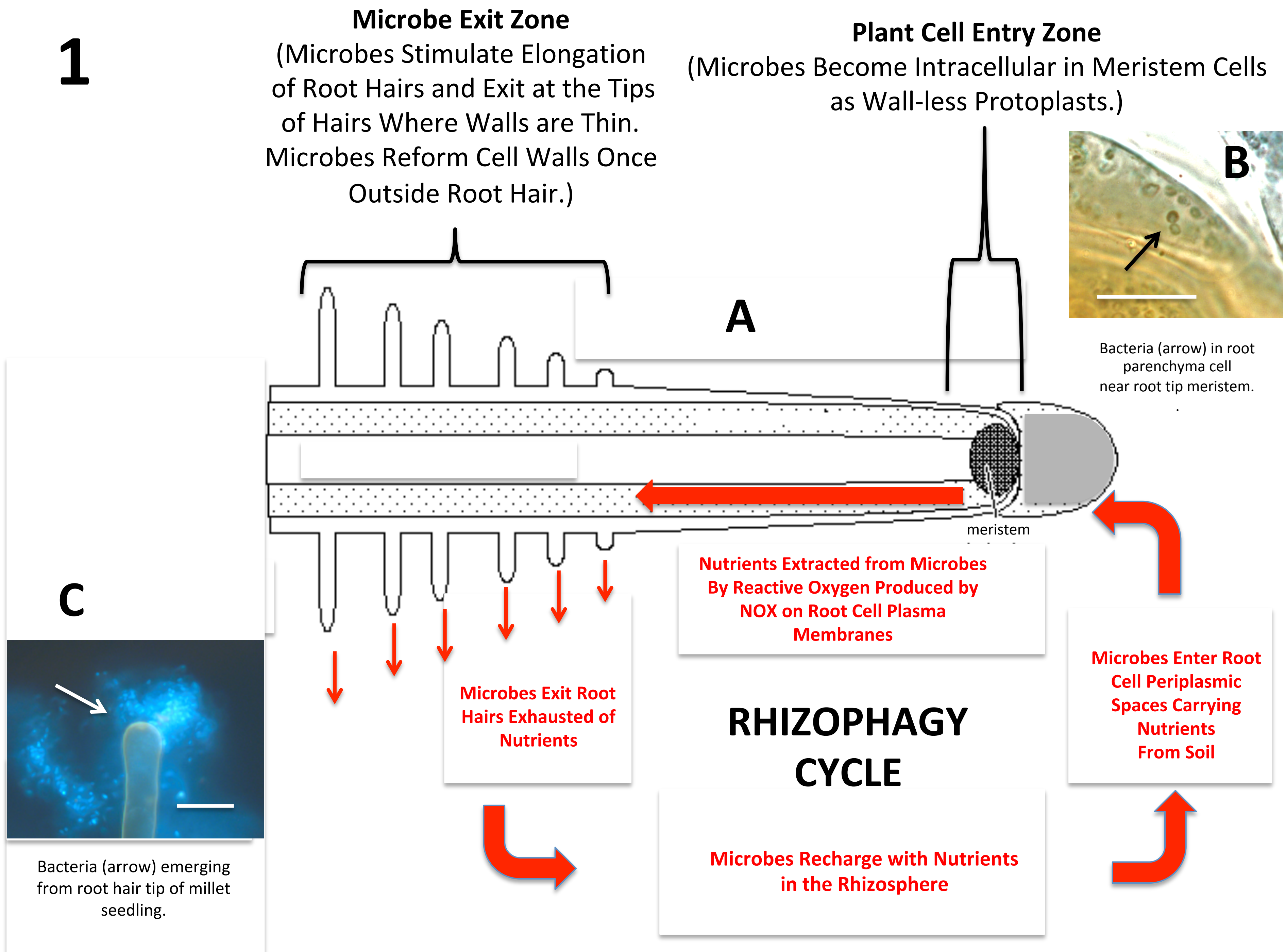
Do plant roots consume bacteria to obtain nutrients?



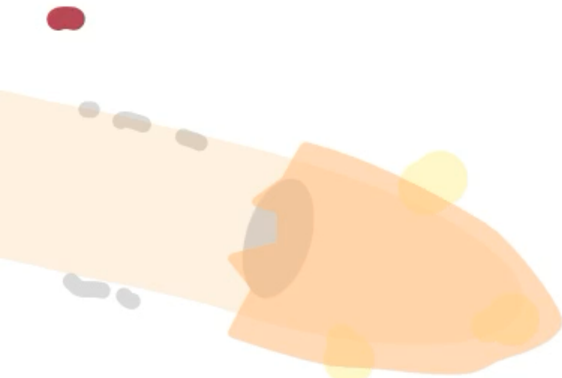
Chany Paungfoo-Lonhienne

Suzanne Schmidt

Paungfoo-Lonhienne C et al. 2010.
Turning theTable: Plants Consume Microbes as a Source of Nutrients.
PLoS ONE 5(7): e11915, doi:10.1371/journal.pone.0011915



1
Microbe Exit Zone
(Microbes Stimulate Elongation of Root Hairs and Exit at the Tips of Hairs Where Walls are Thin. Microbes Reform Cell Walls Once Outside Root Hair.)
Plant Cell Entry Zone
(Microbes Become Intracellular in Meristem Cells as Wall-less Protoplasts.)
A
B
Bacteria (arrow) in root parenchyma cell near root tip meristem.
meristem
Nutrients Extracted from Microbes By Reactive Oxygen Produced by NOX on Root Cell Plasma Membranes
C
Microbes Exit Root Hairs Exhausted of Nutrients
RHIZOPHAGY CYCLE
Microbes Enter Root Cell Periplasmic Spaces Carrying Nutrients From Soil
Microbes Recharge with Nutrients in the Rhizosphere
Bacteria (arrow) emerging from root hair tip of millet seedling.



Dr. Harriet Mella

Grass roots show numerous roots tip meristems. These root tip meristems are the sites of internalization of microbes and extraction of nutrients from microbes in the rhizophagy cycle.

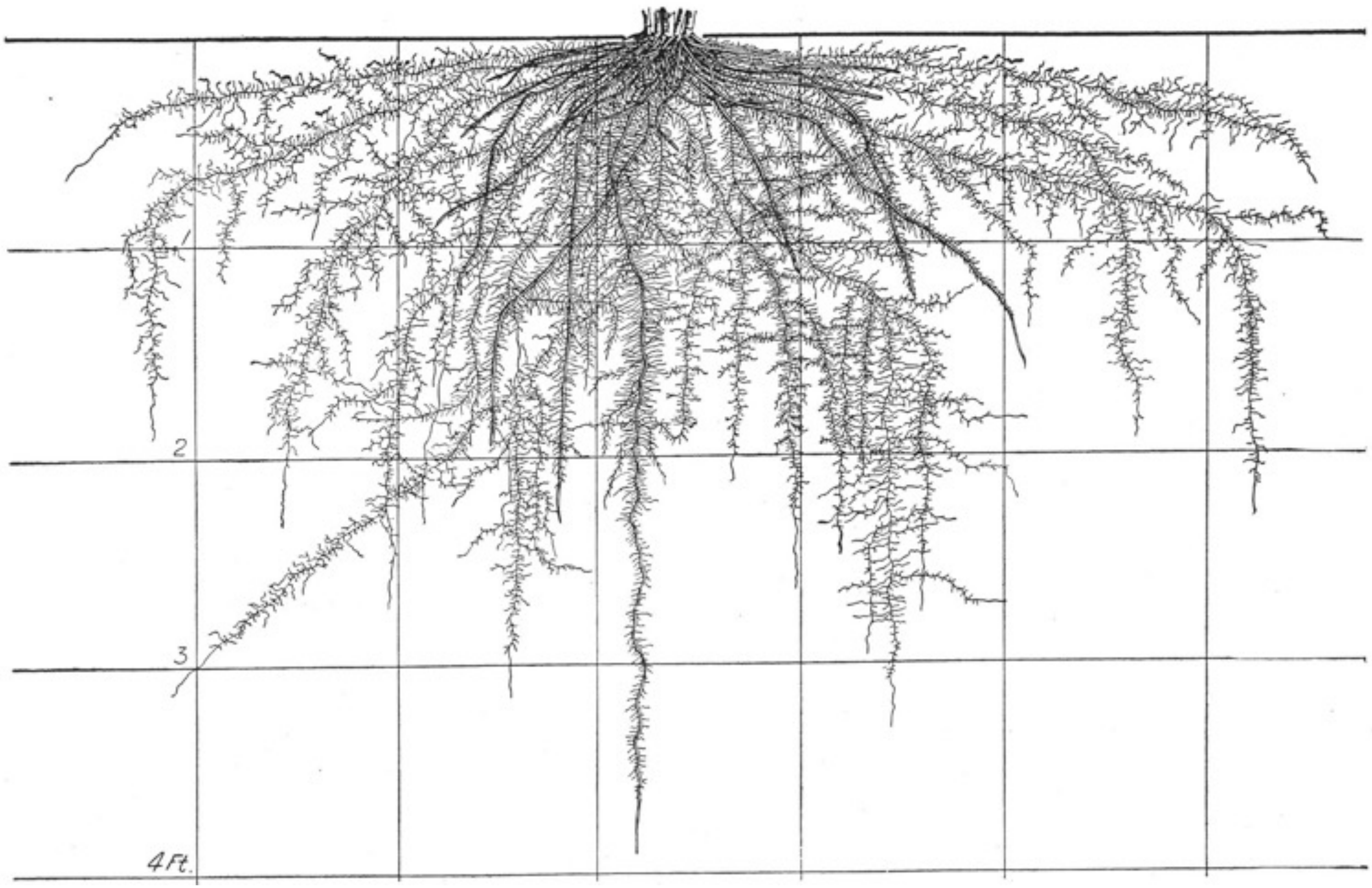
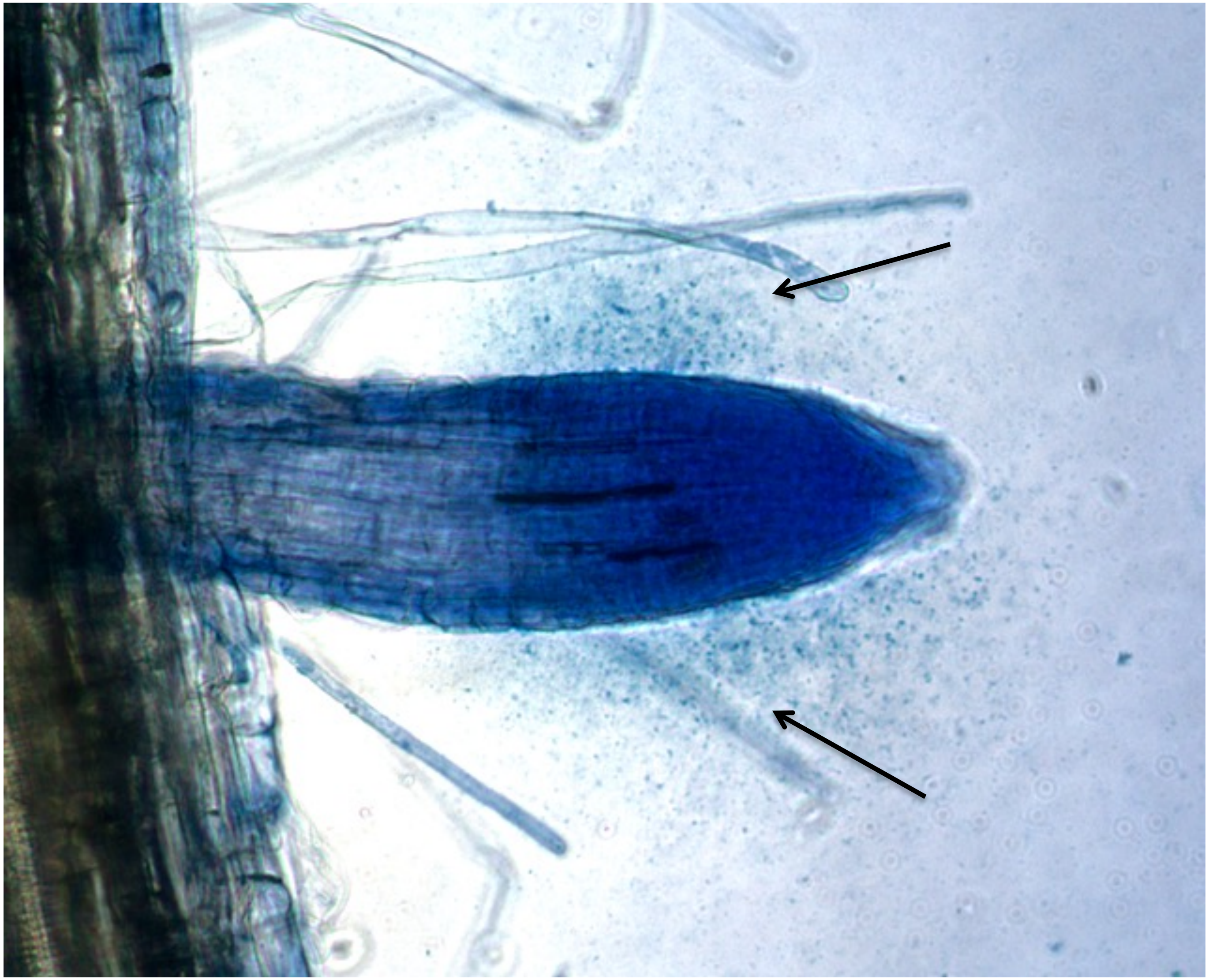


Illustration of a root system of corn (Illustration by Botanist John E. Weaver, 1927)

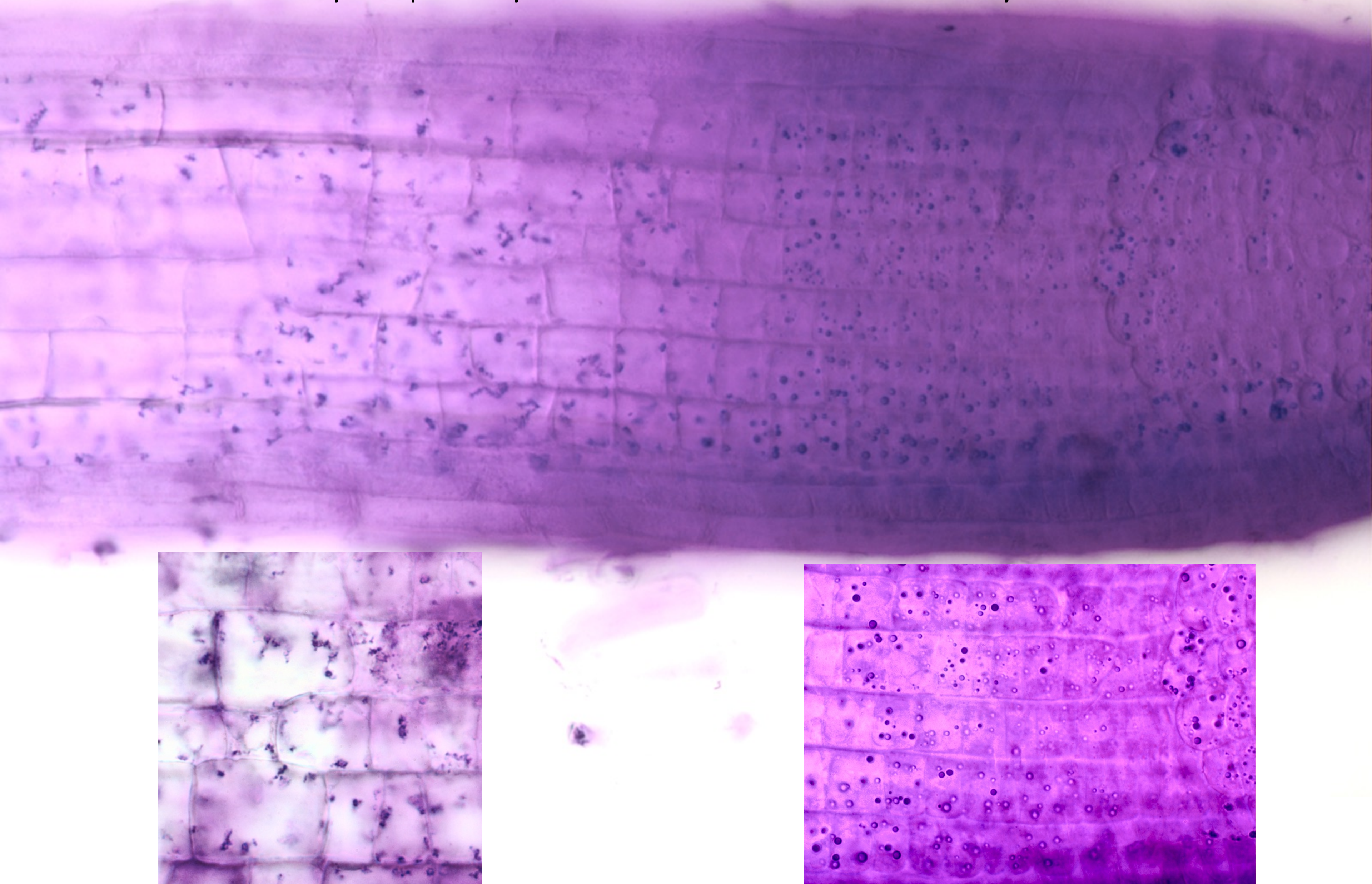
Bacteria entering root epidermal cells in the ‘zone on intracellular colonization’ at the root tip meristem. A cloud of bacteria (arrows) is seen around the root tip meristem where intracellular colonization is occurring. The blue stain is aniline blue.



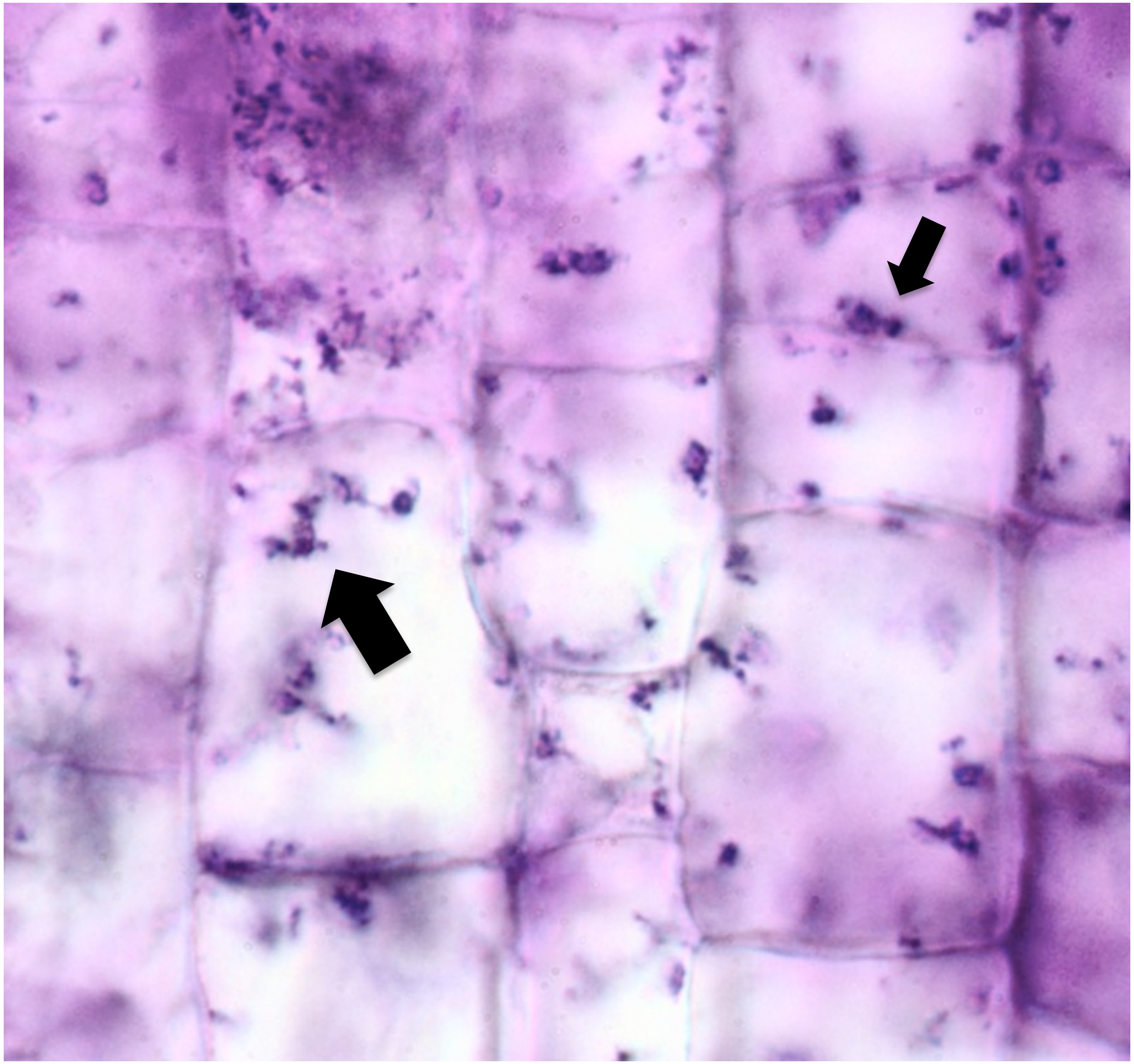
Poa annua root inoculated with *Bacillus* sp. (crystal violet)

Zone of bacterial protoplast replication

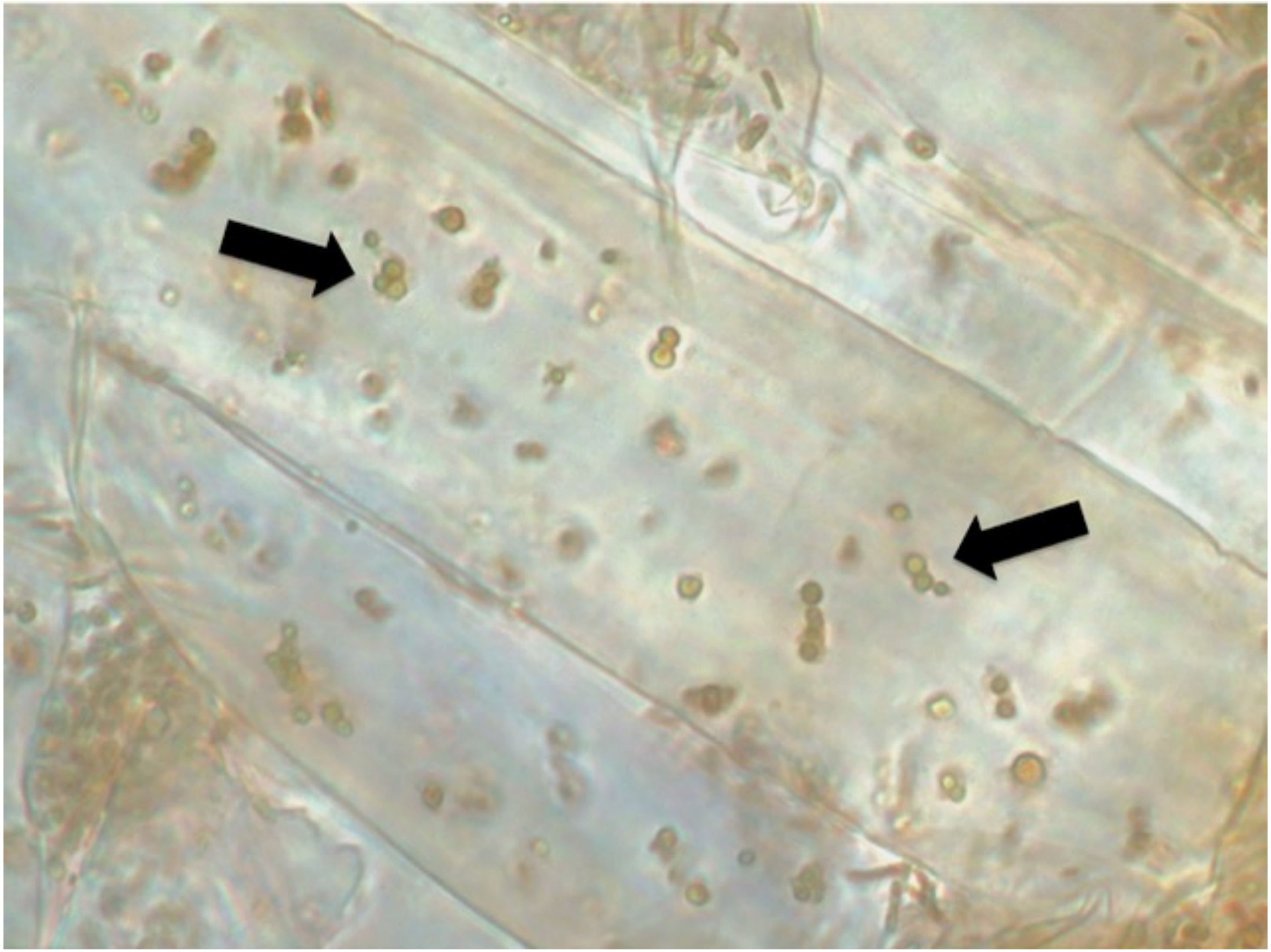
Zone of bacterial entry and cell wall loss



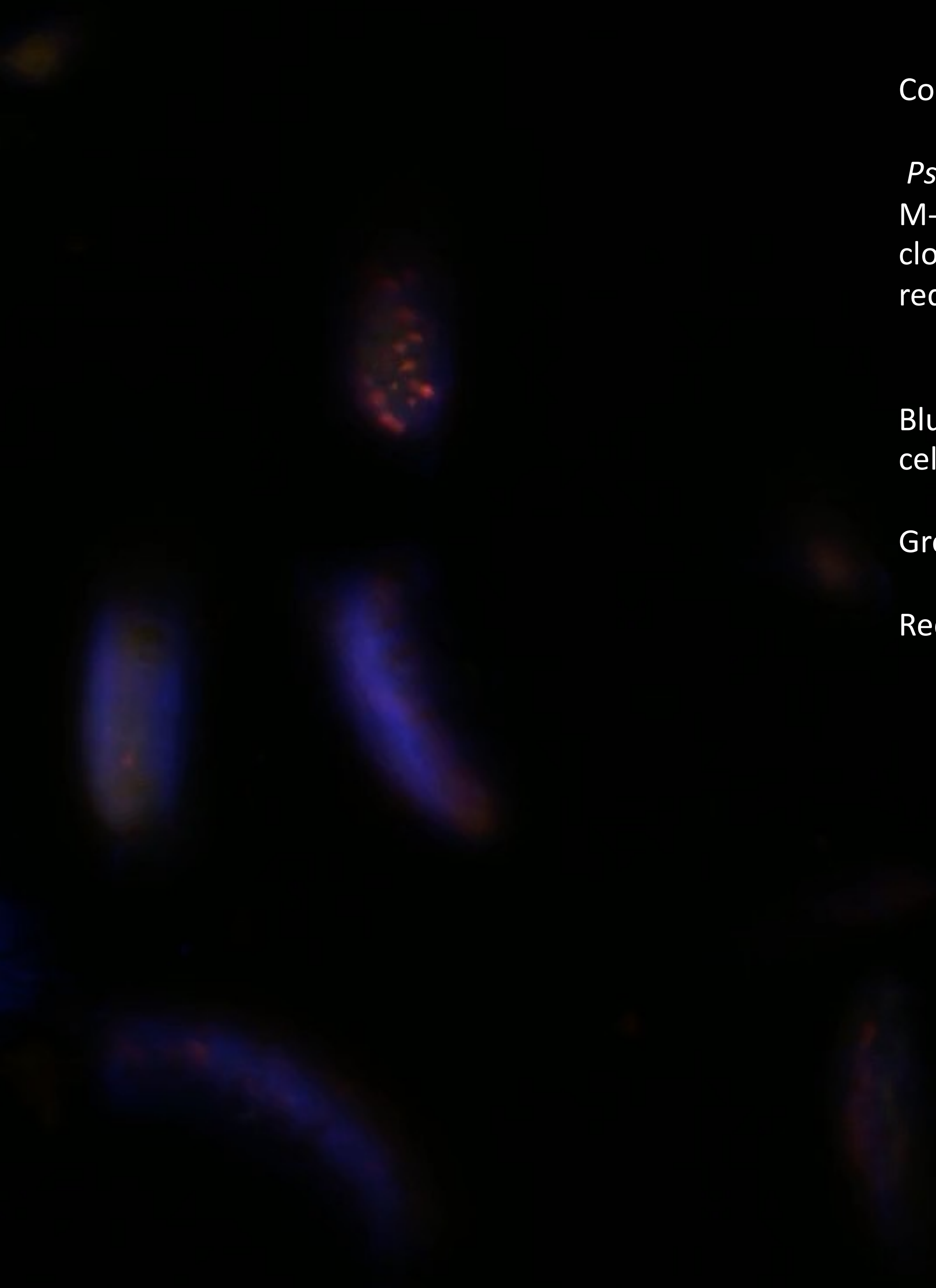
Bacteria lose cell walls after they enter plant cells. Irregular shapes that stain densely with crystal violet are bacterial cell walls (arrows). Bacterial L-forms replicate rapidly in root cells.



Phragmites root stained with diaminobenzidine DAB to visualize reactive oxygen around bacterial protoplasts (arrows). Reactive oxygen is visualizable as brown or red coloration around bacteria. The reactive oxygen is the result of superoxide produced by NADPH oxidases on the root cell plasma membranes. The reactive oxygen extracts nutrients from the bacteria (mostly pseudomonads) that are symbiotic with *Phragmites*.



Celeste Zhang



Confocal Microscopy:

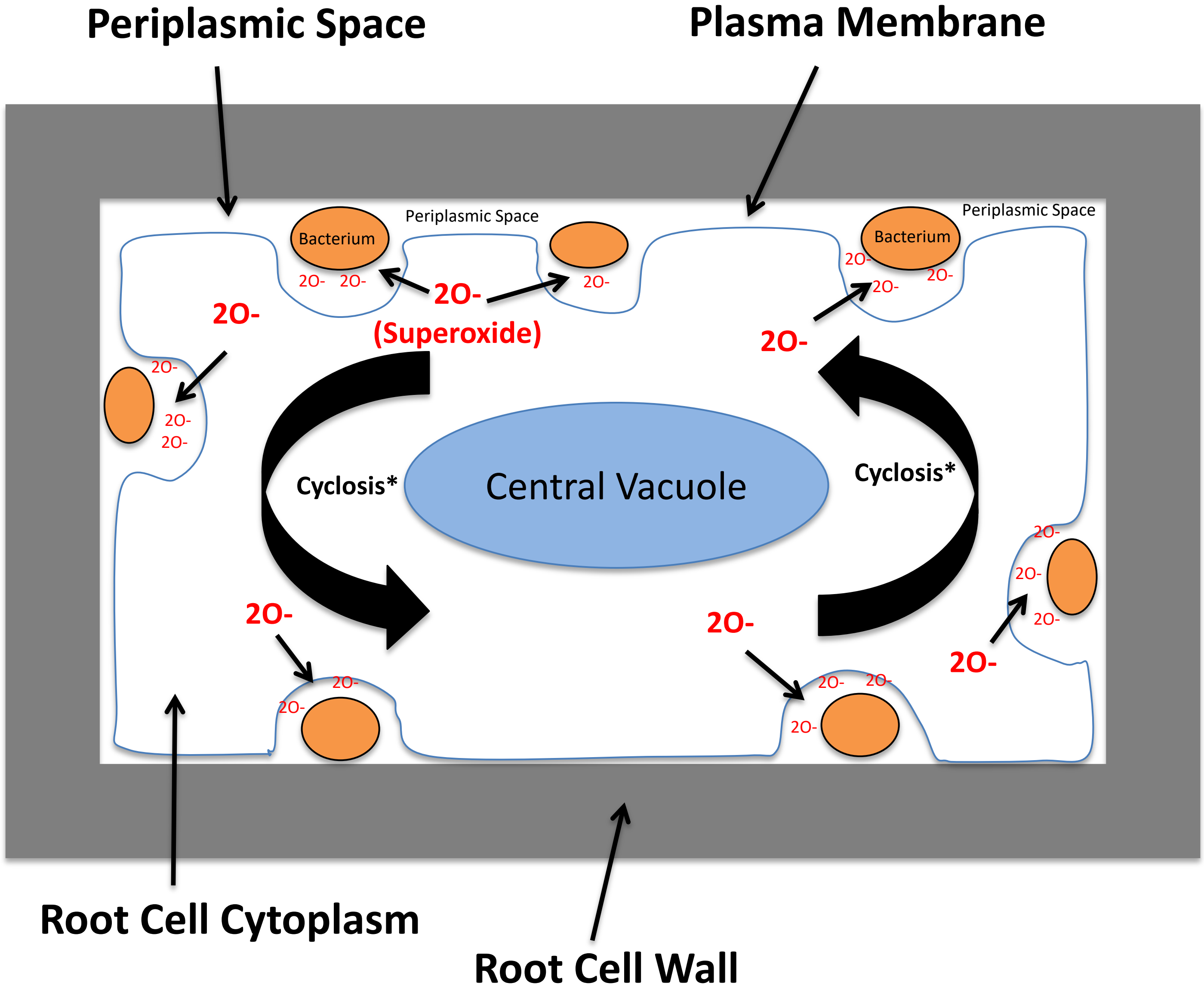
Pseudomonas sp. tagged with M-Cherry and inoculated into clover plants. Bacteria fluoresce red in the root cap cells.

Blue = calcofluor white (plant cell walls)

Green = syto13 (nucleic acid)

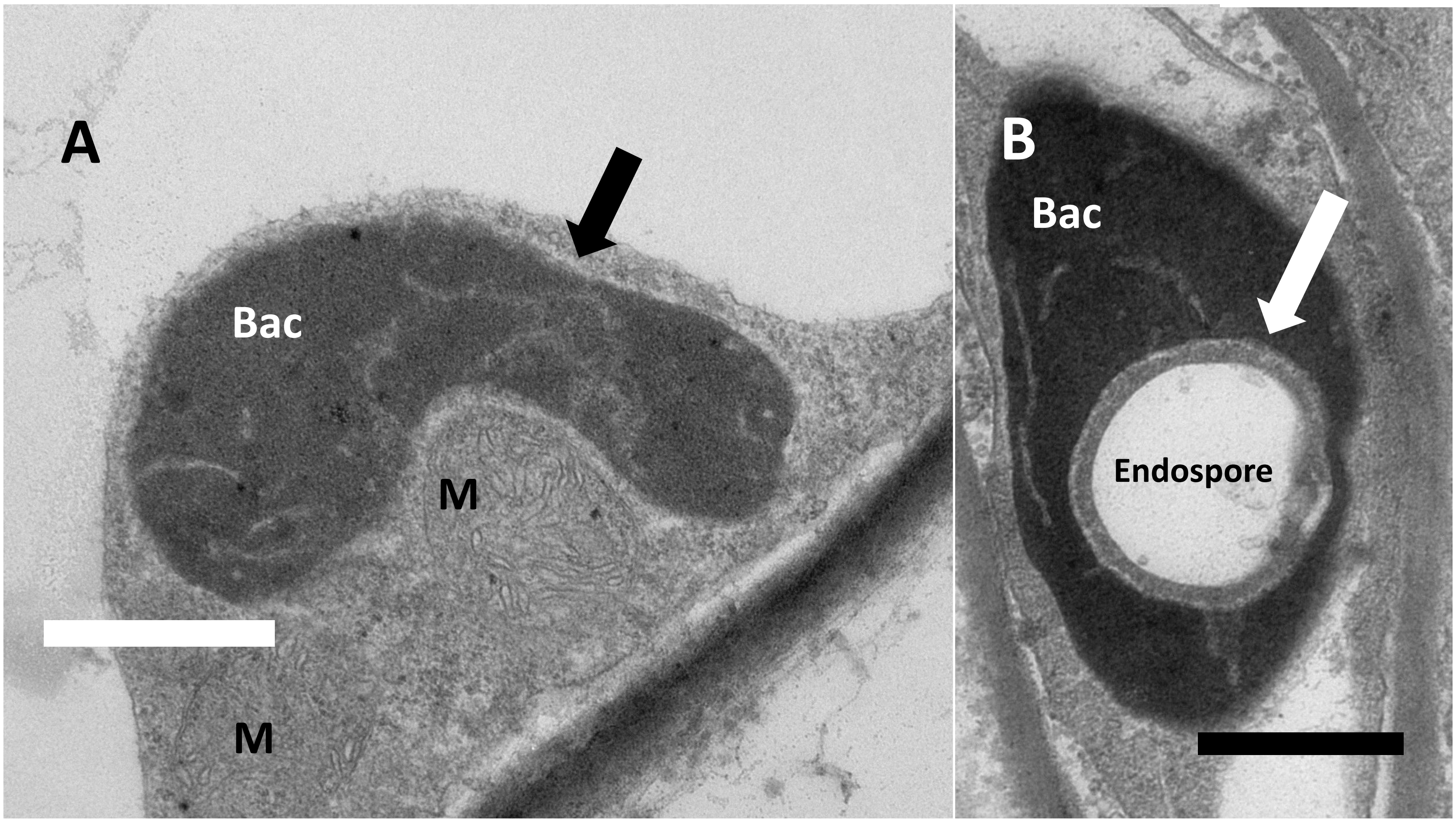
Red = mCherry tagged bacteria

Bacterial Protoplasts in Periplasmic Space are Subjected to Host-Produced Superoxide.

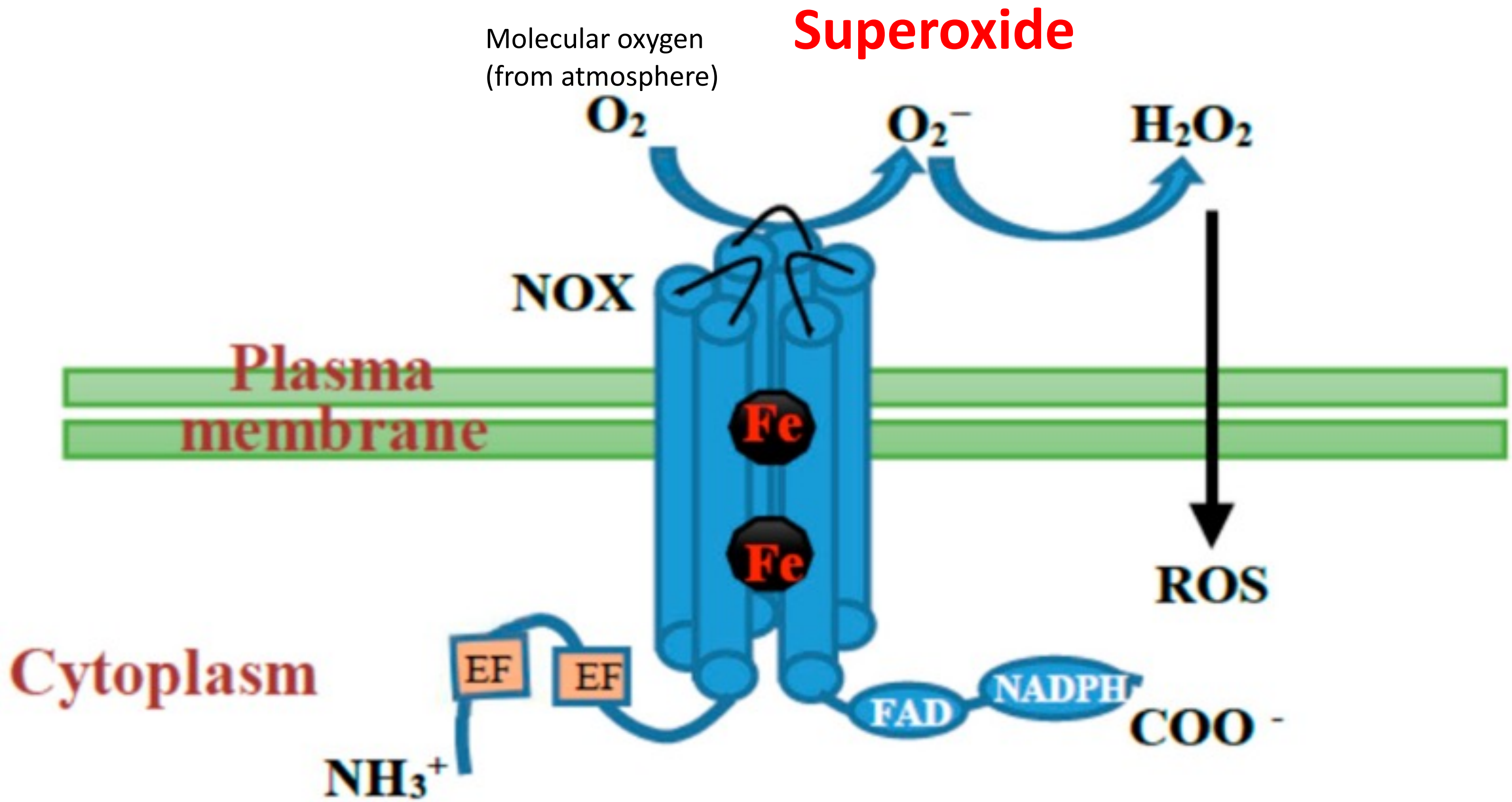


*Cyclosis = Cytoplasmic Movement

Ultrastructural evidence that bacteria also enter the root cell cytoplasm. A. *Bacillus* in root cells of *Amaranthus caudatus* seedlings. B. Bacillus containing an endospore in a root cell.



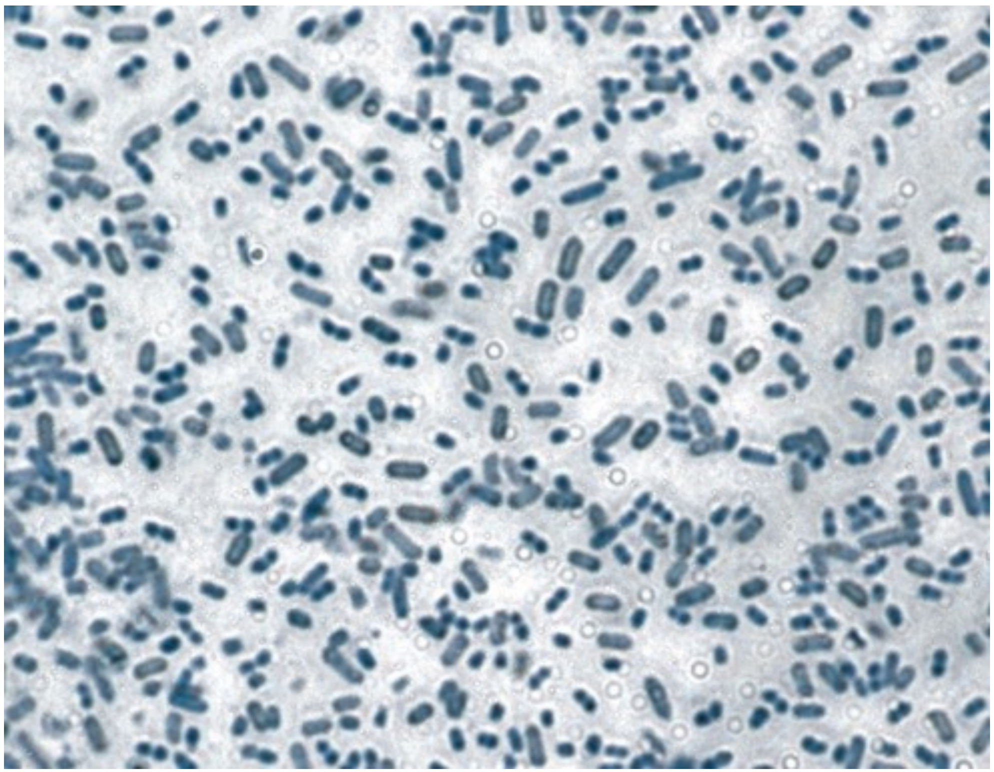
REACTIVE OXYGEN DEFENSE RESPONSE OF THE ROOT CELL INVOLVES MEMBRANE-BOUND NADPH OXIDASES (NOX)



Chemical equation: $NADPH + 2O_2 \xrightarrow{NOX} NADP^+ + 2O_2^- + 2H^+$

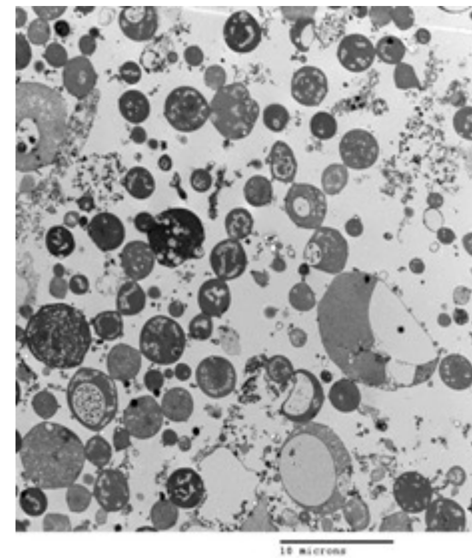
Bacterium *Bacillus subtilis*

Bacteria with cell walls (rods)



Reactive oxygen (superoxide)

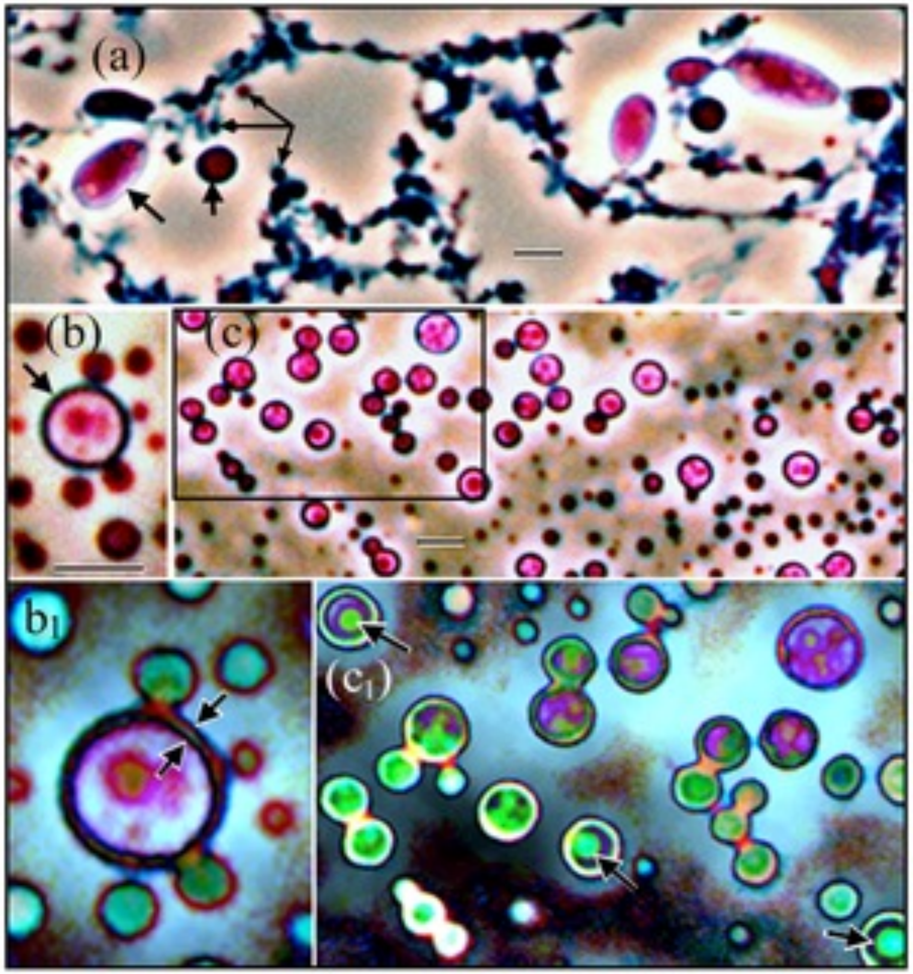
Spherical bacterial protoplasts (no cell walls)

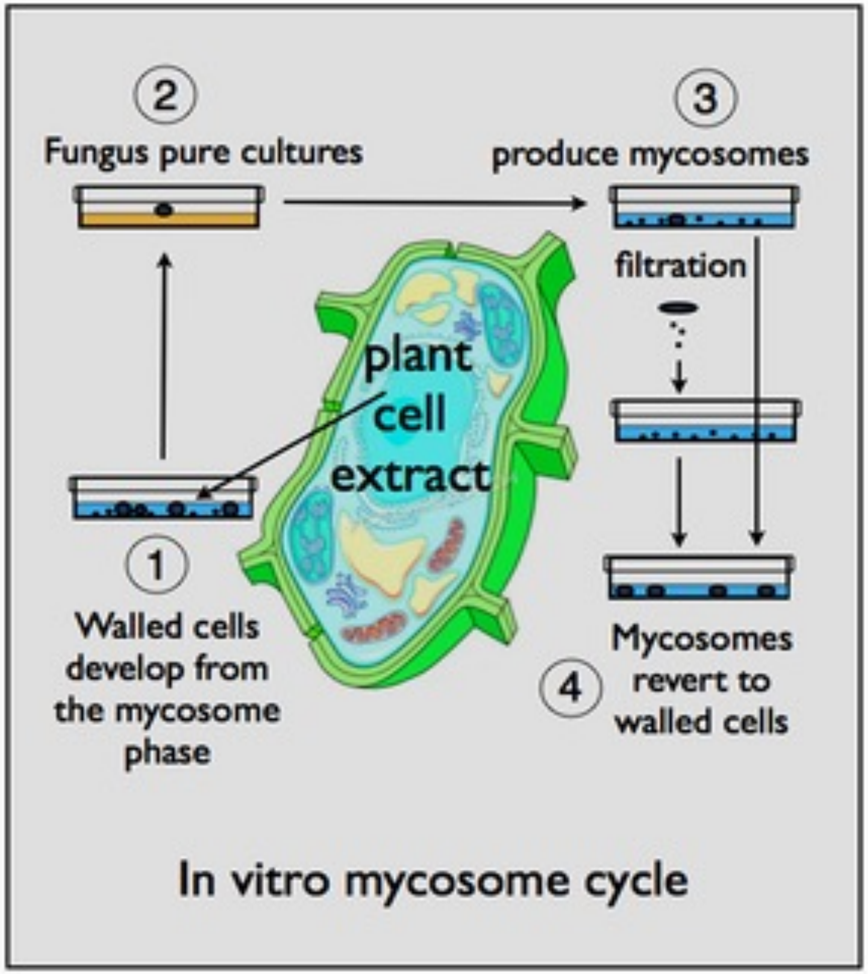


Bacterial protoplasts are called L-forms.

Inside root cells superoxide strips cell walls off of the microbes!

Intracellular phases of fungi may form protoplasts called 'mycosomes'.





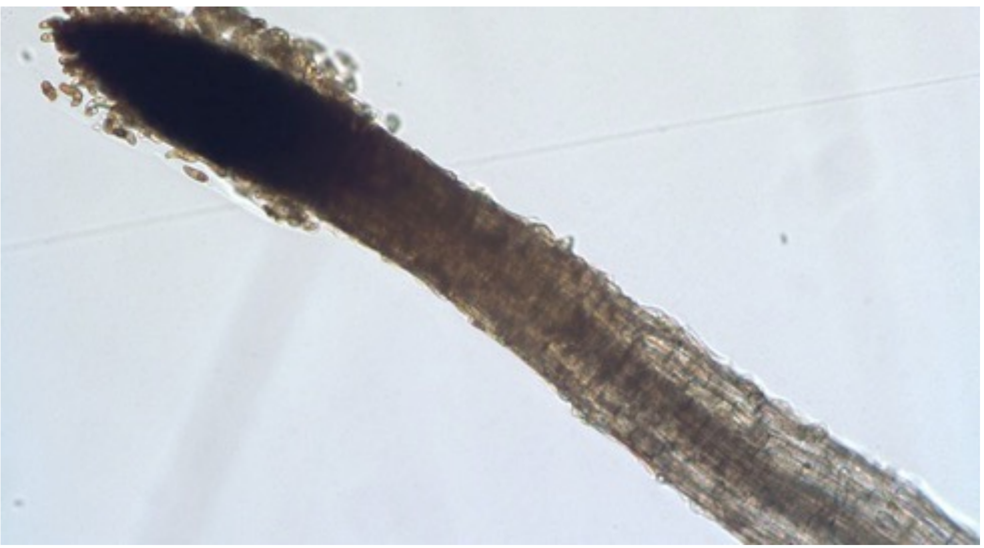
Atsatt PR, Whiteside MD (2014) Novel Symbiotic Protoplasts Formed by Endophytic Fungi Explain Their Hidden Existence, Lifestyle Switching, and Diversity within the Plant Kingdom. PLOS ONE 9(4): e95266. https://doi.org/10.1371/journal.pone.0095266
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0095266

PLOS | ONE

Rhizophagy cycle microbes modulate development of seedlings

- Microbes trigger root hair elongation
- Microbes trigger the gravitropic response in roots
- Microbes increase root branching
- Microbes increase root and shoot elongation

Bermuda grass seedling root in agarose without microbes showing absence of root hairs



Root tip

More developed region of seedling root

Bermuda grass root containing *Pseudomonas* (bacterium)

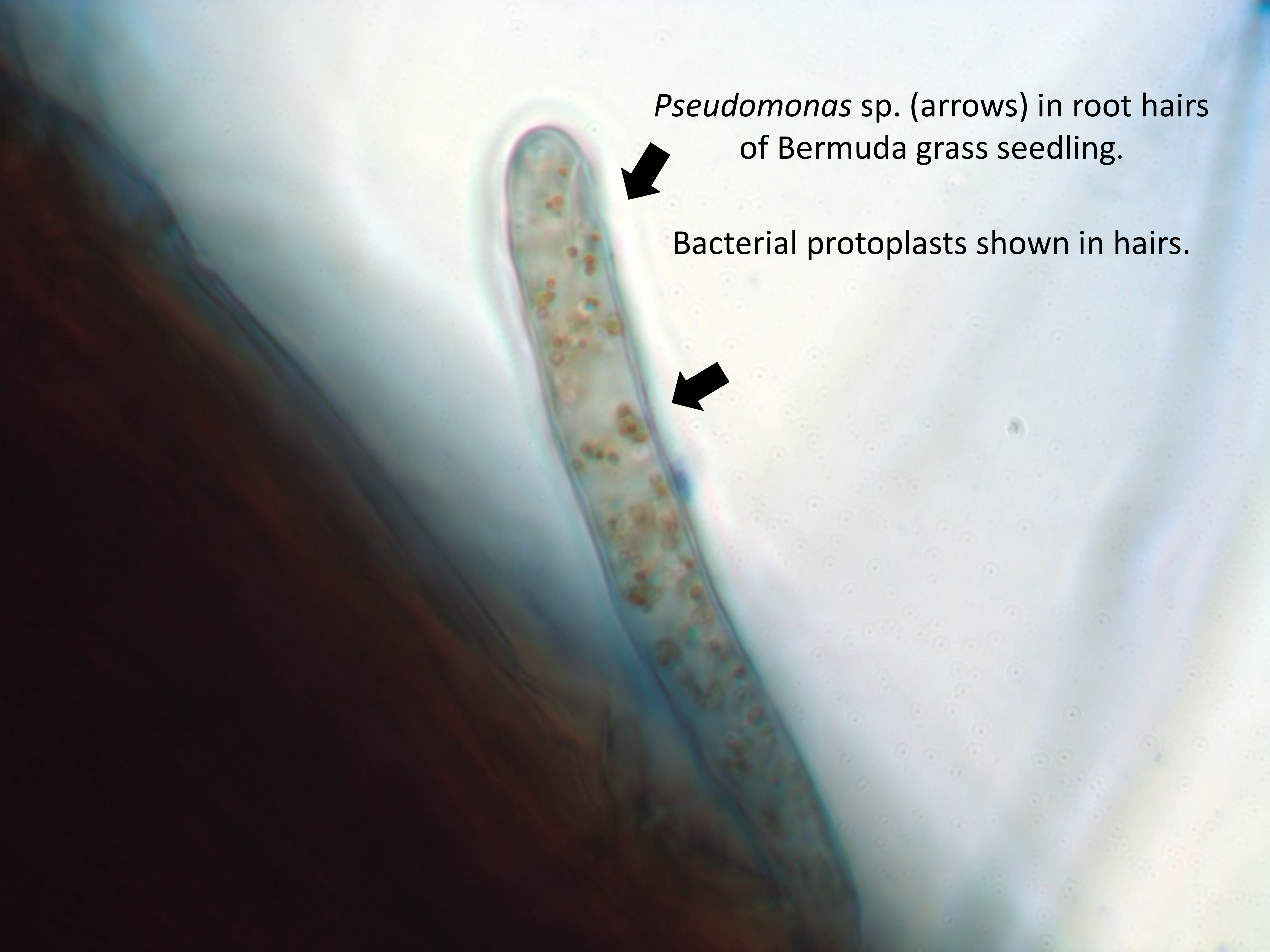
Route of endophyte colonization of root at root tip and reentry to rhizosphere from root hairs

RHIZOPHAGY CYCLE

Bacteria (from seed coat) → Colonize root tip meristem (enter cells) → Intracellular in root parenchyma → Bacteria stimulate root hair formation In root epidermis → Bacteria emerge to surface of hair as the hair elongates → Bacteria colonize soil rhizosphere → Bacteria acquire nutrients in rhizosphere → Colonize root tip meristem (enter cells)

Bermuda grass seedling root containing *Pseudomonas* endophyte.

All brown spots in roots are intracellular bacteria.


Pseudomonas sp. (arrows) in root hairs
of Bermuda grass seedling.
Bacterial protoplasts shown in hairs.

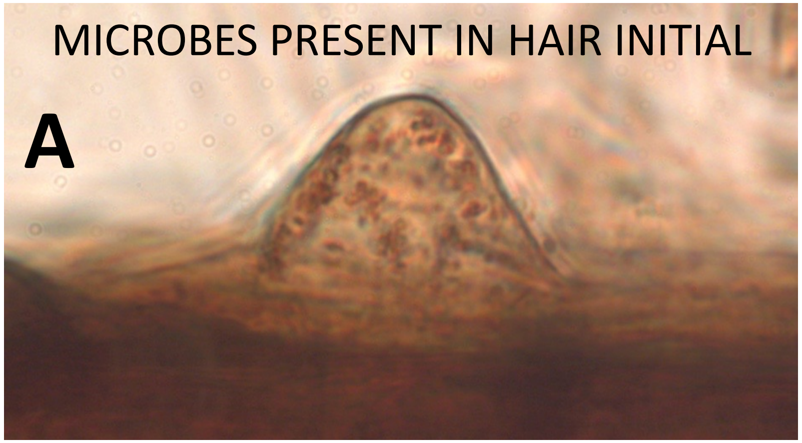
Streptomycin treatment of tomato seedlings

Experiment: All seeds surface disinfected for 20 mins in 4% sodium hypochlorite—then washed.

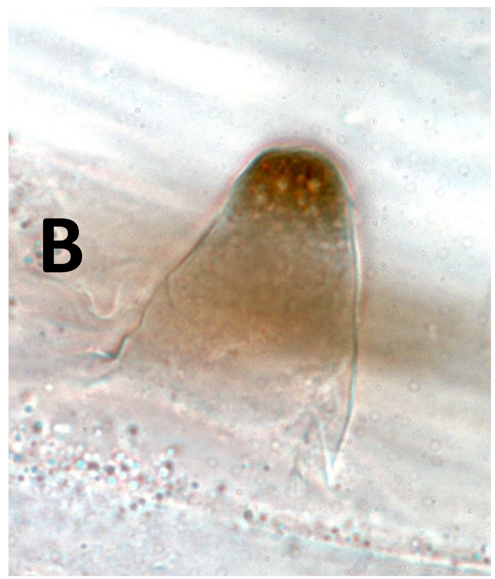
½ seeds treated with streptomycin (100 mg/L) for 24 hours to inhibit growth of endophytic bacteria.

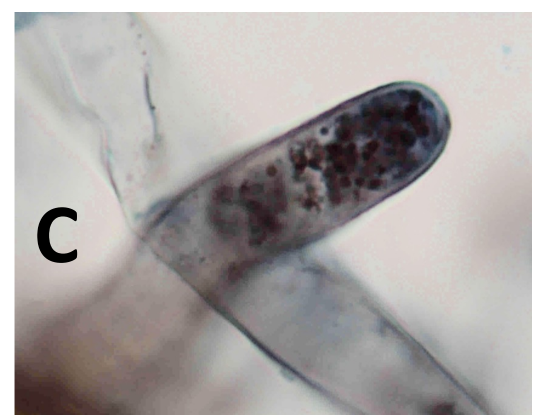
Results: Where bacteria are present I seedlings, tomato seedlings (3-days-old) show root hair formation (arrow); and where antibiotic limits bacterial growth no hairs form.

Mode of action: Streptomycin binds to the small 16S rRNA bacterial ribosome and inhibits protein synthesis.



Tomato Root Hair Initial Without Internal Microbes Do Not Elongate.



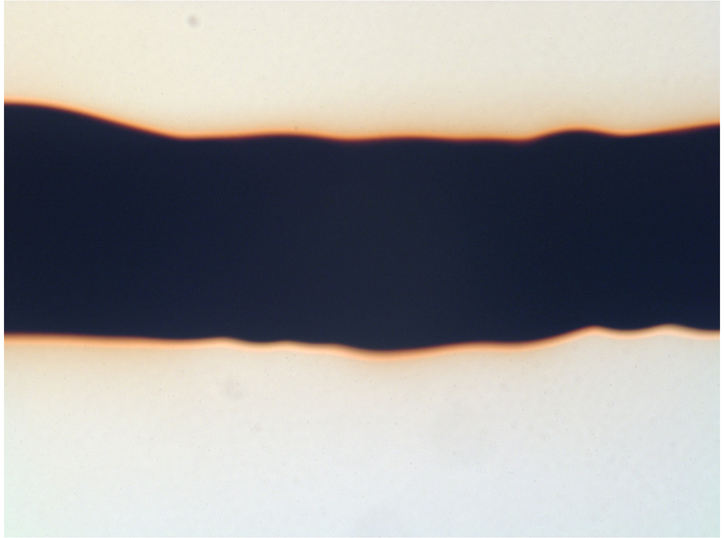


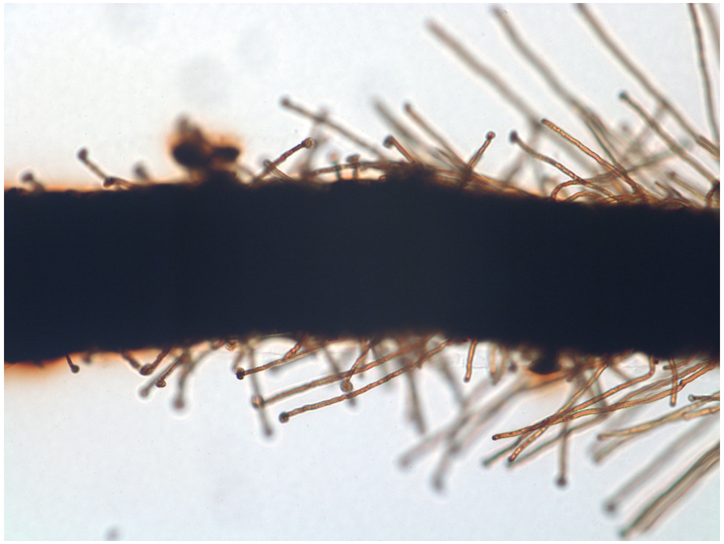
Root hair growth is linked to presence of microbes in hair initials.

Why is root hair growth linked to presence of intracellular bacteria?

Xiaoqian (Ivy) Chang

Experiments to test the 'Microbial Stimulated Cell Growth Hypothesis'





No microbes in seedlings

(Seeds disinfected rigorously.)

Bacterium present

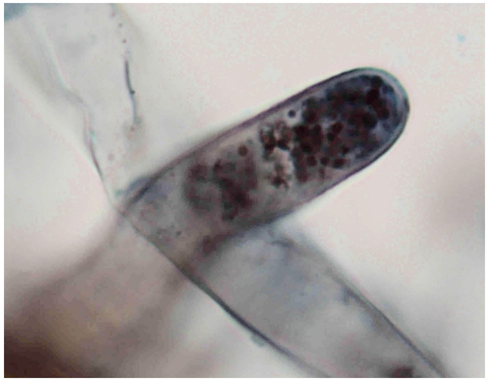
(Pseudomonads fluorescens inoculated onto disinfected seeds.)

What stimulates the plant root hairs to elongate?

Microbe Produced Hormones Hypothesis

Microbial Ethylene and Nitric Oxide Stimulate Root Cell Growth

Microbes in root hair tip
produce ethylene and nitric oxide



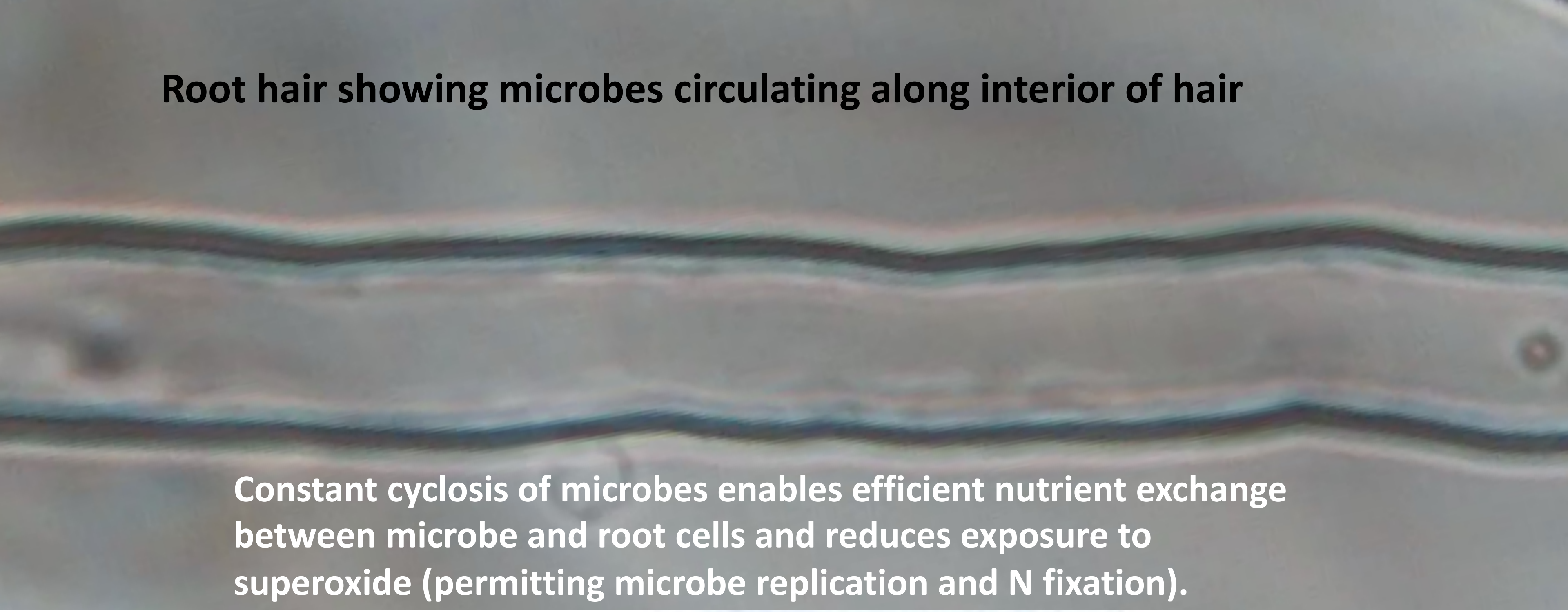
Ethylene and nitric oxide
act as a hormones,
causing root hair to elongate

→ **Ethylene**
Nitric oxide → Root Hair Elongates

Sedge (*Fimbristylis cymosa*)

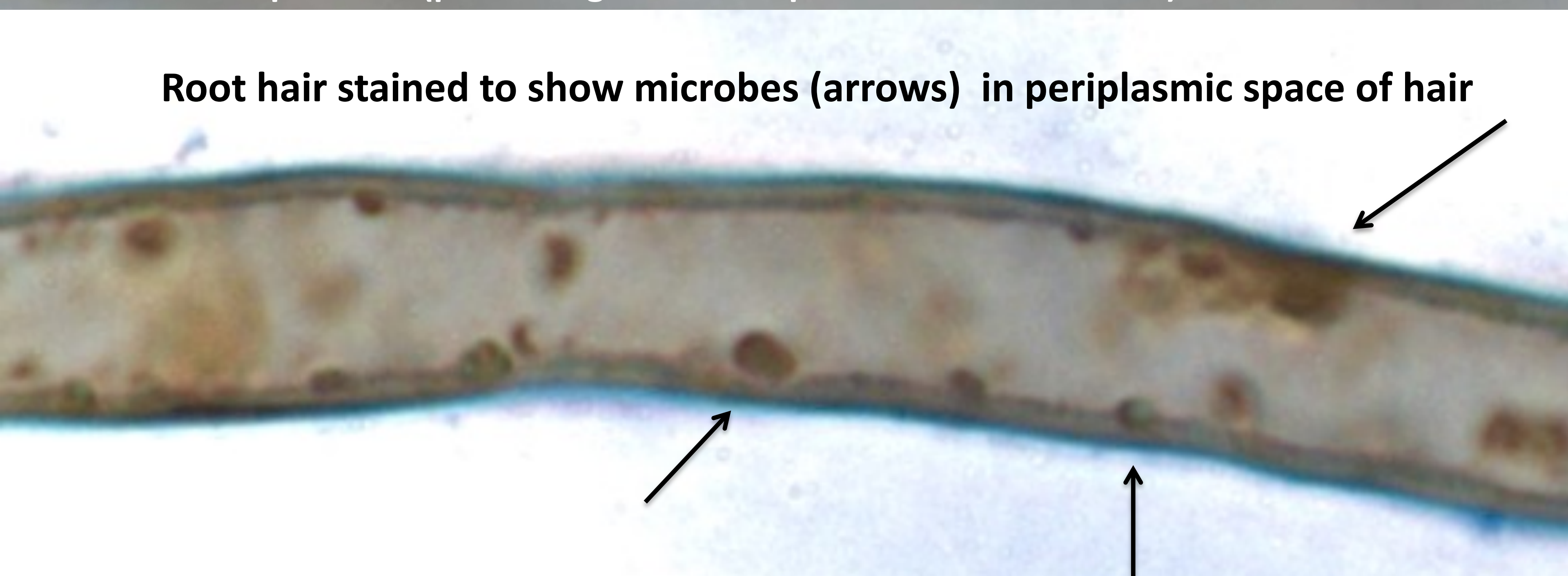
Plant grows in pits and crevices of limestone or in sand along high salt Caribbean shore environments.

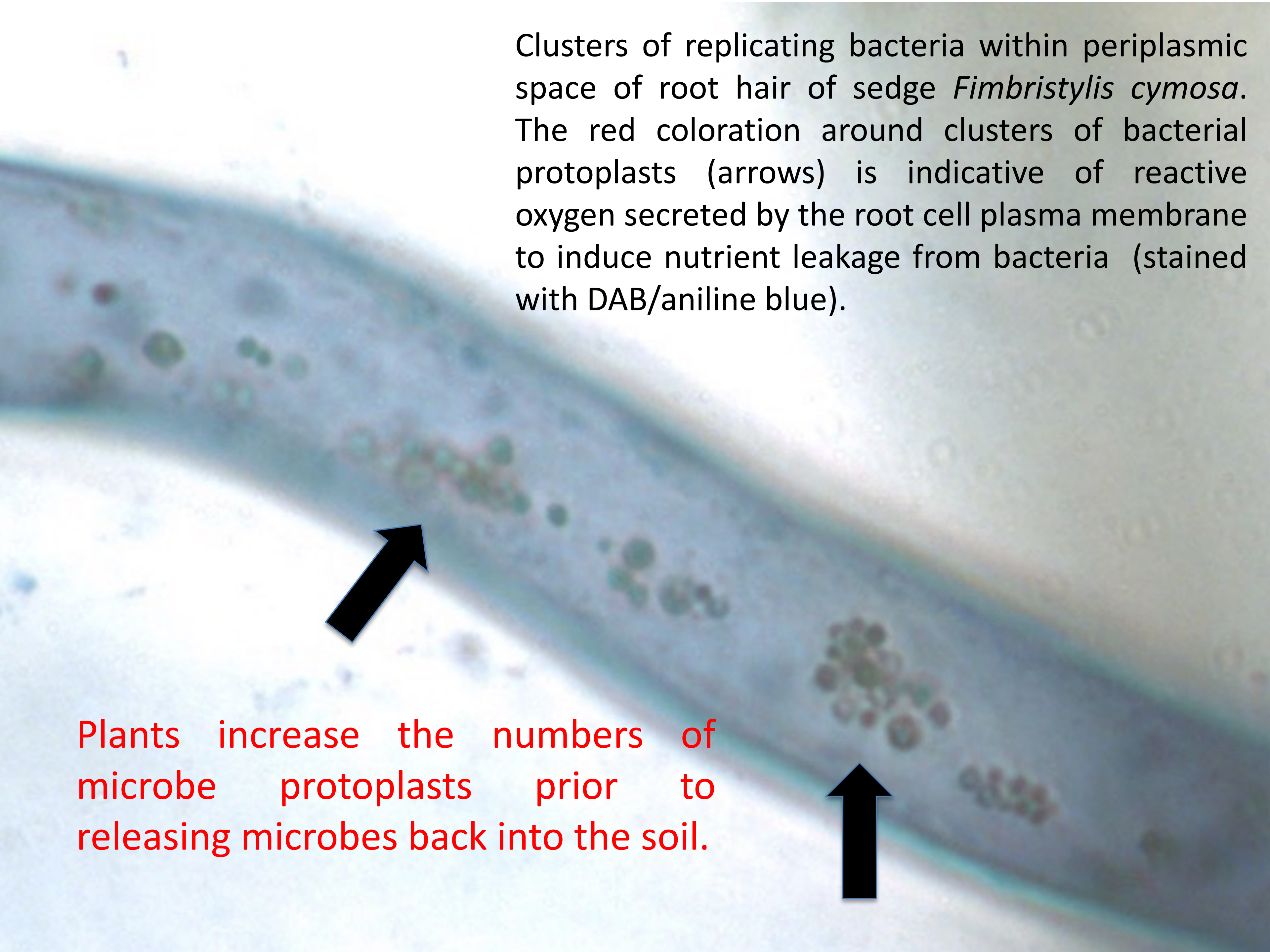
Root hair showing microbes circulating along interior of hair



Constant cyclosis of microbes enables efficient nutrient exchange between microbe and root cells and reduces exposure to superoxide (permitting microbe replication and N fixation).

Root hair stained to show microbes (arrows) in periplasmic space of hair





Clusters of replicating bacteria within periplasmic space of root hair of sedge *Fimbristylis cymosa*. The red coloration around clusters of bacterial protoplasts (arrows) is indicative of reactive oxygen secreted by the root cell plasma membrane to induce nutrient leakage from bacteria (stained with DAB/aniline blue).

Plants increase the numbers of microbe protoplasts prior to releasing microbes back into the soil.

Root hair of sedge *Fimbristylis cymosa*

Cyclosis was measured to move microbes at a rate of 8-11 micrometers/second in root hairs of the sedge *Fimbristylis cymosa*.

Microbes accumulating in hair tip.

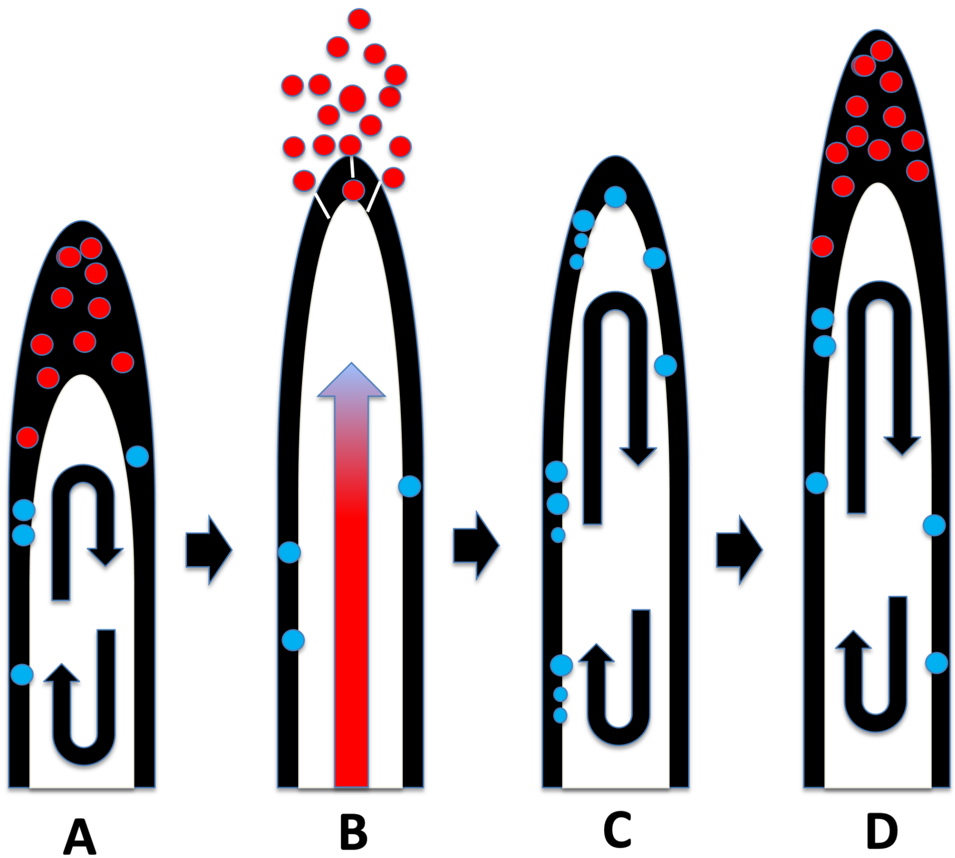
Microbes circulating along length of root hair.

This constant circulation may be a way to induce replication in the microbe protoplasts.

Qiang Chen

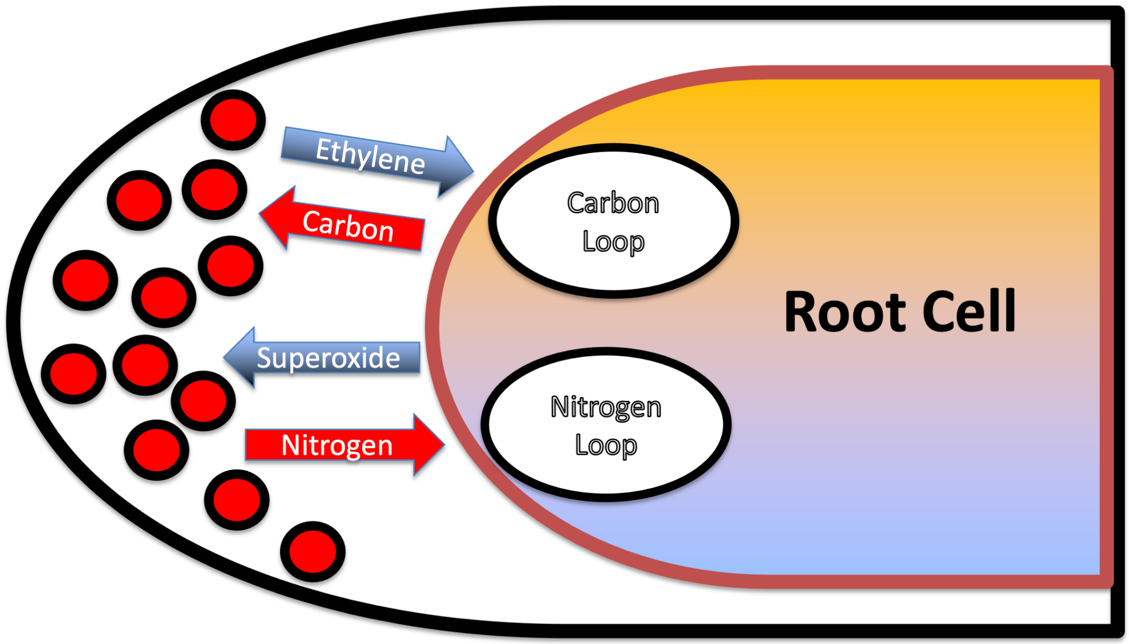
Root hair of sedge (*Fimbristylis cymosa*) showing expulsion of bacteria (large arrow) from the soft-walled hair tip. Red-staining bacterial protoplasts are seen in root hair. A wave of expansion of the hair protoplast propagates from base to tip of hair and this wave forces microbes through pores that form in the hair tip.

Sequence of periodic build-up and ejections of bacteria from root hairs.



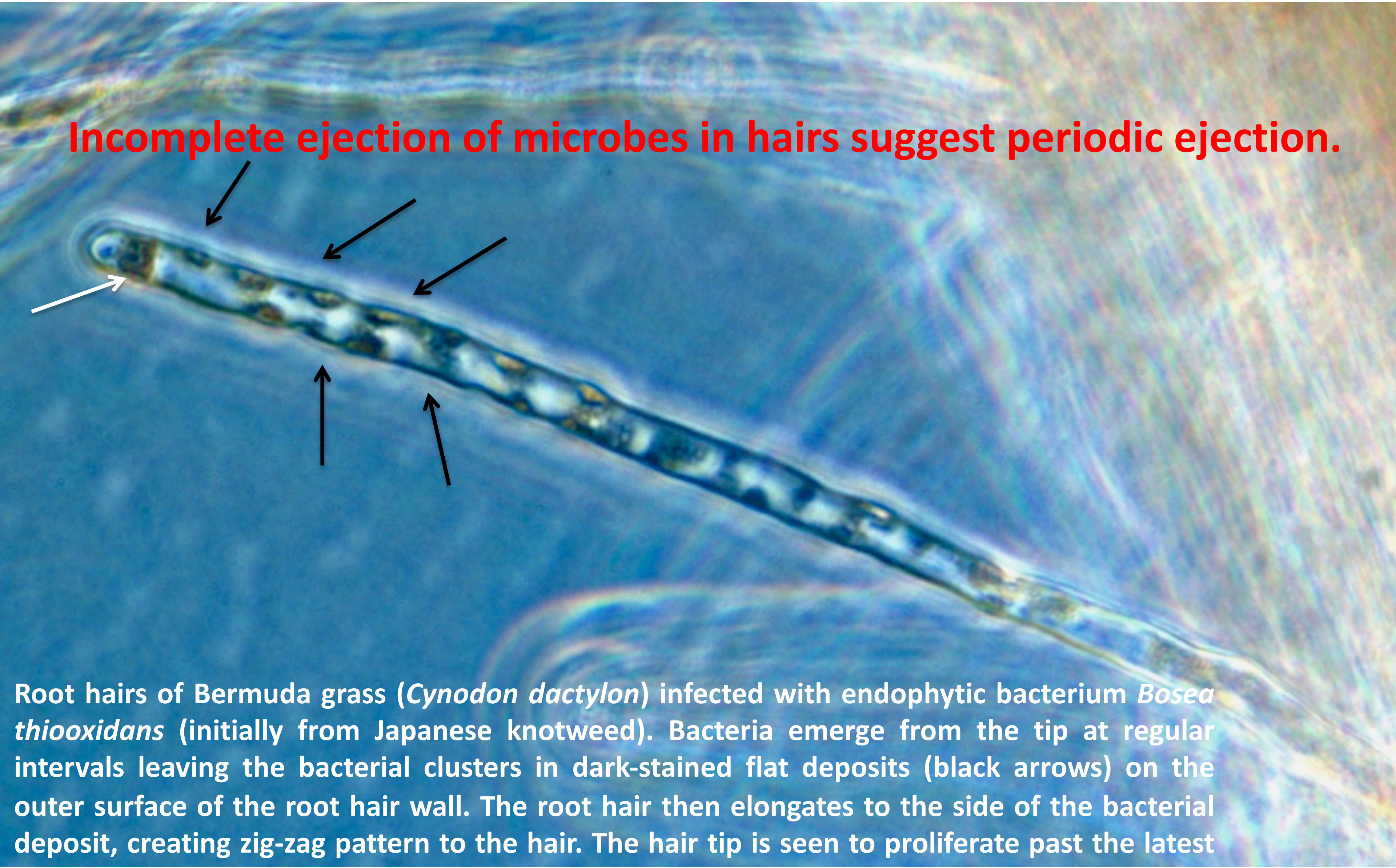
Red bacteria are active in antioxidant nitrogen secretion while blue bacteria are active in nitrogen fixation.

Nitrogen-transfer symbiosis in plant hairs



Nitrogenous antioxidants like nitric oxide are secreted by the bacteria to neutralize superoxide. Nitric oxide combines with superoxide to form nitrate. Nitrate is absorbed directly into the plant.

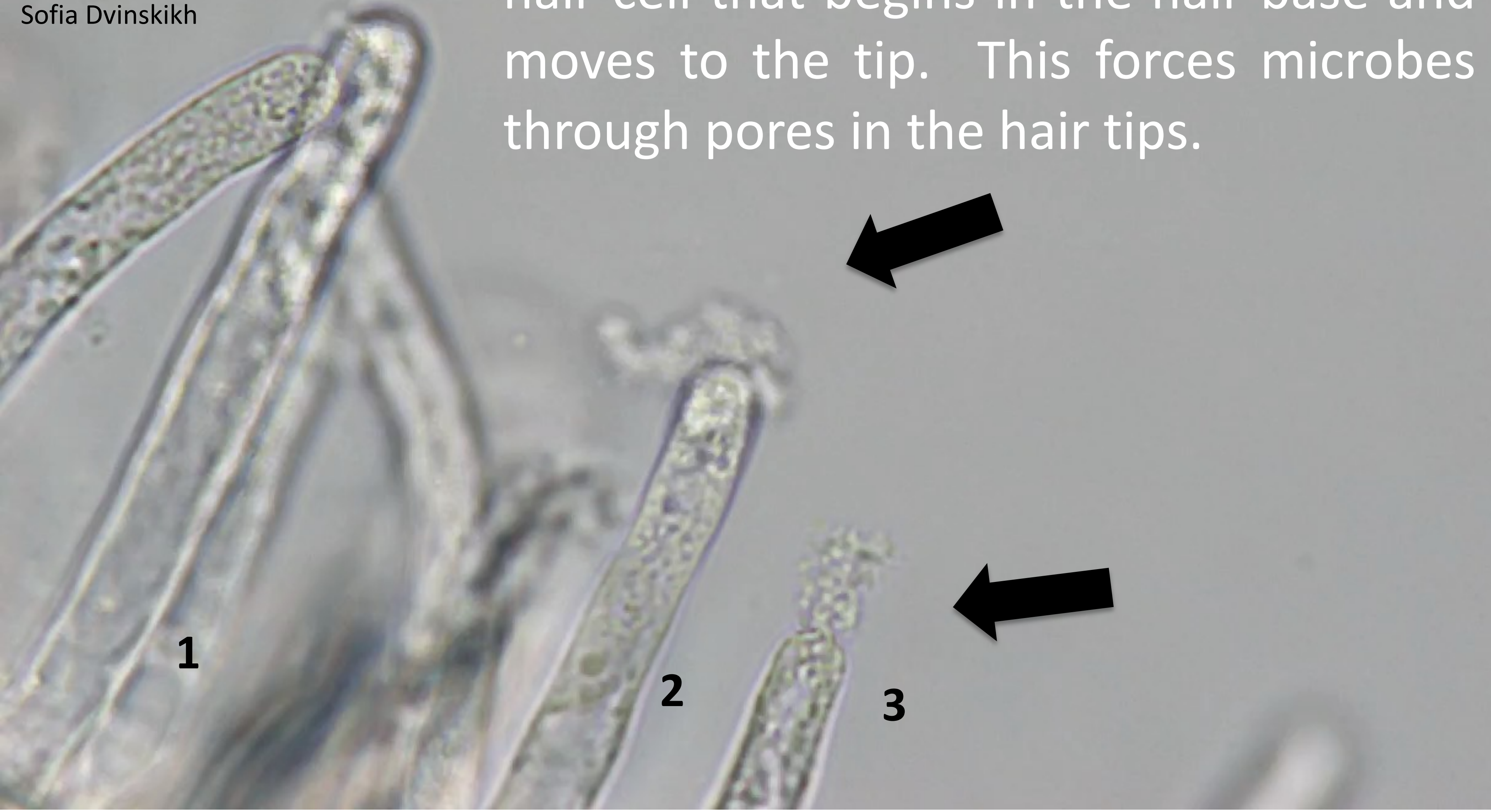
Microbe ejection appears to be periodic rather than continuous. Microbes may be ejected in clusters rather than 1 at a time. This may be the result of ethylene-triggered growth spurts. A growth spurt occurs after a critical mass of bacteria in hair tips secrete enough ethylene to cause hair elongation.



Root hairs of Bermuda grass (*Cynodon dactylon*) infected with endophytic bacterium *Bosea thiooxidans* (initially from Japanese knotweed). Bacteria emerge from the tip at regular intervals leaving the bacterial clusters in dark-stained flat deposits (black arrows) on the outer surface of the root hair wall. The root hair then elongates to the side of the bacterial deposit, creating zig-zag pattern to the hair. The hair tip is seen to proliferate past the latest

Sofia Dvinskikh

This ejection of microbes (arrows) occurs rapidly with a wave of expansion in the hair cell that begins in the hair base and moves to the tip. This forces microbes through pores in the hair tips.



Nutrient Absorption Function of the Rhizophagy Cycle:

Plant nutrient sources

Ivy Chang

1) Nutrients that are dissolved in soil water

2) Nutrients that must be oxidatively extracted from soil microbes within root cells

3) Nutrients obtained from mycorrhizae

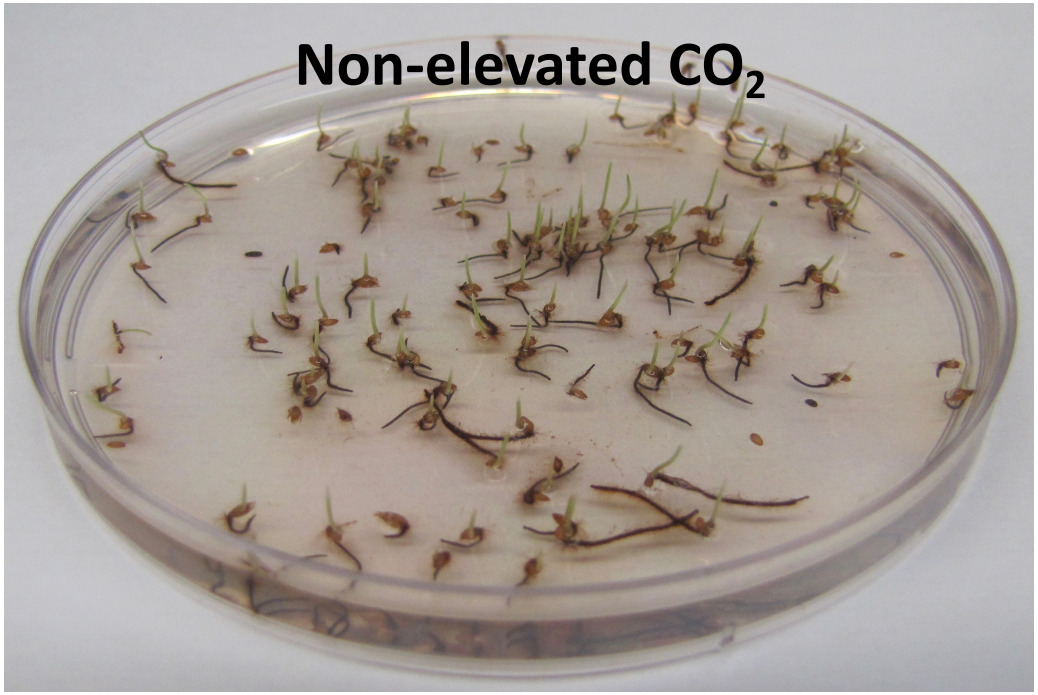
Carbon dioxide inhibits generation of superoxide that plants use to extract nutrients from microbes oxidatively!

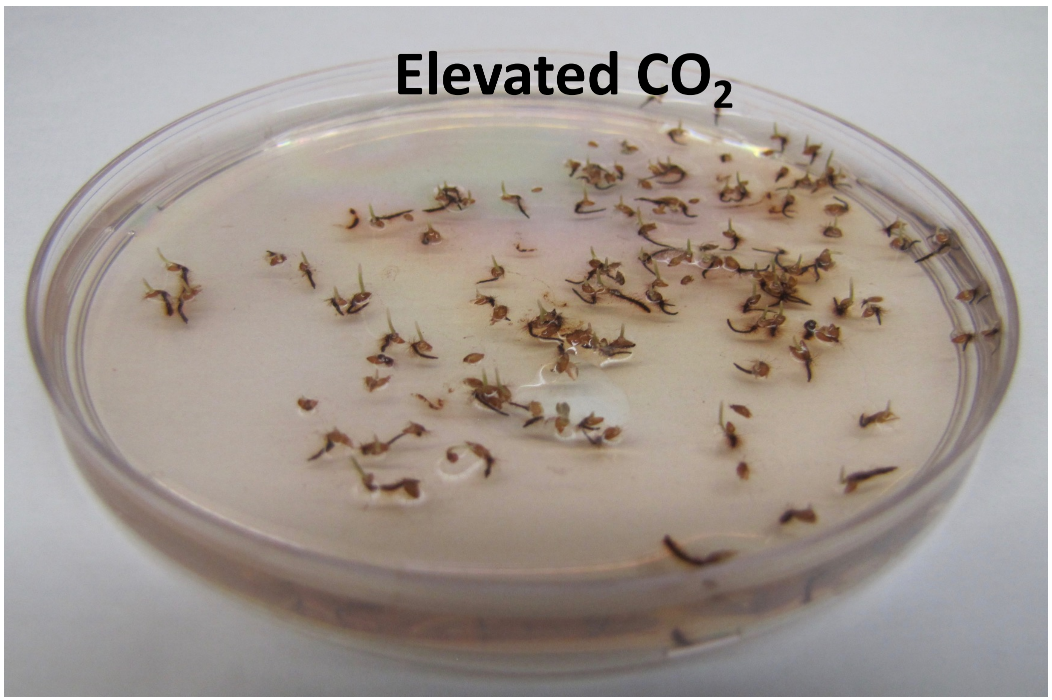
Kogan et al. 1997. Carbon dioxide--a universal inhibitor of the generation of active oxygen forms by cells (deciphering one enigma of evolution). Izvestiia Akademii nauk. Seriia biologicheskaia / Rossiĭskaia akademiia nauk. 1997 Mar-Apr. 204-217.

Bolevick S, et al. 2016. Protective role of carbon dioxide (CO2) in generation of reactive oxygen species. Molecular and Cellular Biochemistry 411: 317-330.

Root superoxide suppression using elevated carbon dioxide gas.

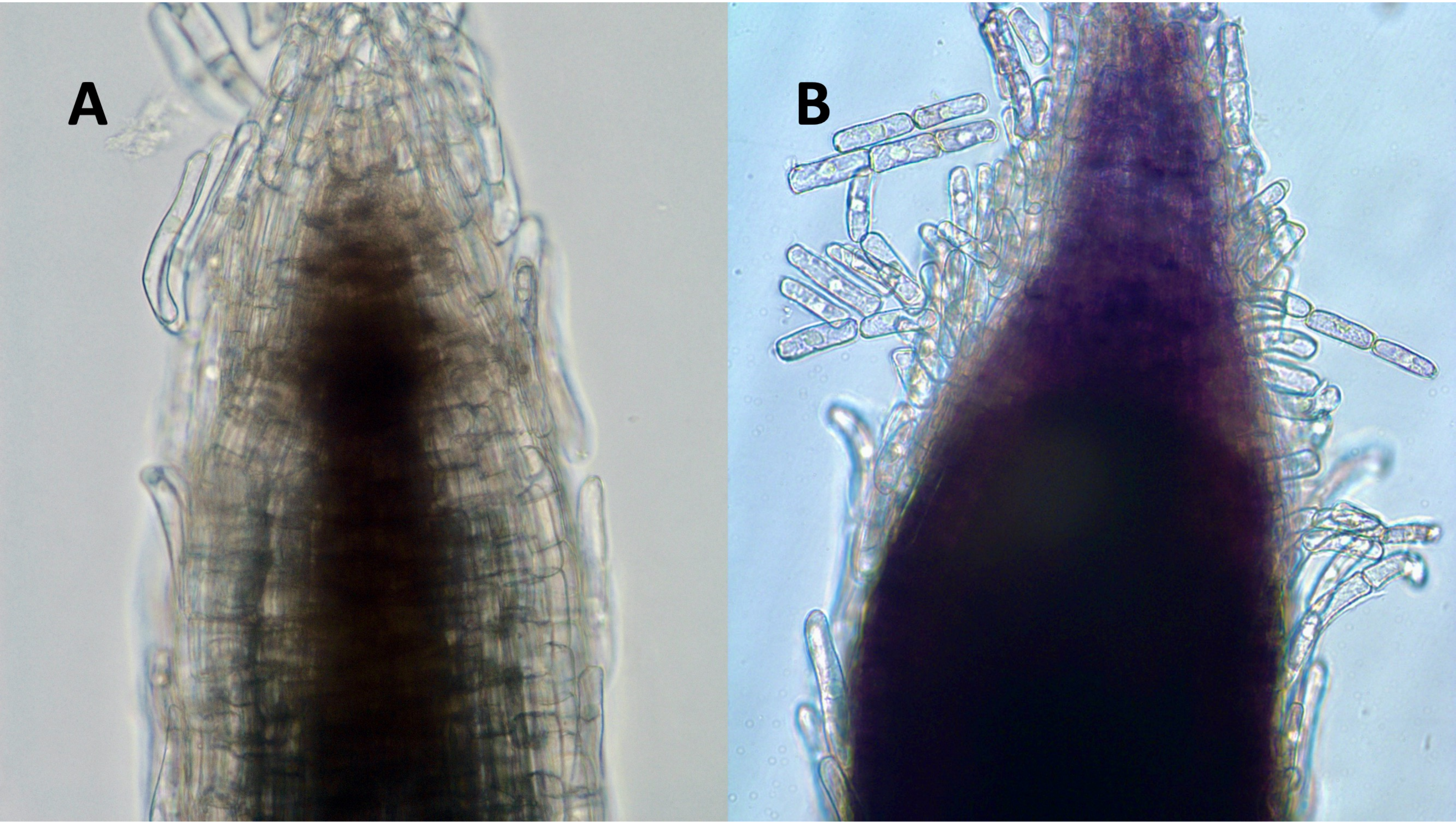
Seedings grown in chambers with elevated CO_2 (1.3 x 10^5 ppm; approximately 0.3 g dry ice/L of air) and chambers with air alone (approx. 410 ppm of CO_2).





Seedlings of *Poa annua* grown on agarose under two levels of atmospheric carbon dioxide. O_2 and N_2 levels were kept constant.

Winter wheat root tips showing: **A.** Repression of superoxide in epidermis layer after elevated CO_2 treatment. **B.** Root tip from non-elevated CO_2 treatment.



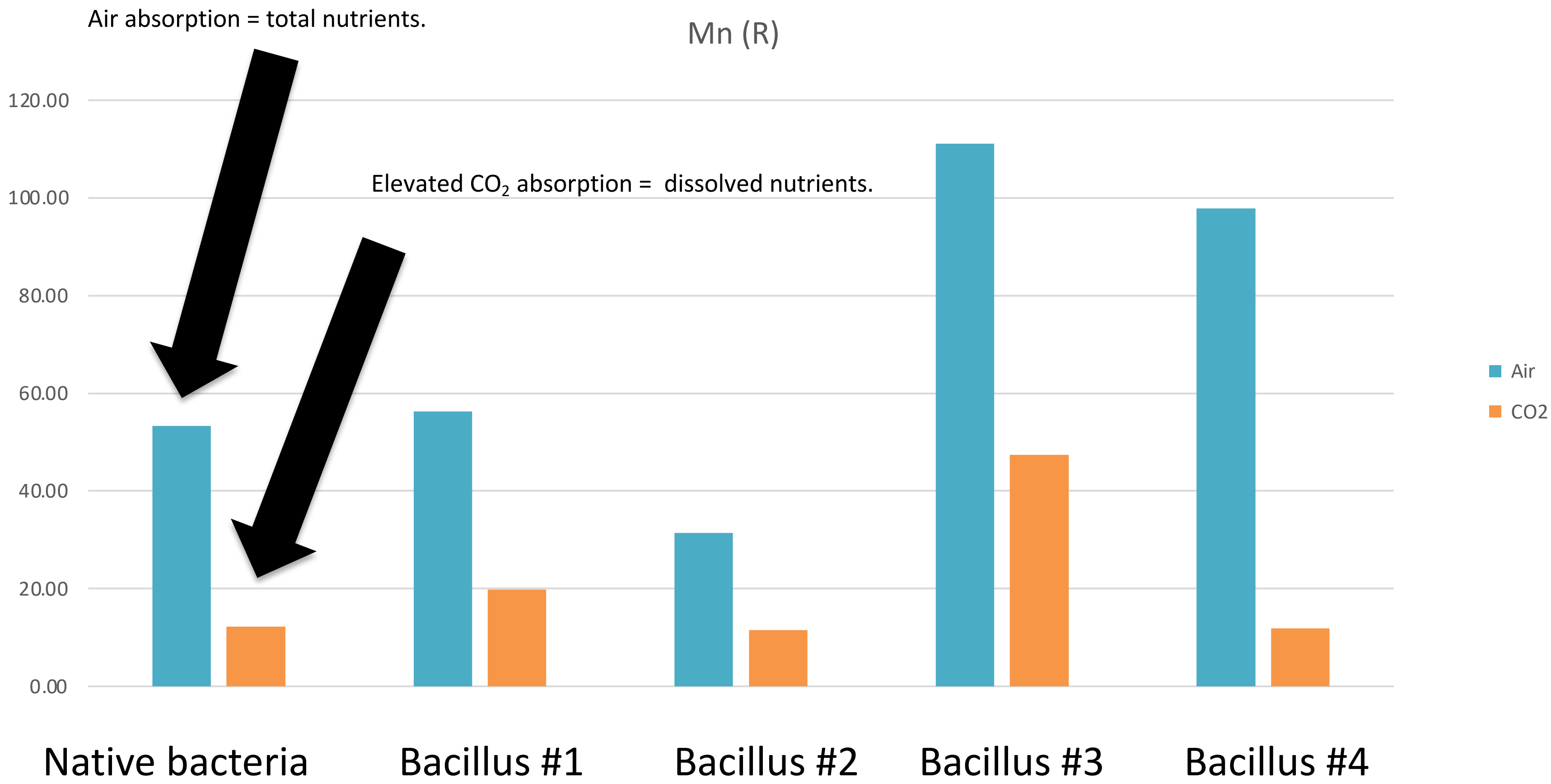
Elevated CO_2 **Non-Elevated CO_2**

Equation to calculate rhizophagy specific nutrients

Oxidatively extracted nutrients from bacteria =

Total nutrients absorbed (air) - dissolved nutrients absorbed (elevated CO_2).

MANGANESE (Mn) in Soybean Roots



Using *Bacillus* spp., the rhizophagy cycle provides 136% to 717% more manganese than absorption of soil dissolved manganese.

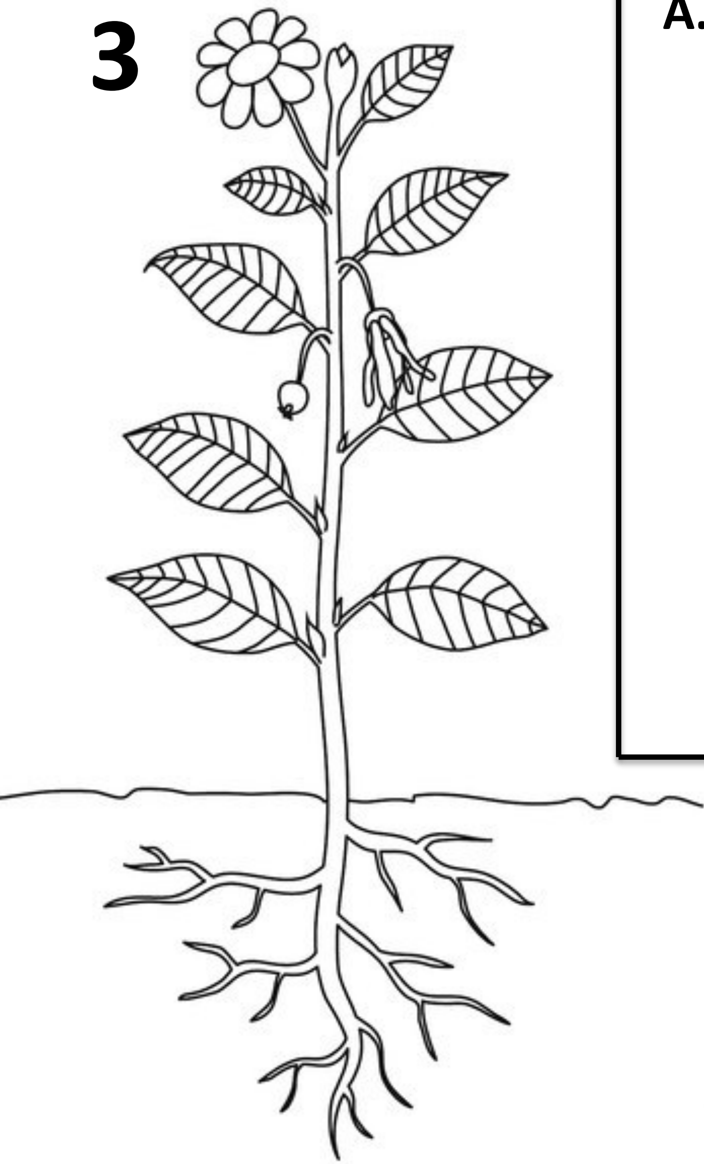
Rhizophagy Nutrients:

Micronutrients tend to be favored in oxidative extraction from bacteria in the rhizophagy cycle.

Sequence of oxidative extraction for nutrients:

Mn > Fe > Ca> Mg > S > Cu > N > Zn > P > K

3



A. Three Beneficial Outcomes of Rhizophagy Symbiosis

1. Plants absorb nutrients from microbes

Rhizophagy microbes enter plant roots with nutrients

2. Increased oxidative stress tolerance in plants

Increased reactive oxygen activity in root cells

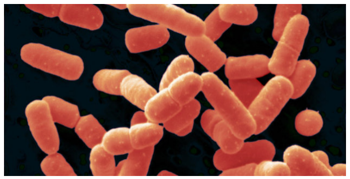
3. Soil fungal pathogens have reduced virulence

Soil fungi drained of nutrients by rhizophagy cycle microbes

B. Nutrient Flow

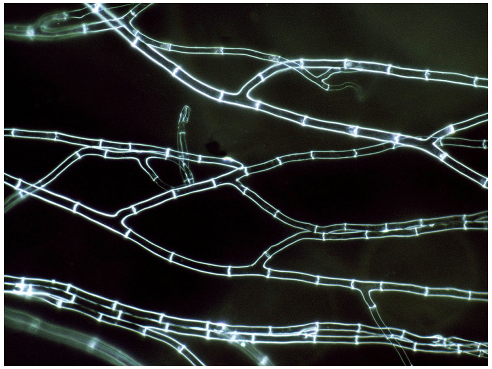
The plant takes nutrients from rhizophagy cycle microbes, and provides photosynthate to soil microbes.

⇄



Rhizophagy cycle microbes take nutrients from microbial community.

⇄



The soil microbial community liberates and absorbs nutrients from soil.

Nitrogen Fixation by Endophytes

The first land plants (Bryophyta) internalized bacteria into their cells (hairs) to obtain nitrogen from them!

In plant hairs plants cultivate and extract nitrogen from nitrogen-fixing bacteria.

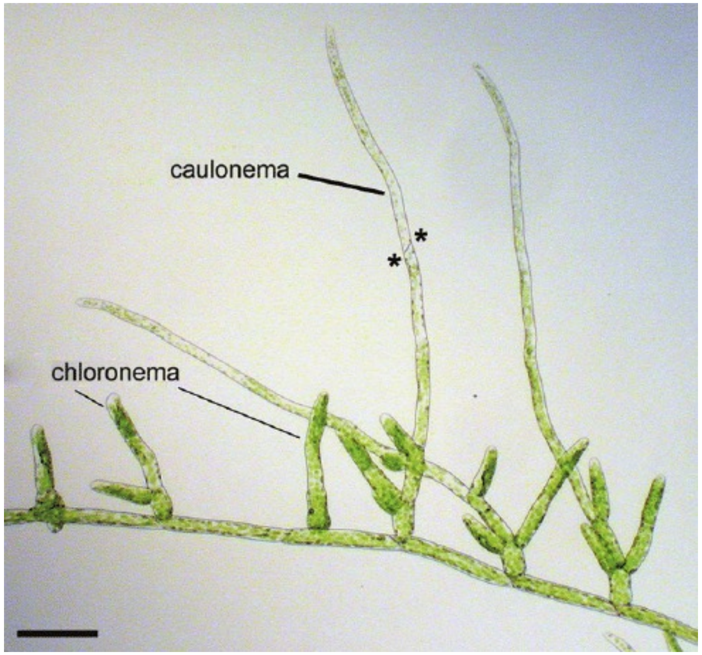

Lena Struwe


Nicole Vaccaro


Blair Young

Moss (*Physcomitrella patens*) gametophytes have chloroplasts and do photosynthesis, but they also have non-photosynthetic tissues where nitrogen-transfer endosymbiosis occurs. Achlorophyllous filaments termed 'caulonemata' contain bacteria that transfer nitrogen to the moss gametophyte.

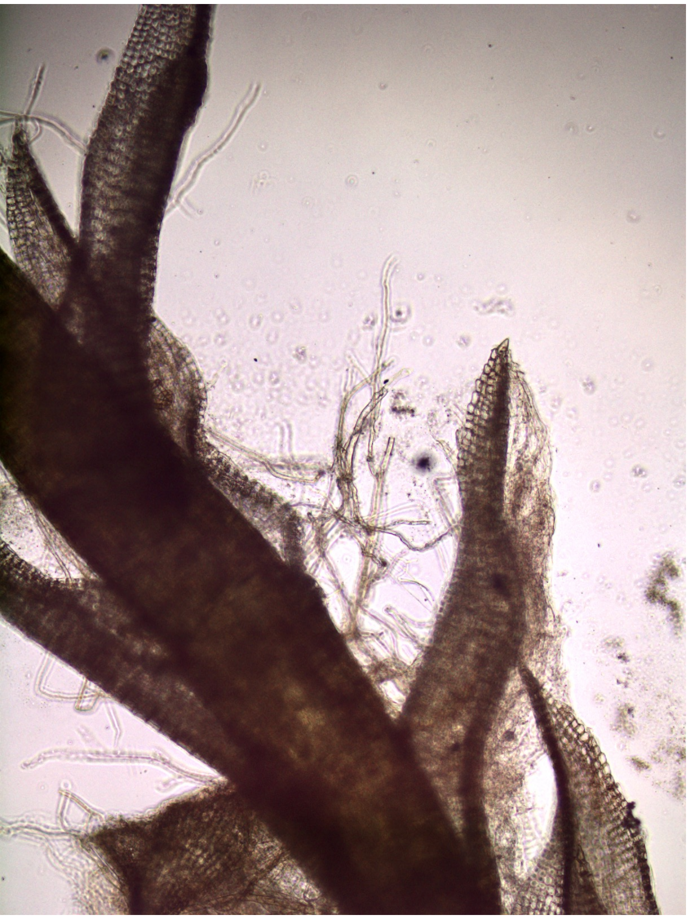

The brown filaments (arrow) in this image are caulonemata.



Chloronemata are photosynthetic filaments; while caulonemata function to fix atmospheric nitrogen and transfer it to the photosynthesizing gametophyte.

Caulonemata of moss stained for ethylene (blue color) around intracellular bacteria (arrows). Stain is ammonium molybdate.

Moss filaments are the earliest versions of plant hairs (trichomes) and they function to extract nitrogen from bacteria.



For histochemical staining protocols see: Chang X, Kingsley KL, White JF. 2021. Chemical Interactions at the Interface of Plant Root Hair Cells and Intracellular Bacteria. *Microorganisms*. 9(5):1041. https://doi.org/10.3390/microorganisms9051041

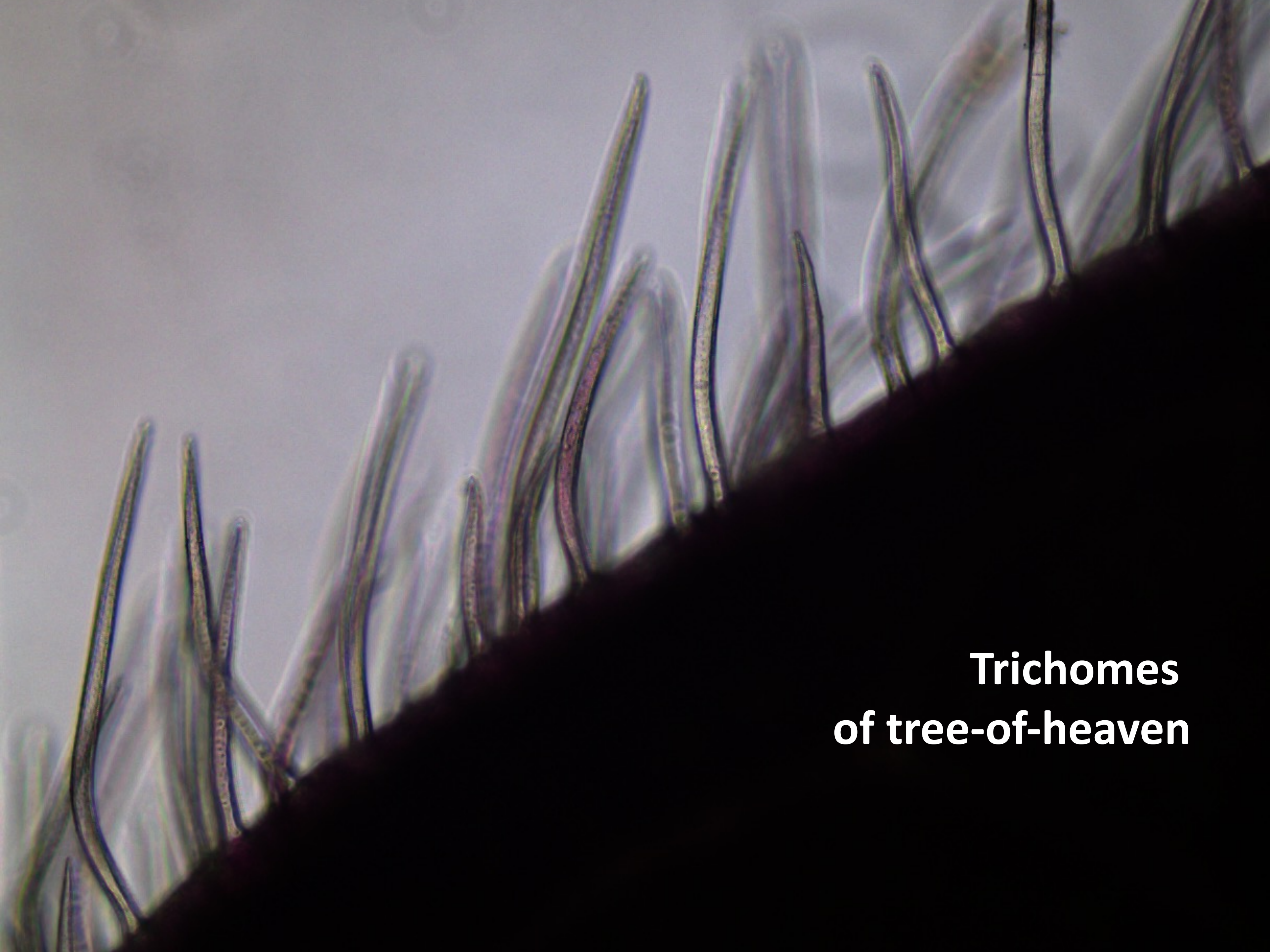
The very first land plants used endophytic microbes for nutrients from the start. These endophytes are about delivering nitrogen to plants.

Liverwort (*Riccia* sp.)

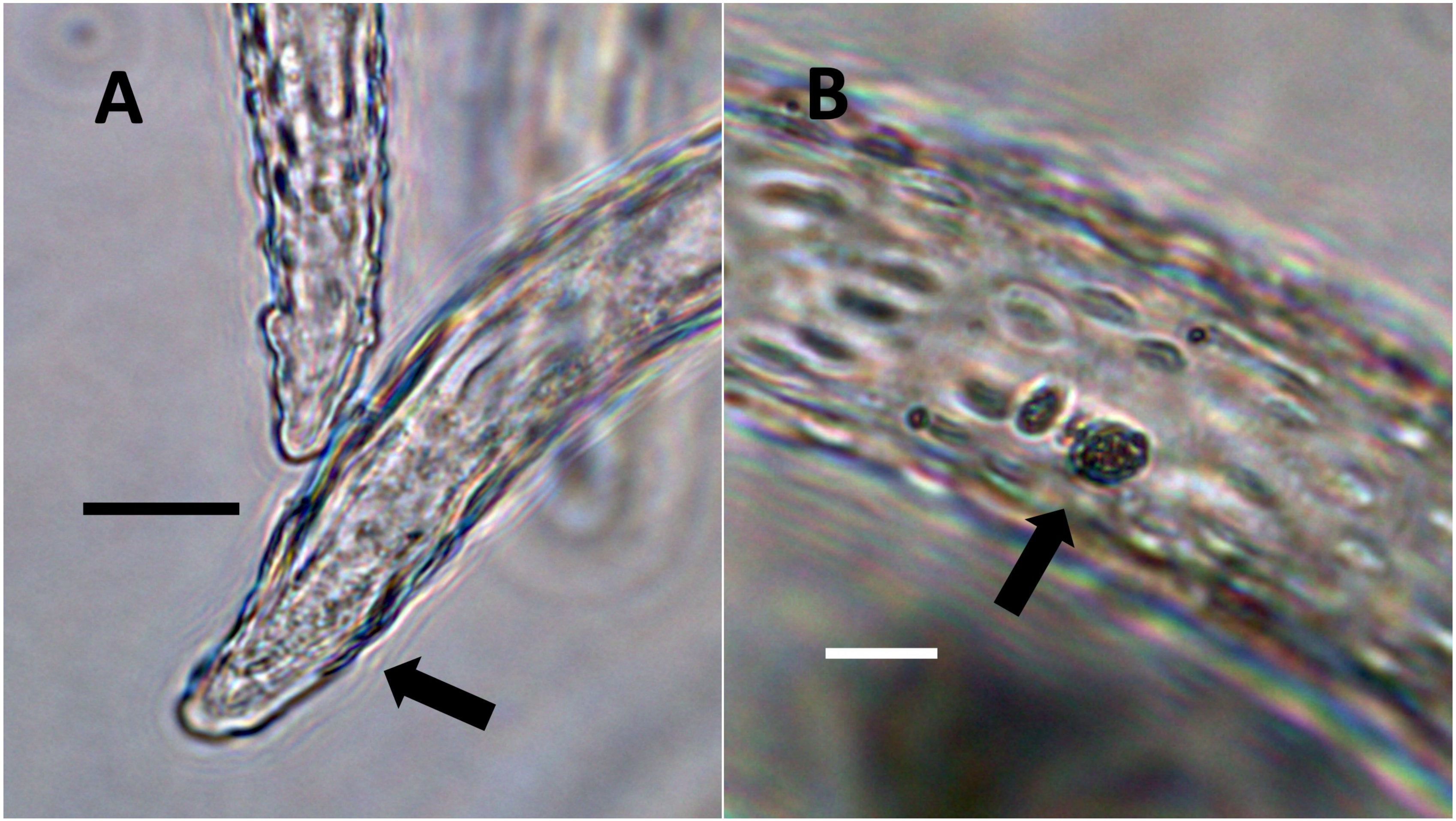
Plant lacks leaves and roots-but has non-photosynthetic filaments that contain bacteria (white arrow).

Nitrogen-fixing endophytes in leaves of plants

Tree-of-heaven (*Ailanthus altissima*)


Trichomes
of tree-of-heaven

Symbiosis in pitted filamentous trichomes of tree-of-heaven



Pitted trichome of tree-of-heaven (*Ailanthus altissima*) showing bacteria. A. Developing trichome stained with acidified diphenylamine showing nitrate (blue color) around bacteria (arrow) in the tip of the trichome (Bar = 10 µm). B. Trichome stained with sulfur monochloride to show bacteria (arrow) emerging from lateral pits in wall (Bar = 10 µm).

Molecular Oxygen (air)

O_2

Arginine

(provided in root exudates)

Microbial Ethylene Synthase

NADPH oxidase (in root cell plasma membrane)

$$2(C_2H_4) + 16O^- \longrightarrow 4(H_2O_2) + 4(CO_2)$$

Ethylene **Superoxide** **Hydrogen Peroxide** **Carbon Dioxide**

Ethylene triggers cell growth and superoxide secretion.

Superoxide secreted onto microbes in periplasm causes loss of cell walls and nutrient leakage from microbe protoplasts.

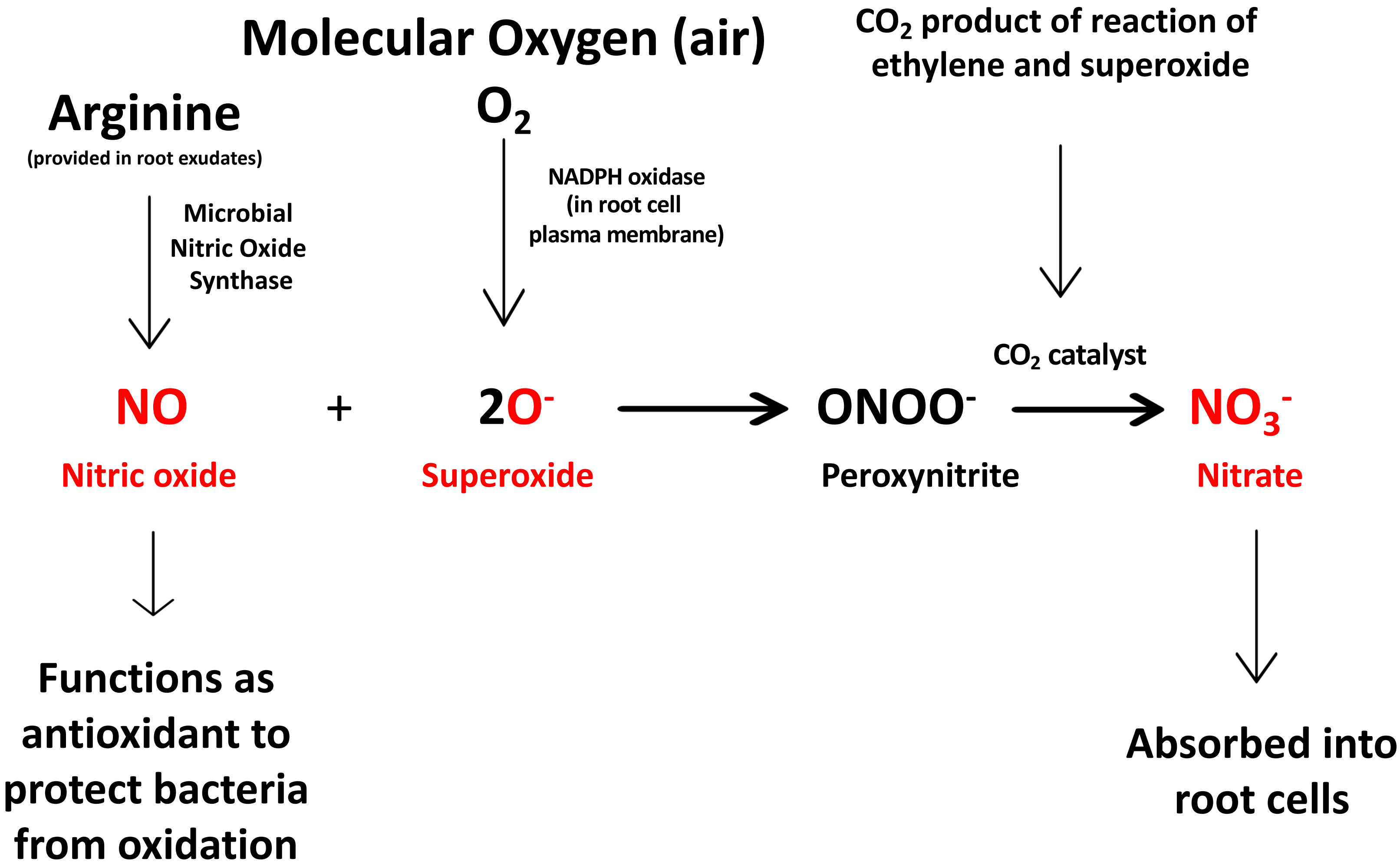
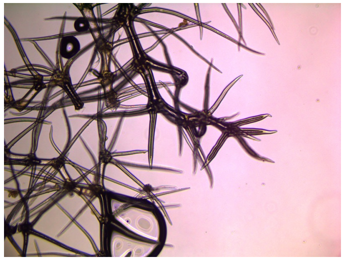
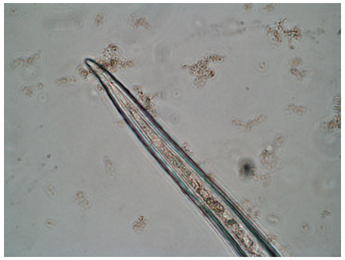

Molecular Oxygen (air)
CO2 product of reaction of ethylene and superoxide
Arginine
(provided in root exudates)
O2
Microbial Nitric Oxide Synthase
NADPH oxidase (in root cell plasma membrane)
CO2 catalyst
NO + 2O- → ONOO- → NO3-
Nitric oxide
Superoxide
Peroxynitrite
Nitrate
Functions as antioxidant to protect bacteria from oxidation
Absorbed into root cells

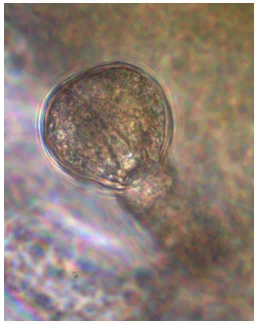
Table. Histochemical analyses of bacterial endophytes in leaves and fruits of plants



Dendroid trichome
Hairy mullein



Filamentous trichome
Tree-of-heaven



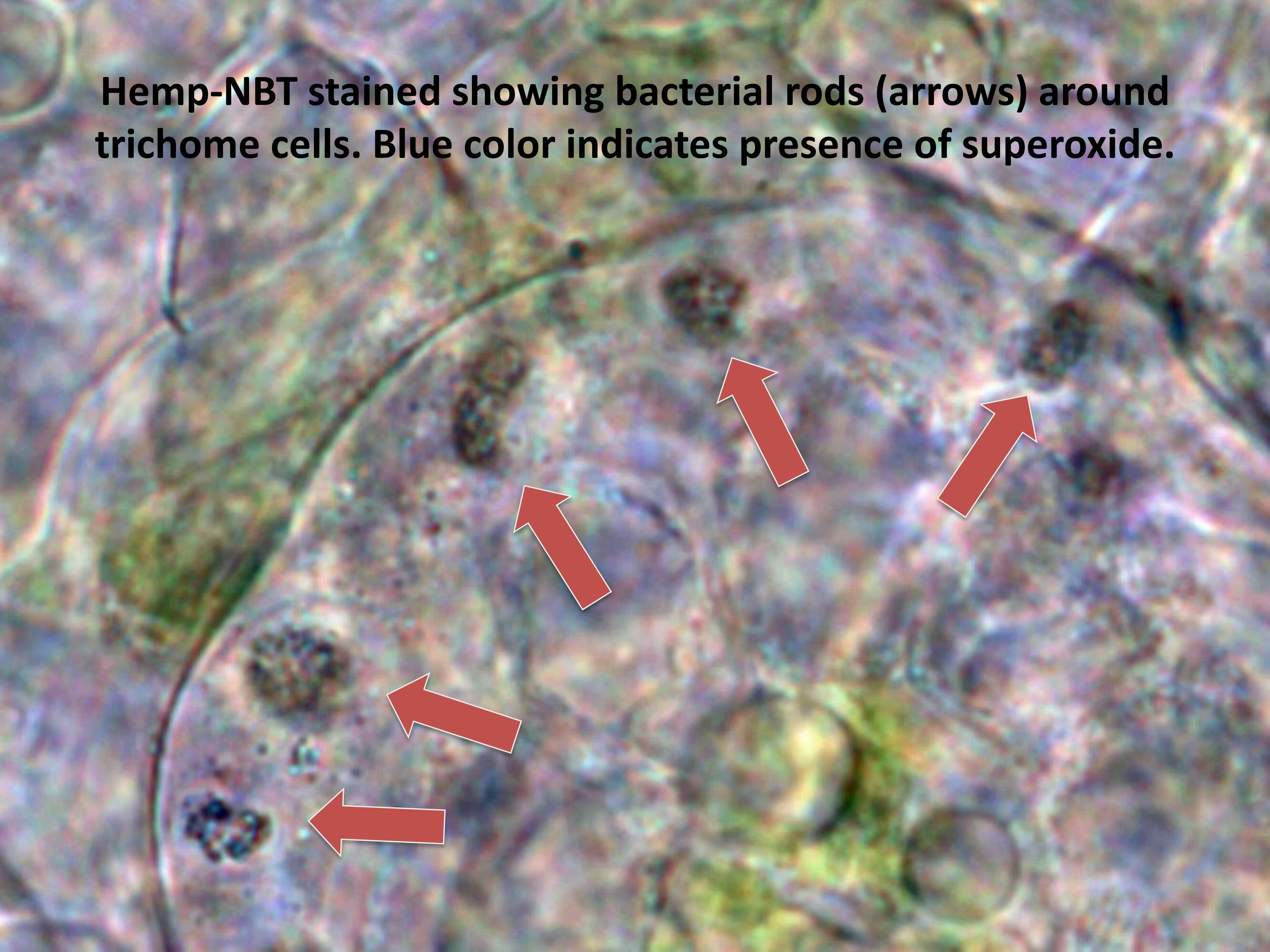
Glandular trichome
Hemp and hops

Species	Family	Trichome Type		Ethylene			Reducing Sugar	Superoxide	H_2O_2	Nitric Oxide	Nitrate	Comments
		F	G	AM	SM	PP	CS	NBT	DAB	FS	DS	
Ailanthus altissima	Simaroubaceae	√		√	√	√	√	√	√	√	√	
Eupatorium purpureum	Asteraceae	√										
Humulus lupulus	Cannabaceae	√	√	√	√	√	√	√	√	√	√	Glandular trichomes on leaf veins
Glycine max	Fabaceae	√		√		√	√	√	√	√	√	
Solanum dulcamara	Solanaceae	√	√	√	√	√	√			√	√	Glandular tichomes on leaf veins
Rhus glabra	Anacardiaceae	√	√	√		√		√		√	√	
Celtis occidentalis	Ulmaceae	√		√							√	
Thespesia populnea	Malvaceae	√	√			√		√		√	√	Glandular and peltate trichomes
Verbascum thapsis	Scrophulariaceae	√				√		√		√	√	Dendroid trichomes and glandular
Cannabis sativa	Cannabaceae	√	√			√		√	√	√	√	Glandular and peltate trichomes
Euphorbia maculatum	Euphorbiaceae		√	√				√			√	Enations/glandular trichomes at leaf serrations
Apocynum cannabinum	Apocynaceae										√	
Monotropa uniflora	Ericaceae	NA	NA	√			√	√			√	Bacteria in nuclei and in cytoplasm of leaf epidermal cells

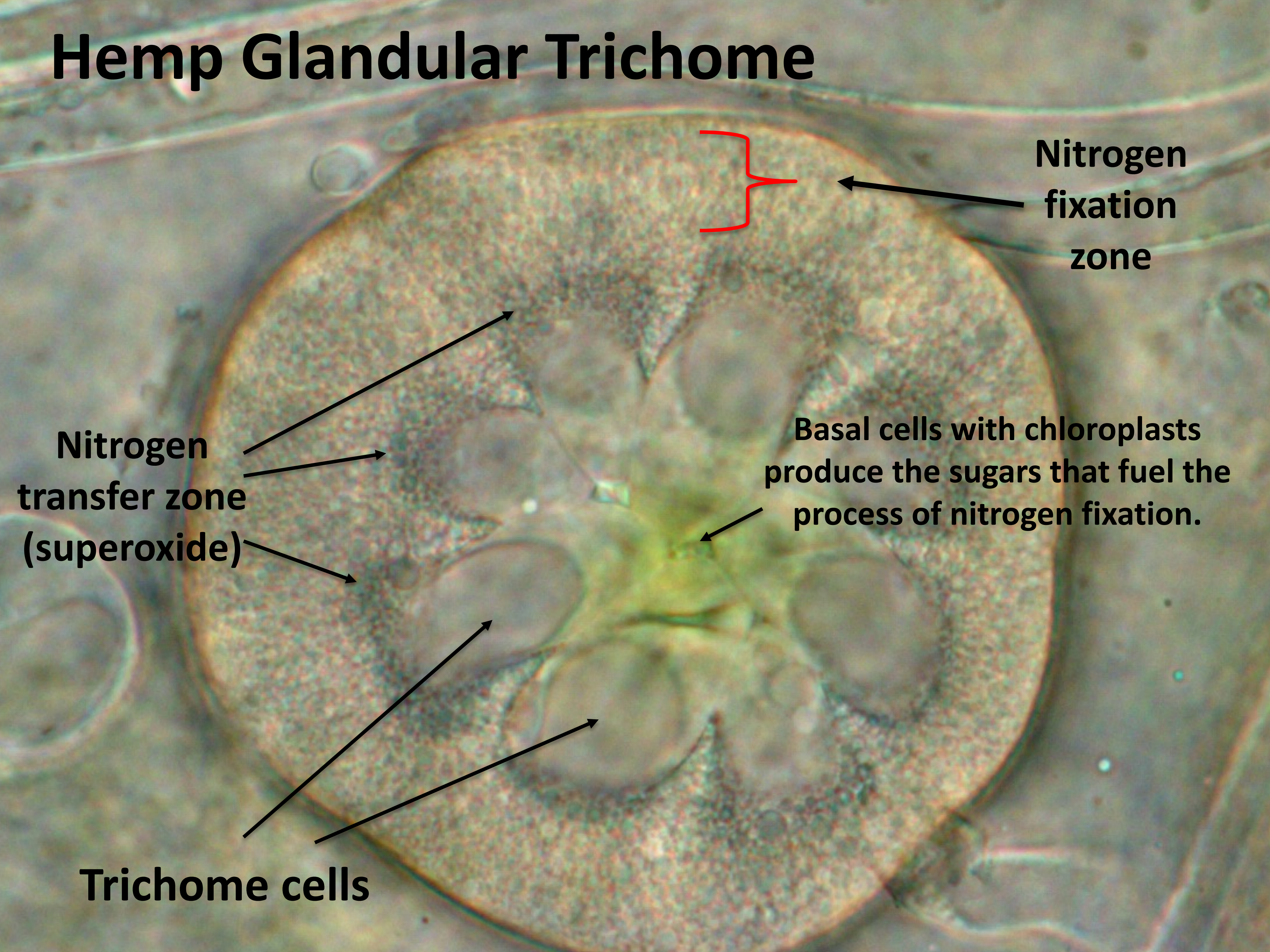
Staining reagents are as follows: AM= ammonium molybdate, SM= sulfur monochloride, PP= potassium permanganate, CS= copper sulfate, NBT= nitroblue tetrazolium, DAB= daiminobenzidine tetrahydrochloride, FS= ferric (II) sulfate, DS= diphenylamine sulfate

Hemp leaves bear trichomes (leaf hairs) that contain endophytic bacteria.

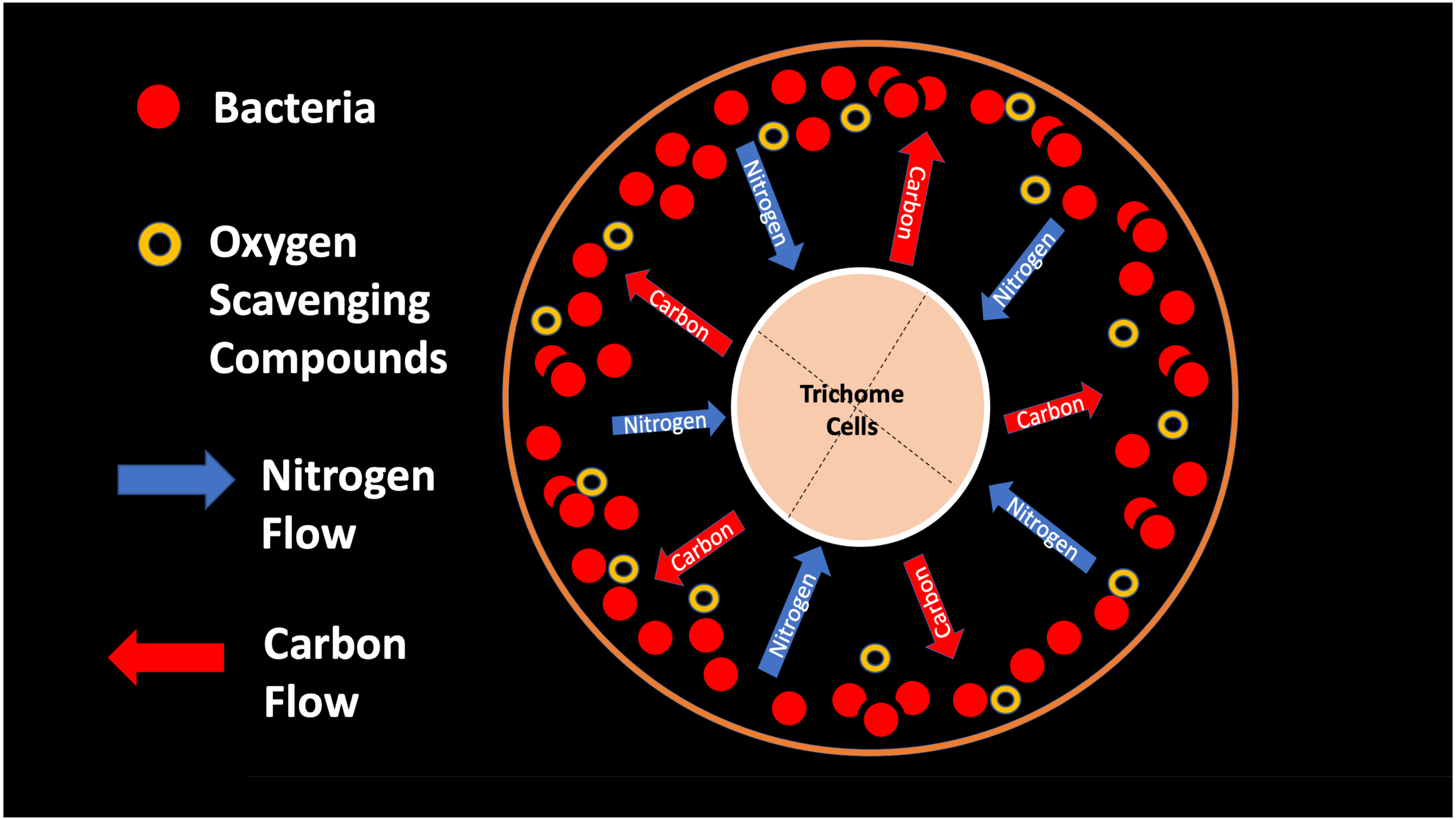
Hemp glandular trichomes unstained


Hemp-NBT stained showing bacterial rods (arrows) around
trichome cells. Blue color indicates presence of superoxide.

Hemp Glandular Trichome

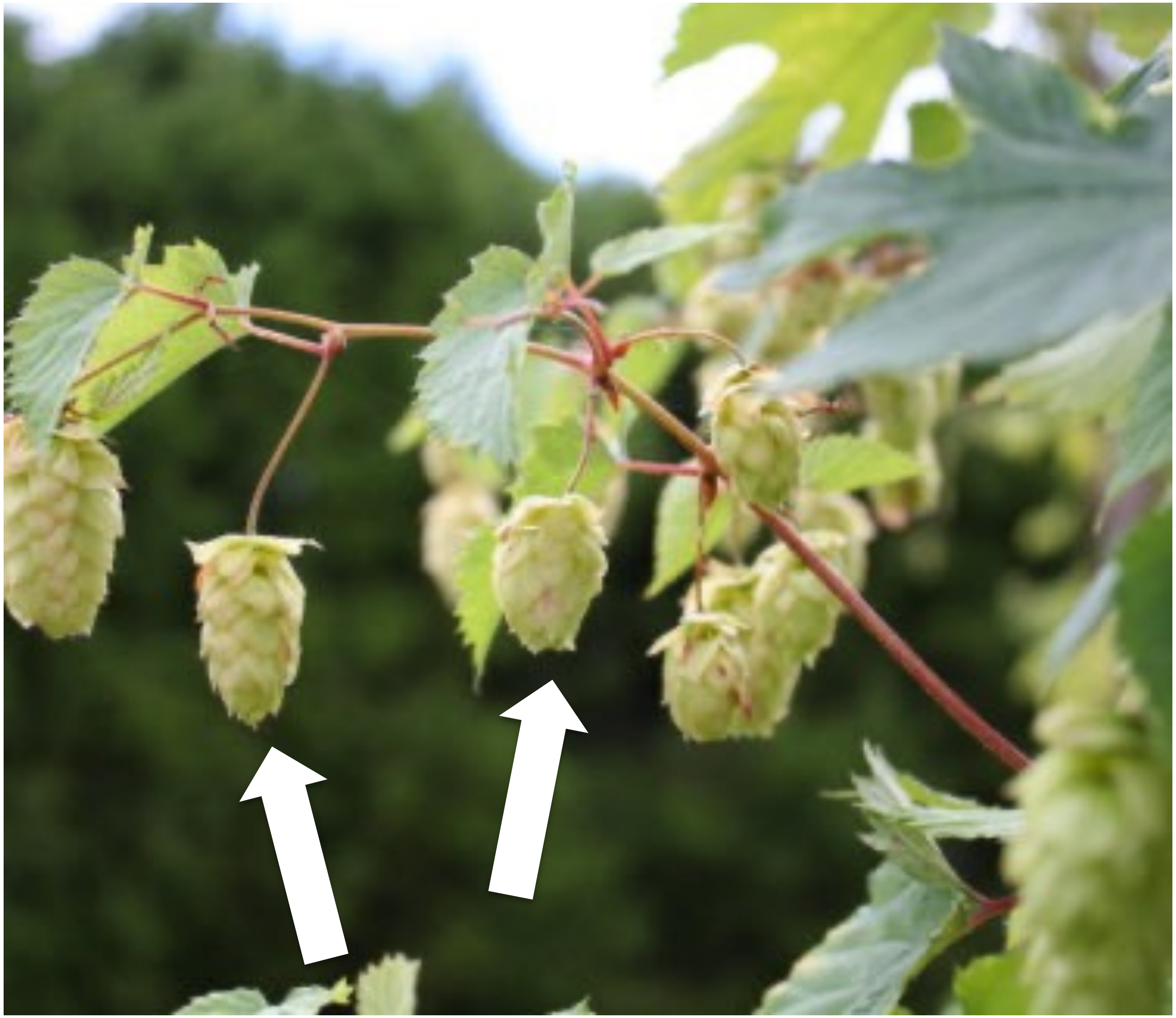


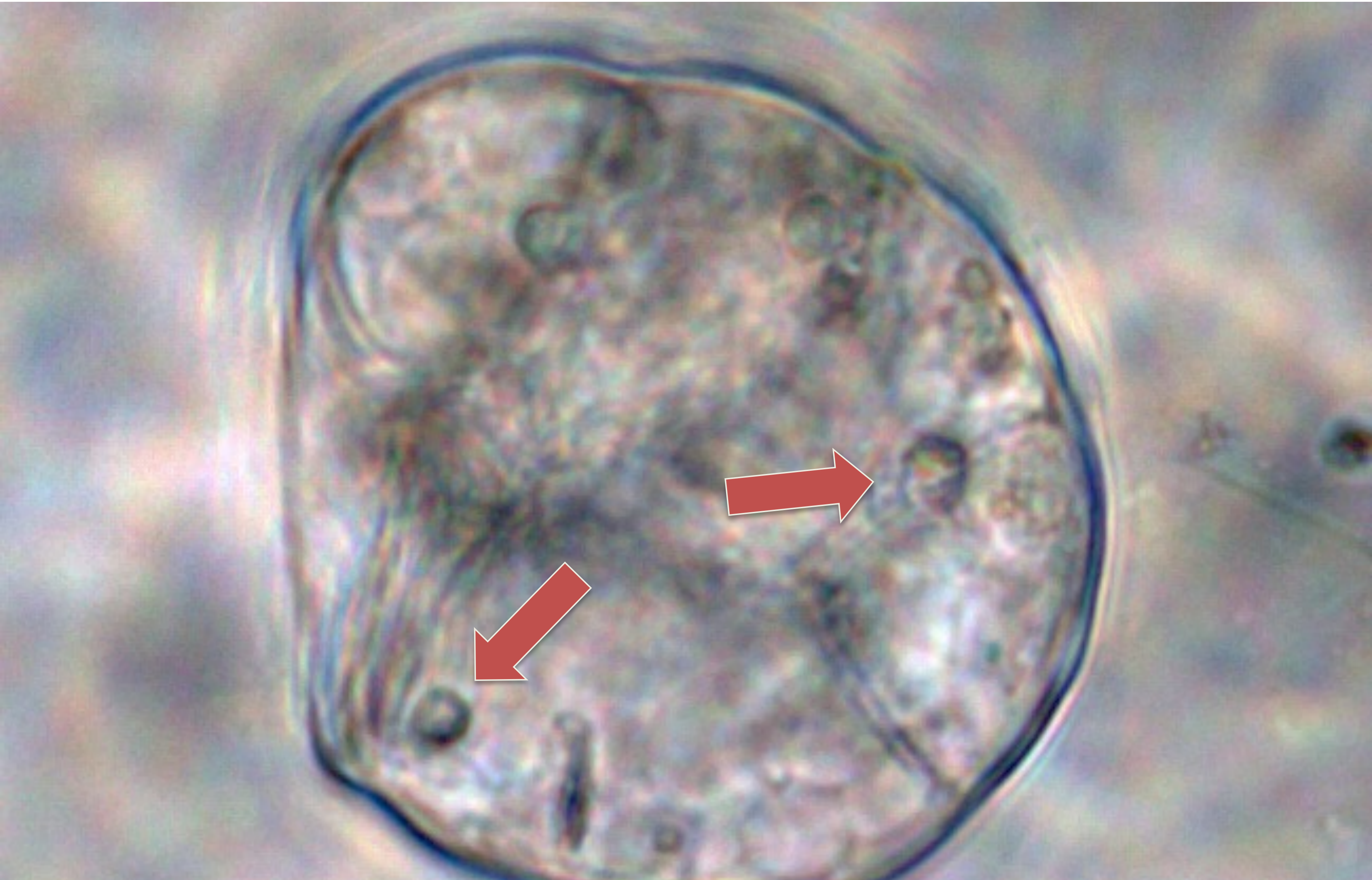
Model for how glandular trichomes work



Hops photo showing inflorescences (arrows) composed of whorls of bracts that bear trichomes.

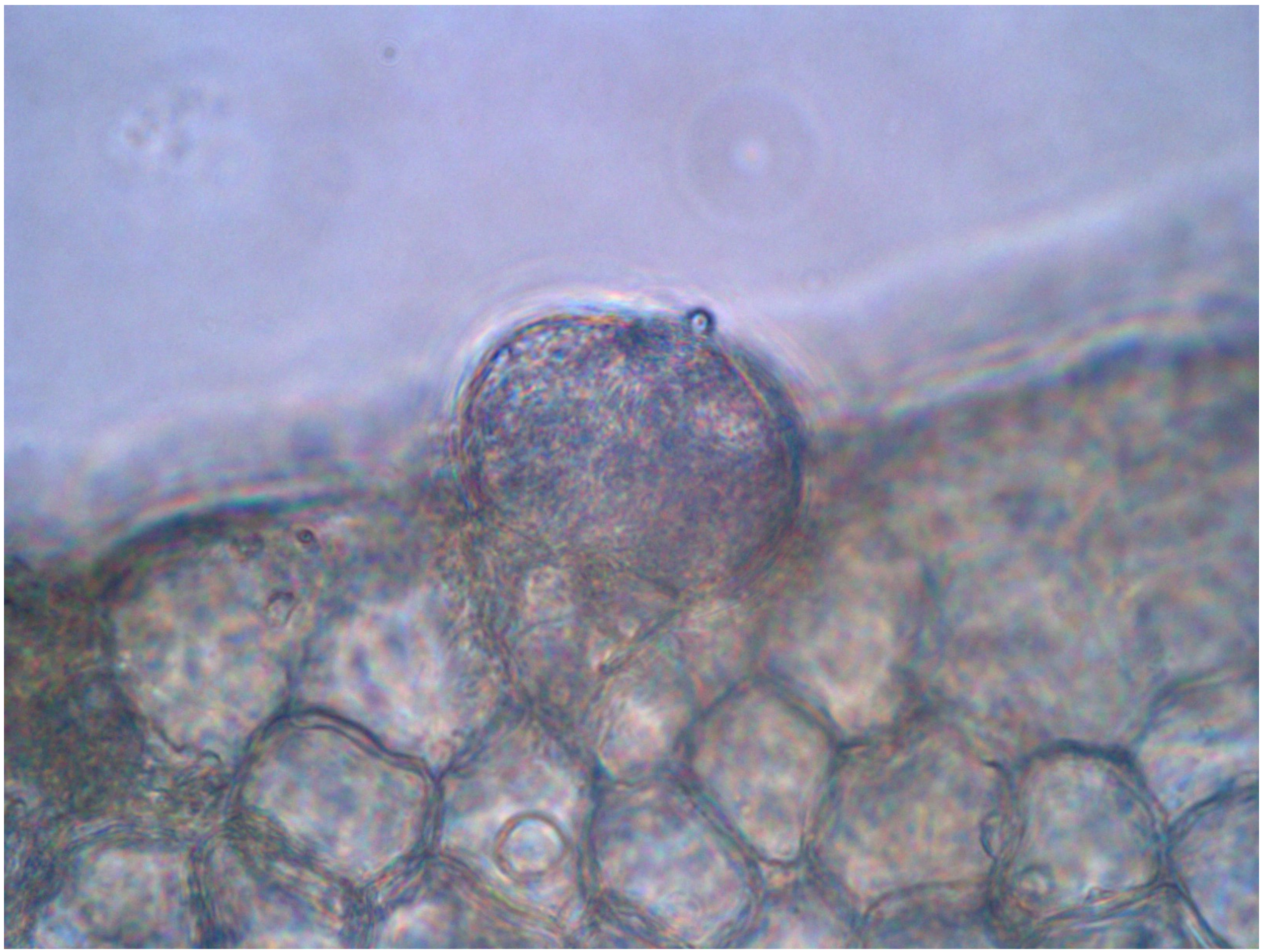
April Micci





Hops stained with nitro blue tetrazolium to show superoxide (blue) around bacteria (arrows).

Hops glandular trichome stained for nitrate.



Developing N-fixing crops by microbiome transfer from land races and wild species.

Dr. Walter Goldstein (Plant Breeder)
Mandaamin Institute, WI

Nitrogen-fixing Peruvian landrace ‘piricinco’.

Raquele Strickland
Rutgers University

Fig. Cob leaves dissected from a cob of a nitrogen-fixing Piricinco (from Walter Goldstein; Mandaamin Inst., Wisconsin).

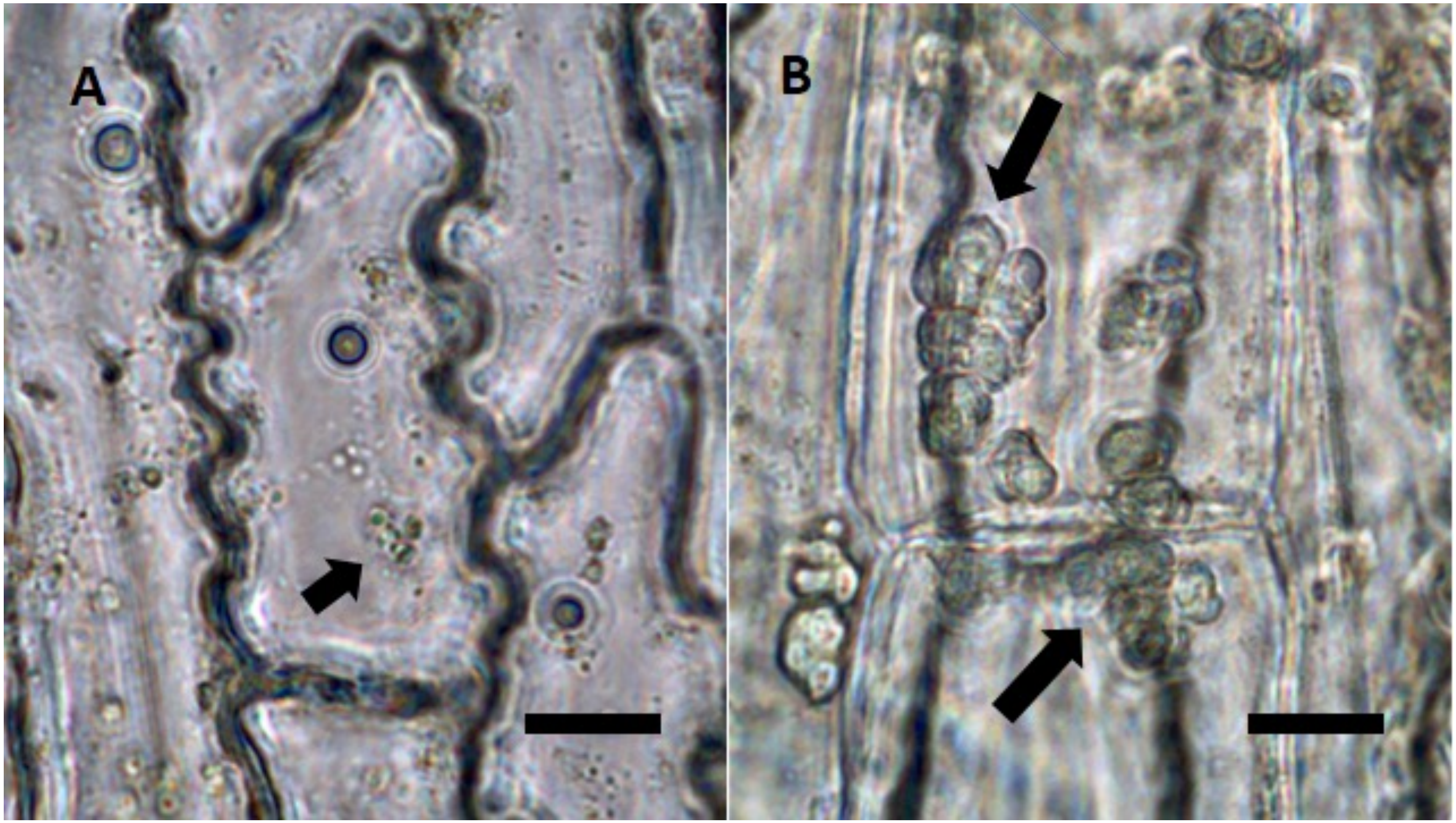
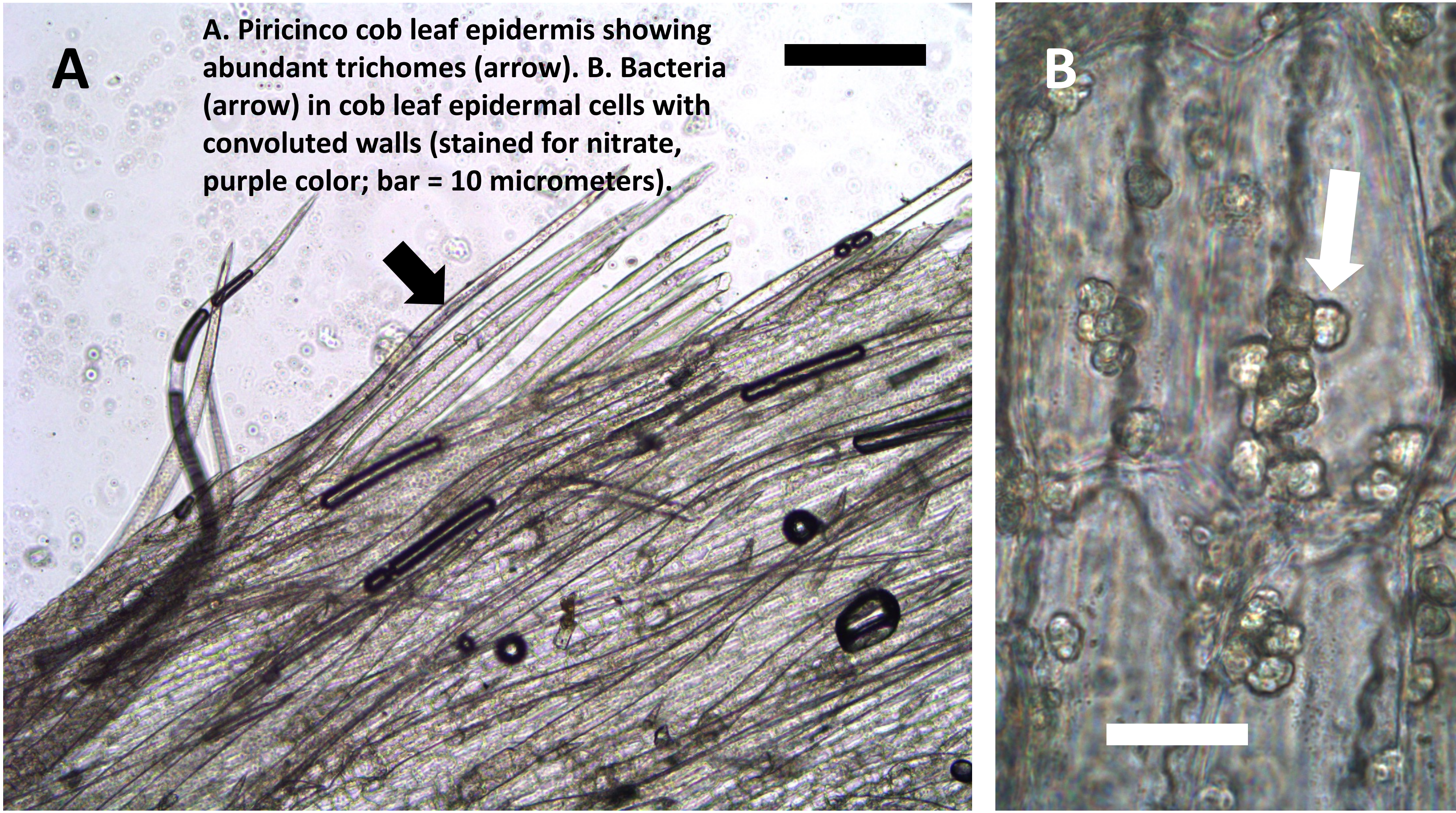
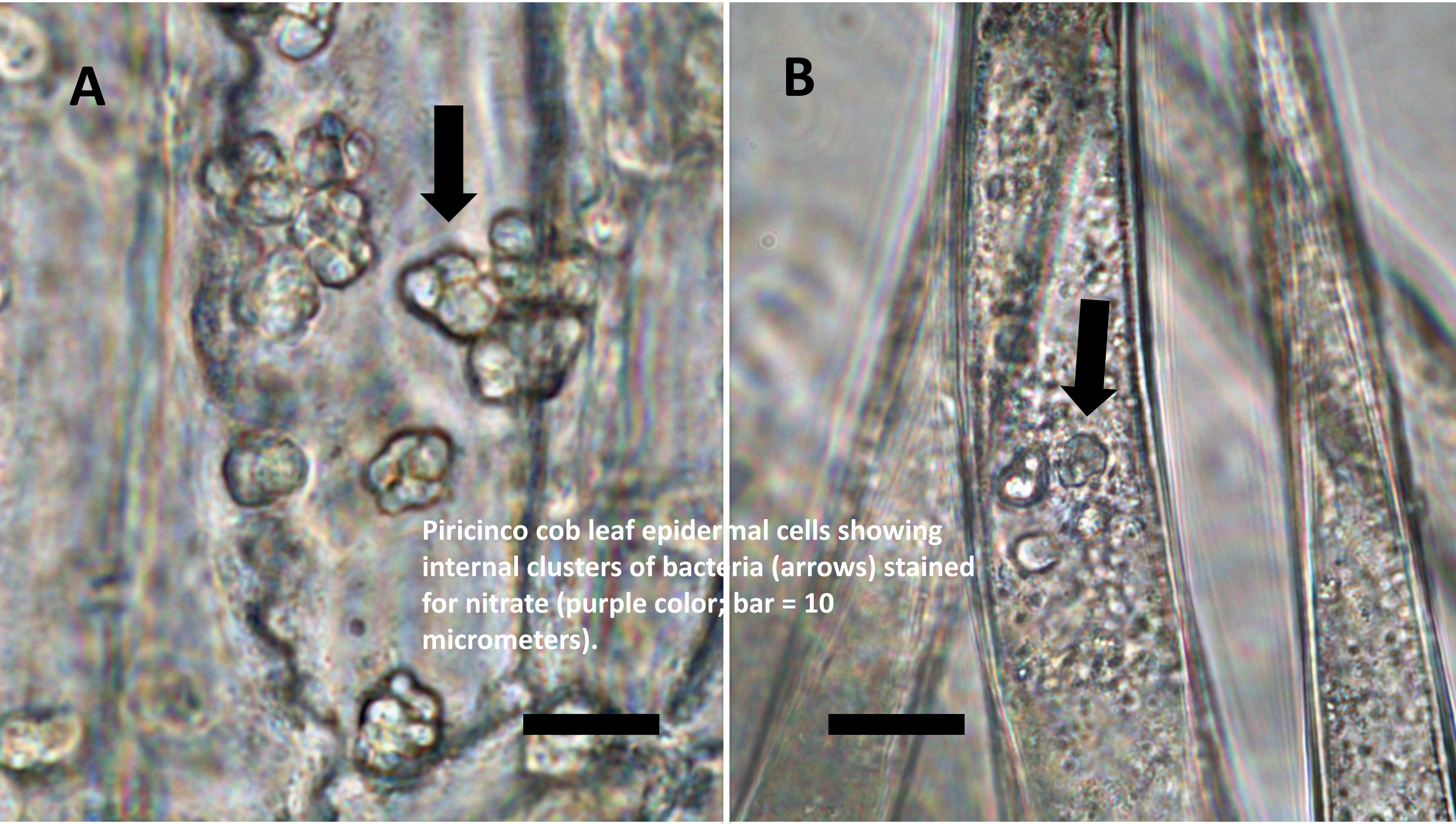


Fig. The microbial community in the husk leaves of a conventional sweet corn variety (A) and in the husk leaves of a nutrient dense, nitrogen efficient Mandaamin Institute cultivar derived from Peruvian 'Piricinco' (B). The arrows show the clusters of bacteria in the epidermal cells (Bar = 10 micrometers).



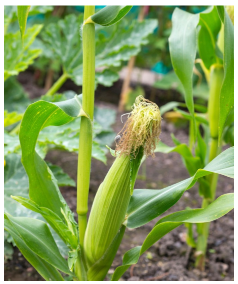
A. Piricinco cob leaf epidermis showing abundant trichomes (arrow). B. Bacteria (arrow) in cob leaf epidermal cells with convoluted walls (stained for nitrate, purple color; bar = 10 micrometers).



Piricinco cob leaf epidermal cells showing internal clusters of bacteria (arrows) stained for nitrate (purple color; bar = 10 micrometers).

Protocol for microbiome transfer from corn to *Poa reptans*

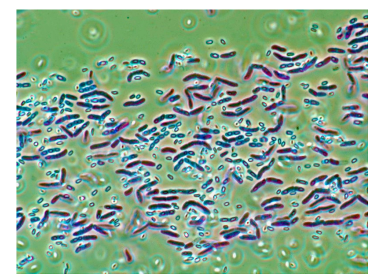
We transfer endophytes between plants using variations of the technique used by the Iroquois Indians in their 'Iroquois corn medicine'.



1) Microbiome source
(A = cob Leaves; B = roots)

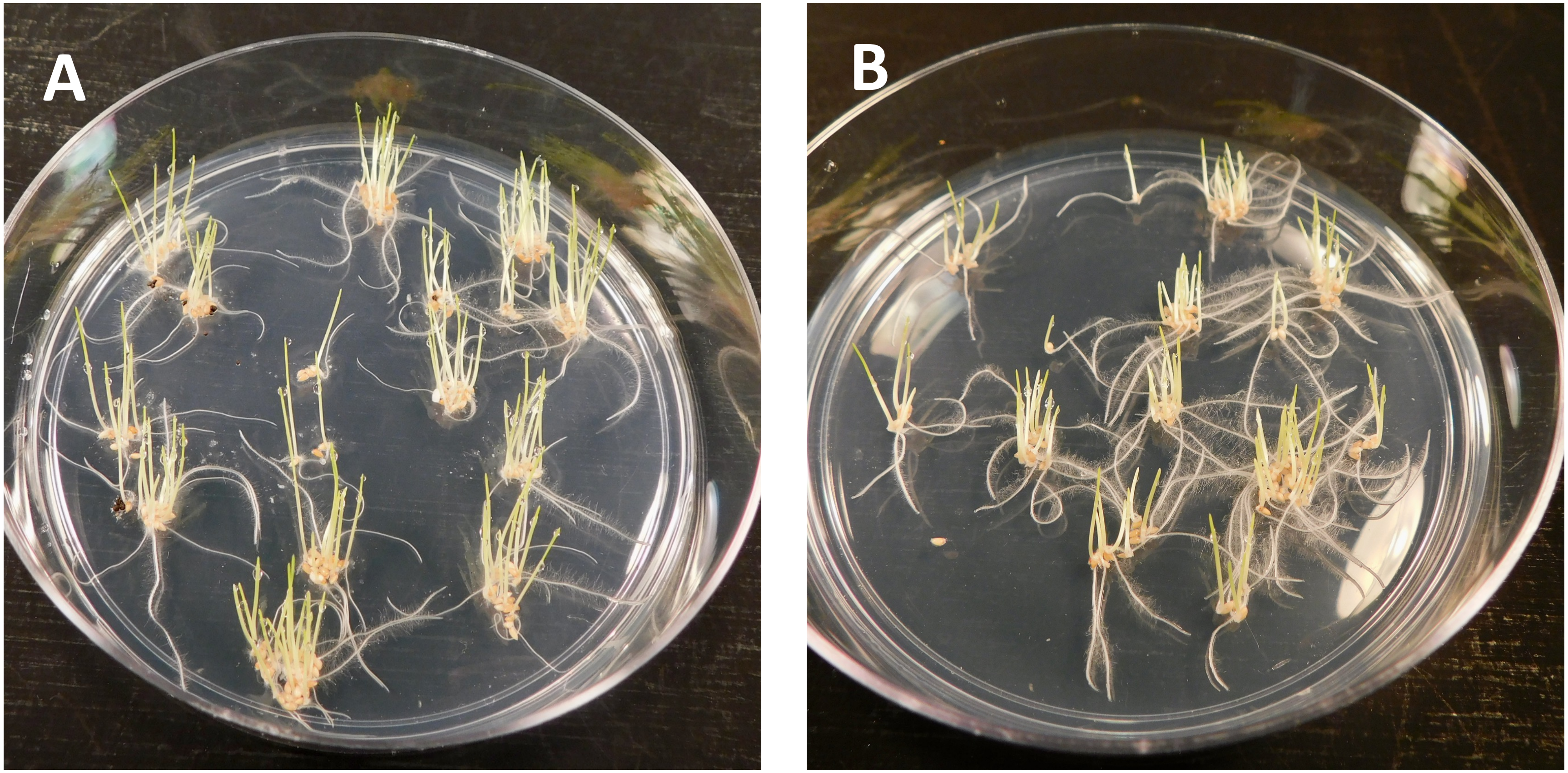
2) Separate out cob leaves

3) Triturate young cob leaves
In H_20 to release bacteria



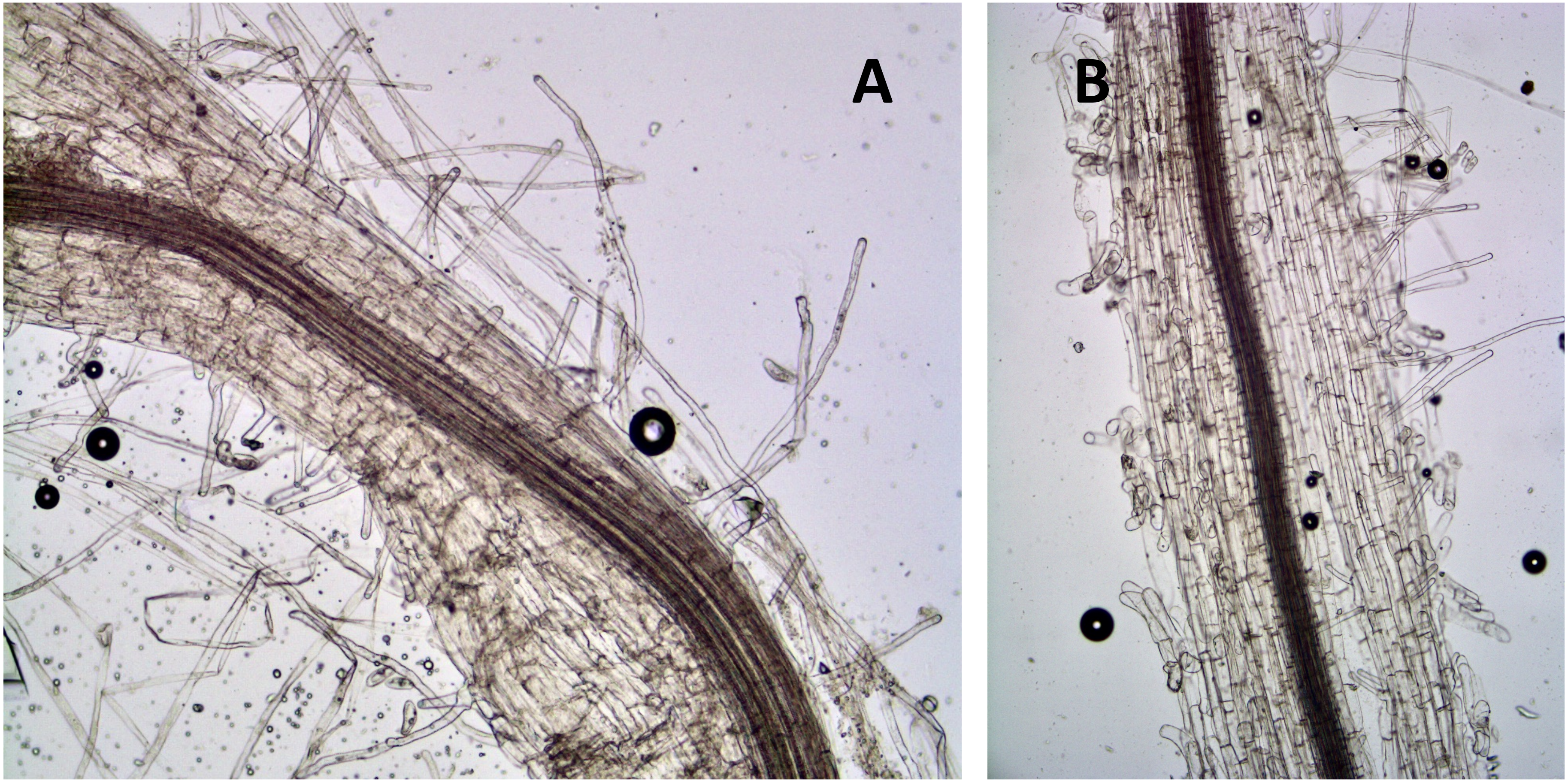
4) Strain out large tissue pieces of plant tissues

5) Inoculate cleaned seeds on agarose using filtrate



5-day-old *Poa reptans* seedlings: A. Seedings from seeds inoculated with microbiome extracted from ‘piricinco’ selection. B. Seedlings from seeds that were treated with water.

Plants with more nitrogen-fixing endophytes in them have more and longer root hairs and trichomes.



Figs. A. Roots of Poa seedlings from piricinco-inoculated seeds; B. Root from non-inoculated control.

Using a nitrate stain (acidified diphenylamine: purple color) more nitrate can be seen around bacteria (black arrows) in plant cells.

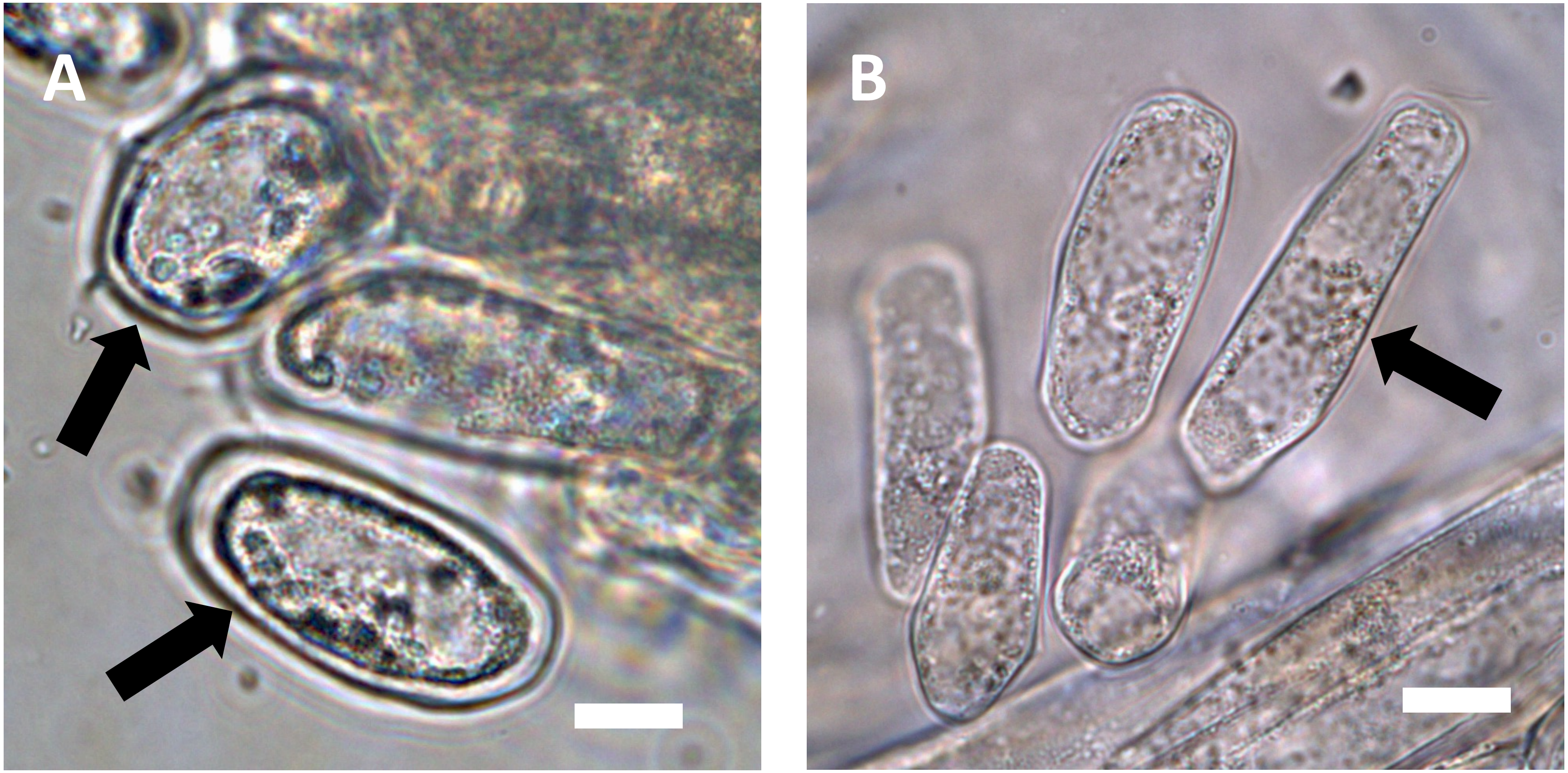


Fig. Root cap cells from 'Piricinco' treated and untreated *Poa reptans*. A. Cells showing dense nitrate staining (purple) of bacteria in cells; B. Cells from untreated seedlings showing absence of dark-staining masses of bacteria (bar = 10 microns).

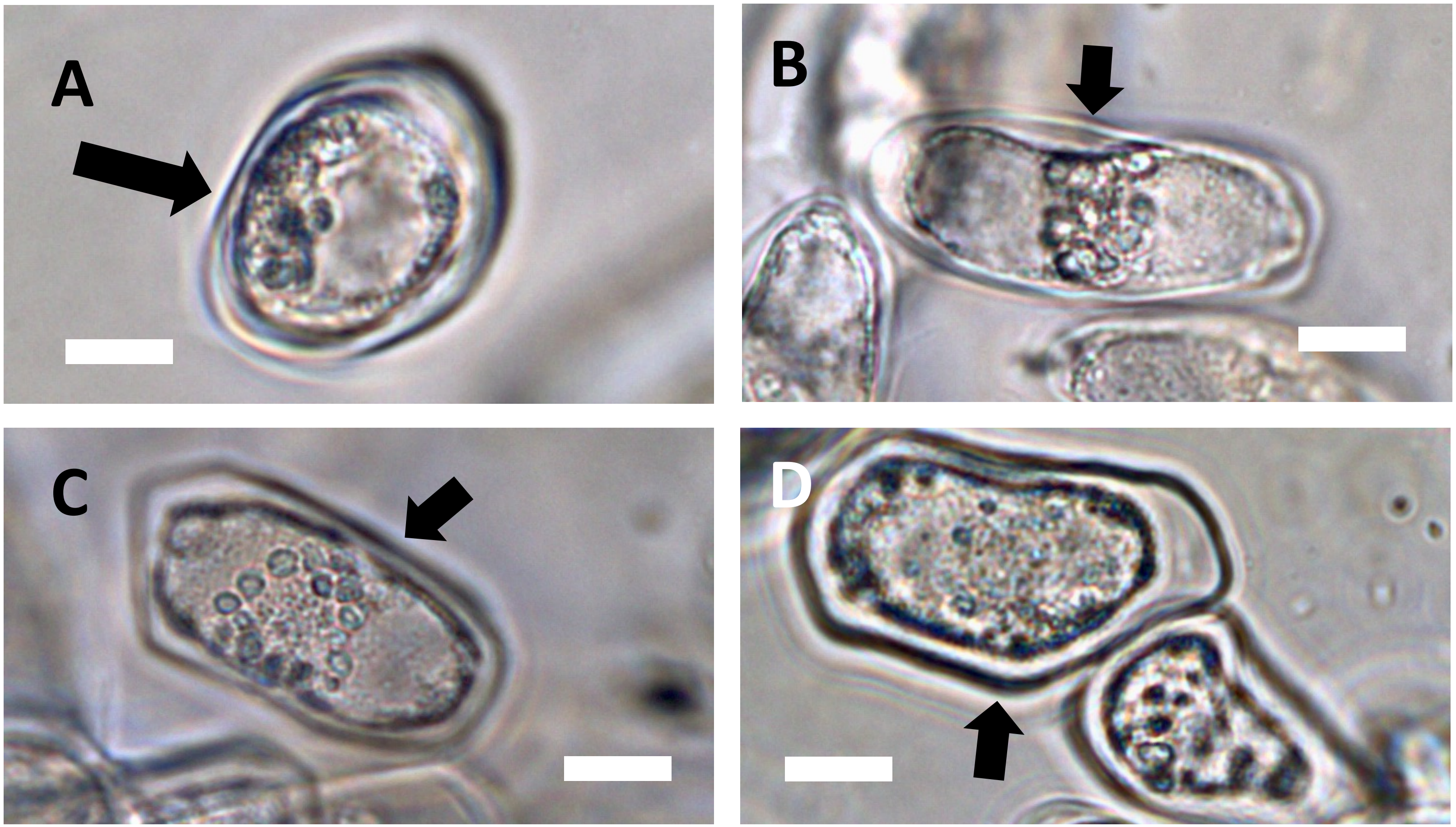


Fig. Root cap cells from 'Piricinco' treated *Poa reptans*. A-D. Cells showing dense nitrate staining (purple) of bacteria in cells (bar = 10 microns).

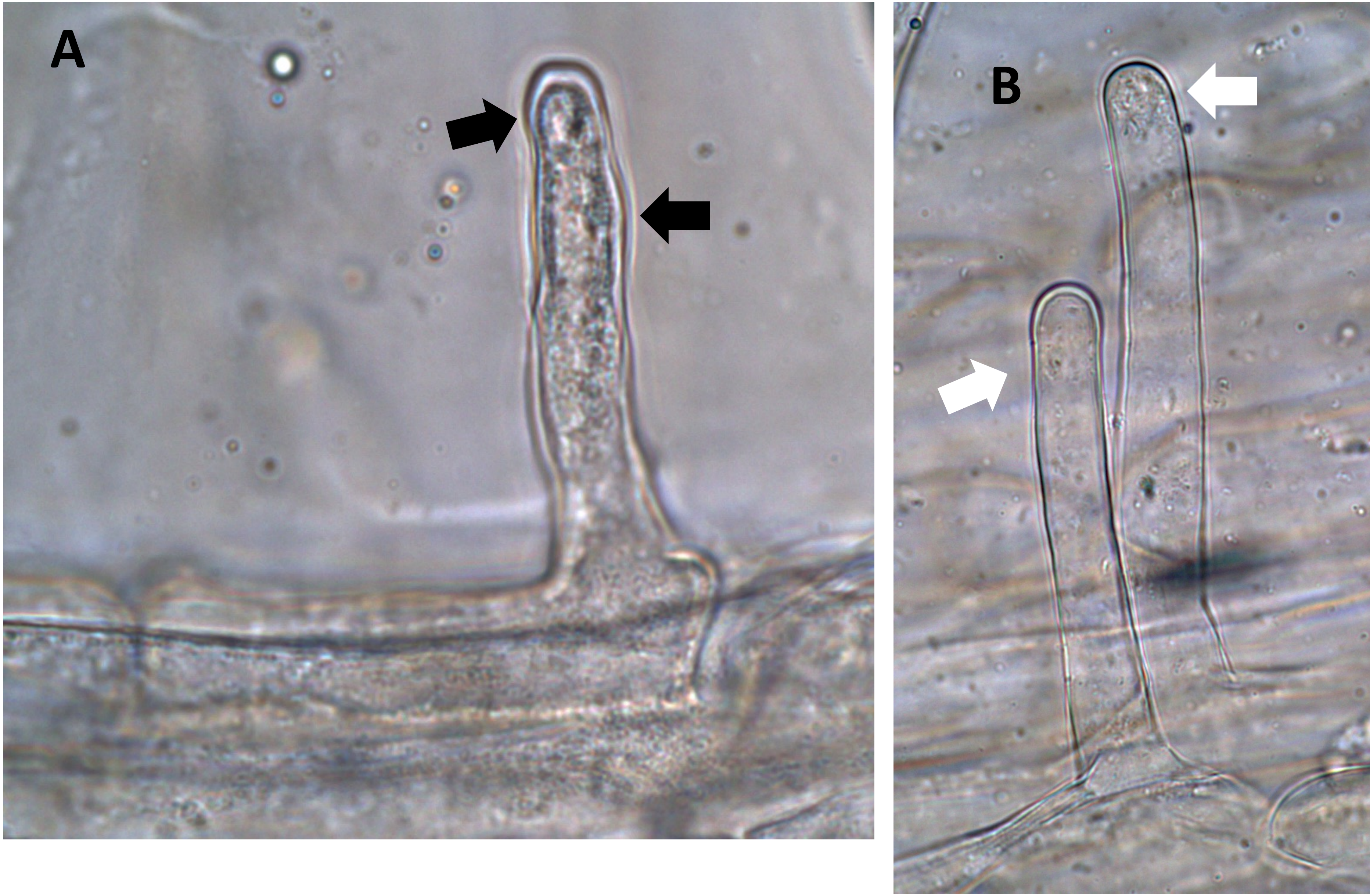
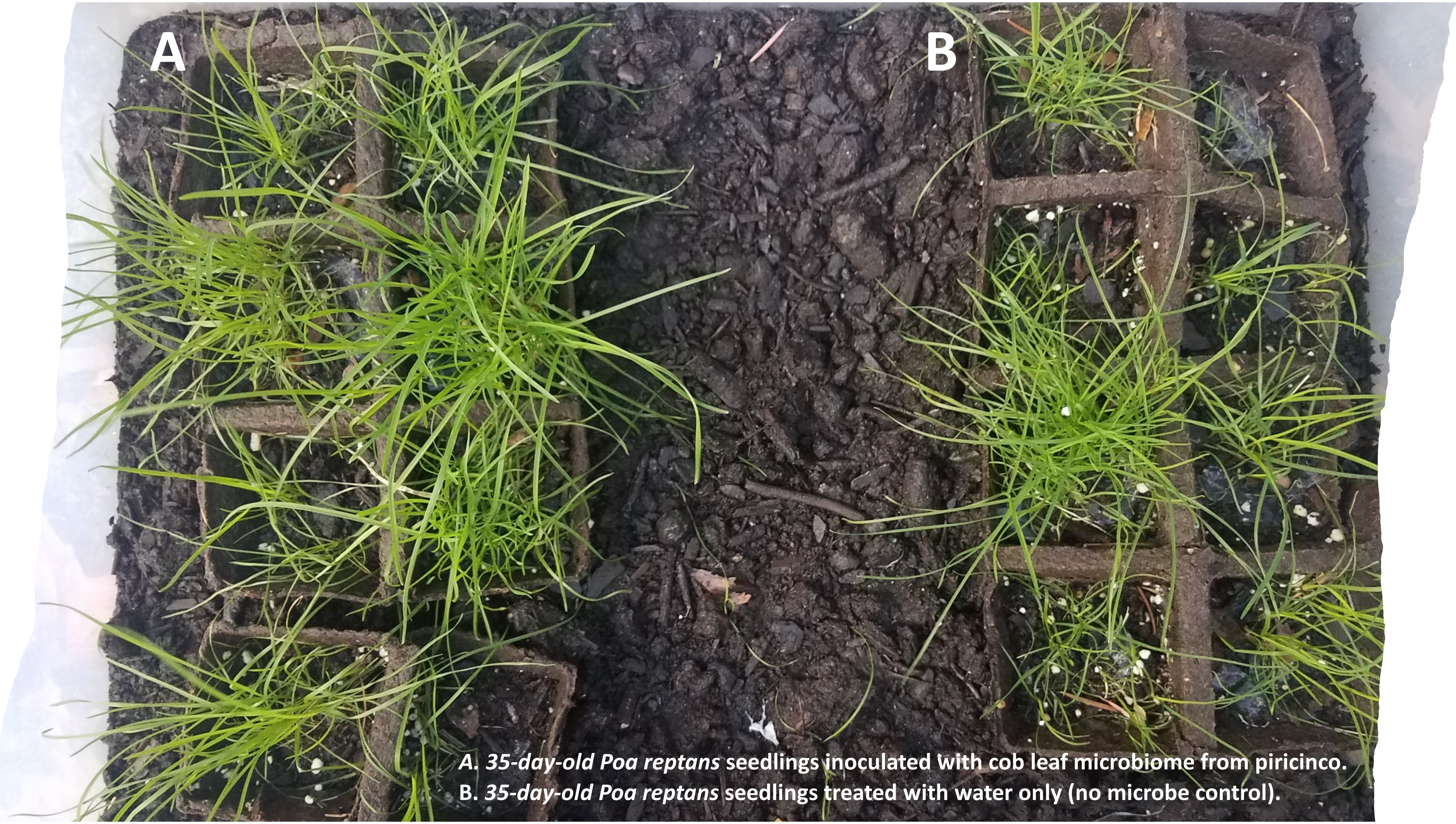


Fig. Root hairs (arrows) from piricinco-treated (A) and untreated control (B). Intense nitrate staining (purple color) can be seen in the piricinco treated seedlings (bar = 10 microns).

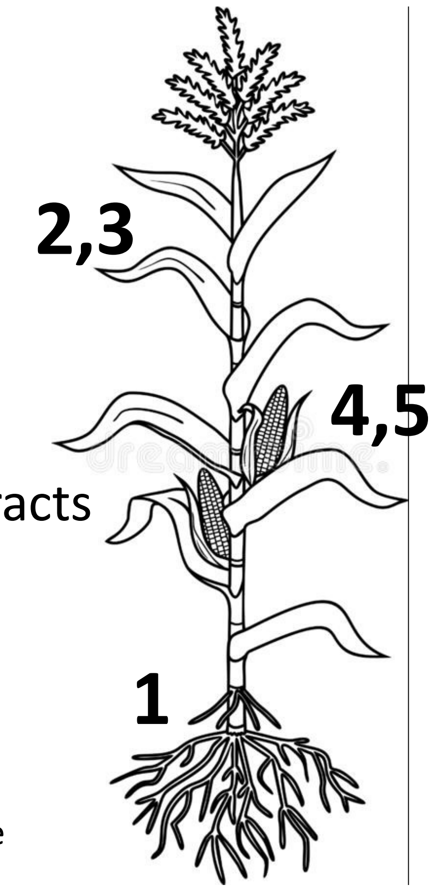


A. 35-day-old Poa reptans seedlings inoculated with cob leaf microbiome from piricinco.

B. *35-day-old Poa reptans* seedlings treated with water only (no microbe control).

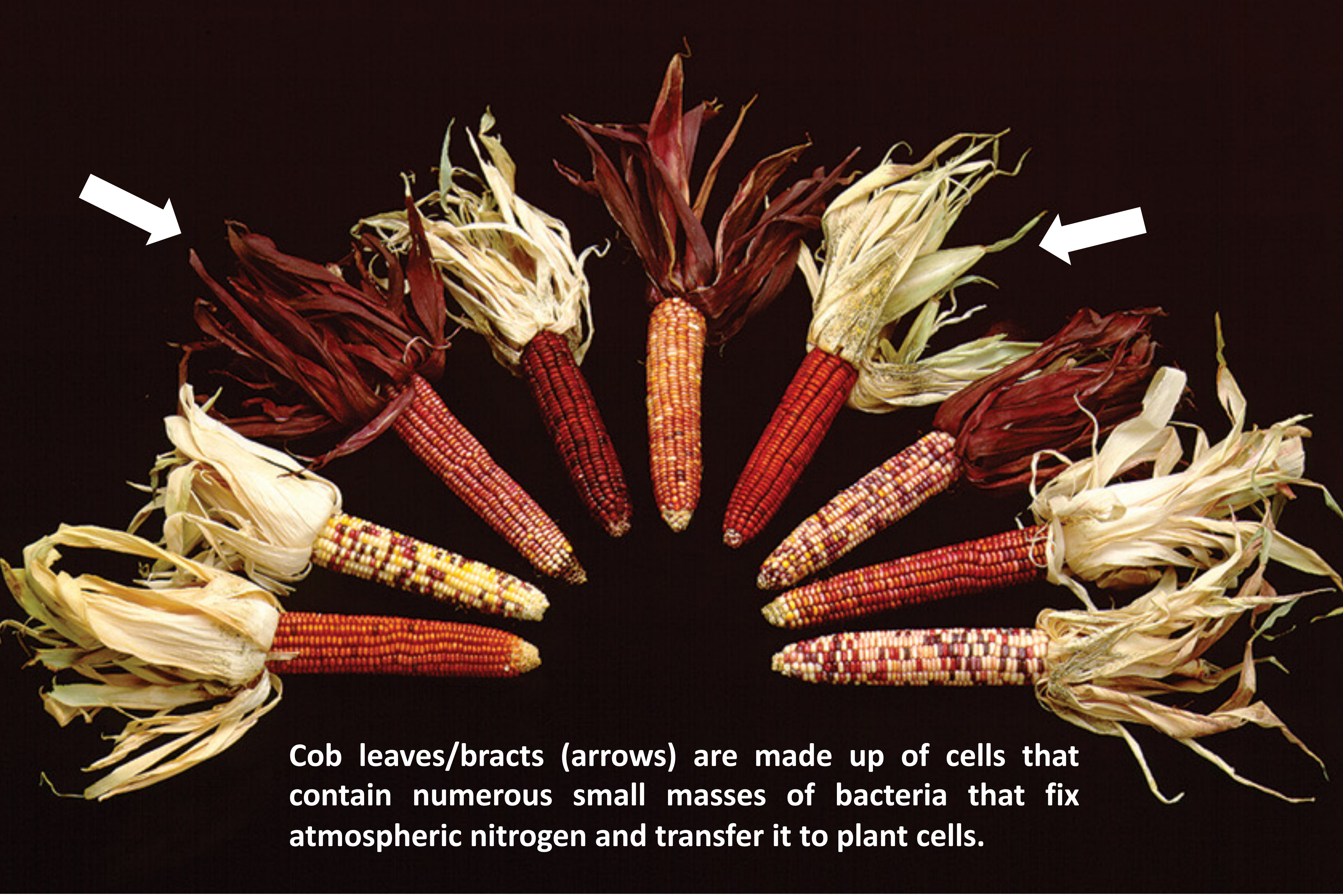
Endosymbiosis Stacking in Corn

1) Rhizophagy cycle in roots
2) Convoluted wall epidermal cell symbiosis in stem leaves
3) Trichome endosymbiosis on stem leaves
4) Trichome endosymbiosis in cob leaves/bracts
5) Convoluted epidermal wall symbiosis in the cob leaves/bracts

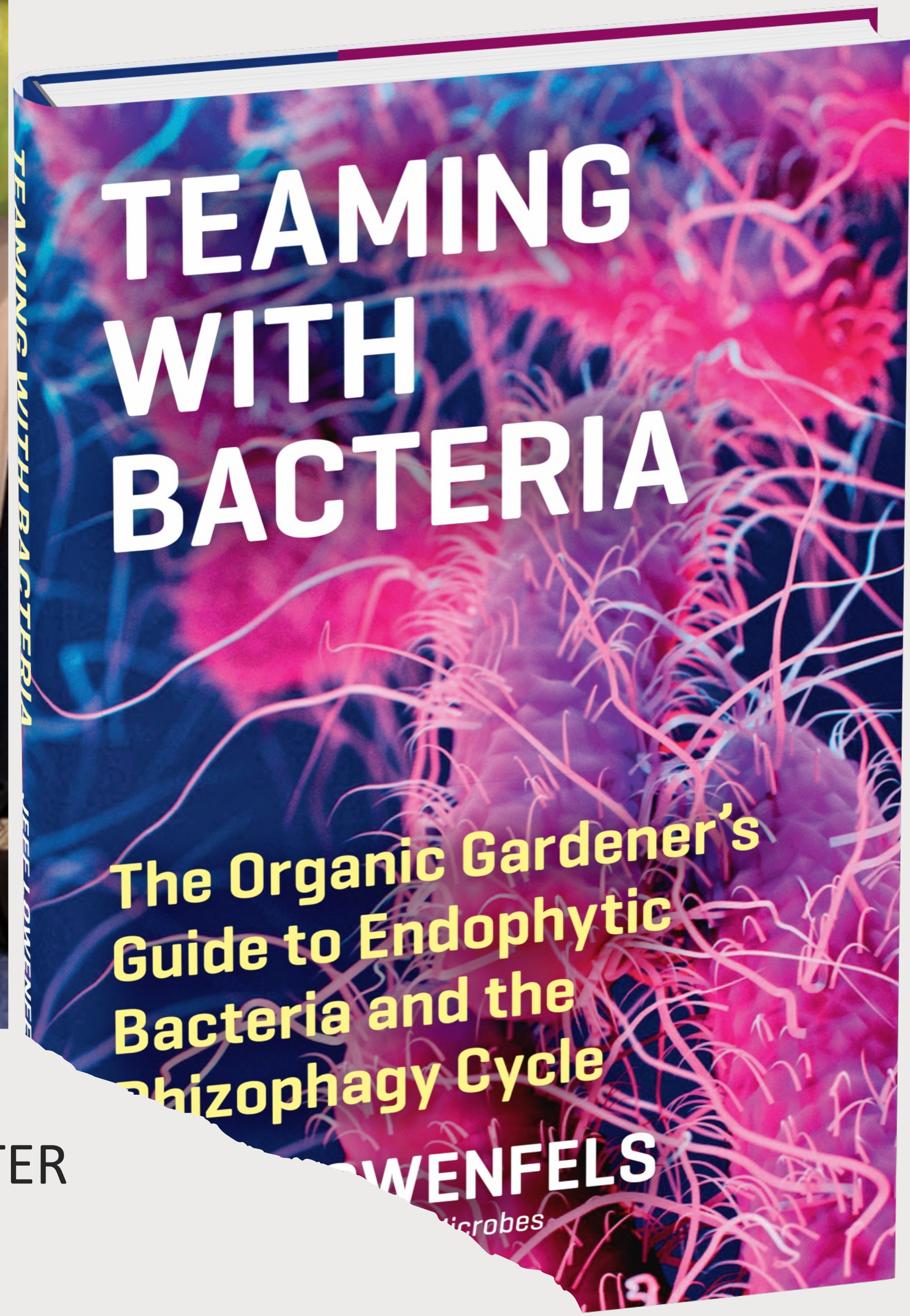


In corn plants without damage to endosymbiotic systems, microbes in several tissues provide nutrients to support growth. Rhizophagy cycle in roots delivers nutrients that are extracted oxidatively from microbes in root cells. As leaves grow, bacteria within the convoluted-walled epidermal cells and in trichomes deliver nitrogen to plants. In the cob leaves/bracts convoluted-walled epidermal cells and trichomes deliver nitrogen to plant cells that provides nitrogen for the developing ovaries/kernels on the cob. Use of chemical fertilizers may negate or damage these endosymbiosis in corn plants.

Reference: Micci, A.; Zhang, Q.; Chang, X.; Kingsley, K.; Park, L.; Chiaranunt, P.; Strickland, R.; Velazquez, F.; Lindert, S.; Elmore, M.; Vines, P.L.; Crane, S.; Irizarry, I.; Kowalski, K.P.; Johnston-Monje, D.; White, J.F. Histochemical Evidence for Nitrogen-Transfer Endosymbiosis in Non-Photosynthetic Cells of Leaves and Inflorescence Bracts of Angiosperms. *Biology* **2022**, *11*, 876. https://doi.org/10.3390/biology11060876



Cob leaves/bracts (arrows) are made up of cells that contain numerous small masses of bacteria that fix atmospheric nitrogen and transfer it to plant cells.



Jeff Lowenfels with MicroBIOMETER founder Judy Fitzpatrick

Summary Conclusion

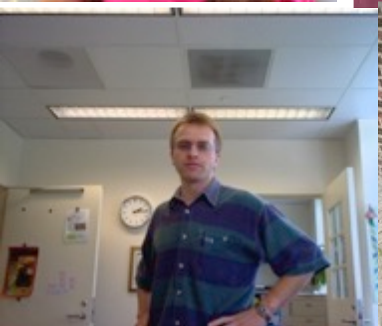
<u>All plants</u> ‘absorb’ <u>soil microbes</u> into their cells and ‘manipulate’ them using reactive oxygen to obtain nutrients.

Plants are absorbers and manipulators of soil microbes.







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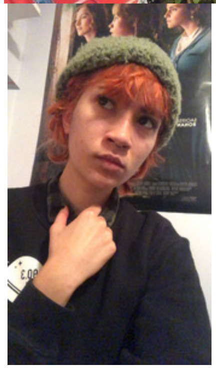
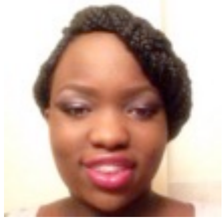
USGS-Rutgers U (CESU Study Agreement)









Questions?