Increasing atmospheric CO 2 levels may reduce extraction of nutrients from soil microbes in plant roots

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Increasing atmospheric CO_2 levels may reduce extraction of nutrients from soil microbes in plant roots

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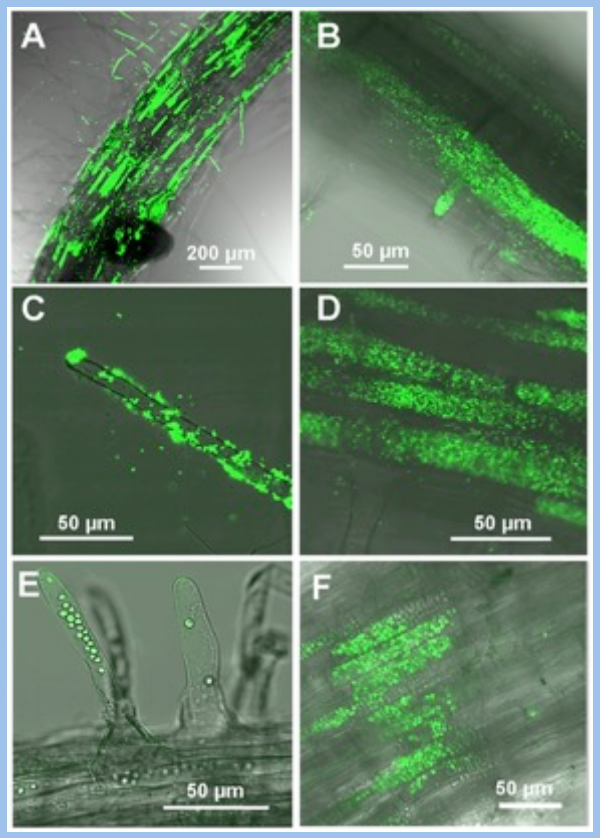
Figure 1. Roots of axenically grown Arabidopsis and tomato were incubated with E coli or yeast expressing green fluorescent protein (GFPE. coli or GFPyeast).

“Rhizophagy”

Do plant roots consume bacteria to obtain nutrients?



‘Turning the Table: Plants Consume Microbes as a Source of Nutrients’

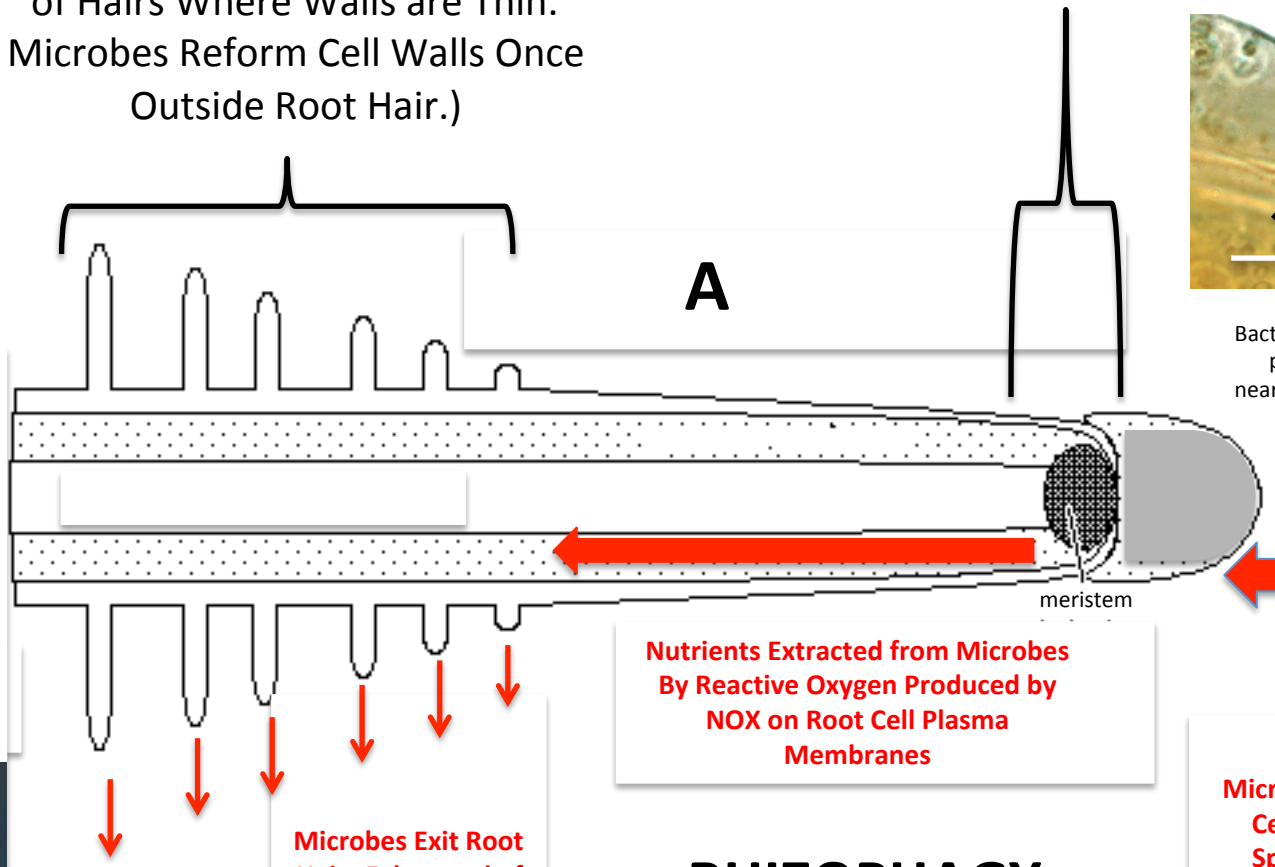
Chany Paungfoo-Lonhienne

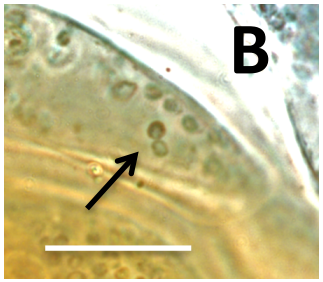
Paungfoo-Lonhienne C et al. 2010.
Turning theTable: Plants Consume Microbes as a Source of Nutrients.
PLoS ONE 5(7): e11915, doi:10.1371/journal.pone.0011915

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.)

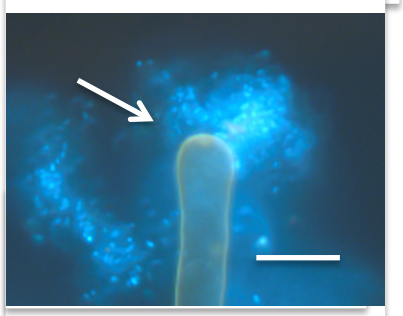
Plant Cell Entry Zone
(Microbes Become Intracellular in Meristem Cells as Wall-less Protoplasts.)





Bacteria (arrow) in root parenchyma cell near root tip meristem.

C



Bacteria (arrow) emerging from root hair tip of millet seedling.

Nutrients Extracted from Microbes By Reactive Oxygen Produced by NOX on Root Cell Plasma Membranes

Microbes Enter Root Cell Periplasmic Spaces Carrying Nutrients From Soil

Microbes Exit Root Hairs Exhausted of Nutrients

RHIZOPHAGY CYCLE

Microbes Recharge with Nutrients in the Rhizosphere

Controlling Plant Growth Through Control of Plant Microbiomes!

Our work is focused on understanding the functions of the plant microbiome, and controlling it in order to reduce invasiveness in plants like the Common Reed Grass (*Phragmites australis*).

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.

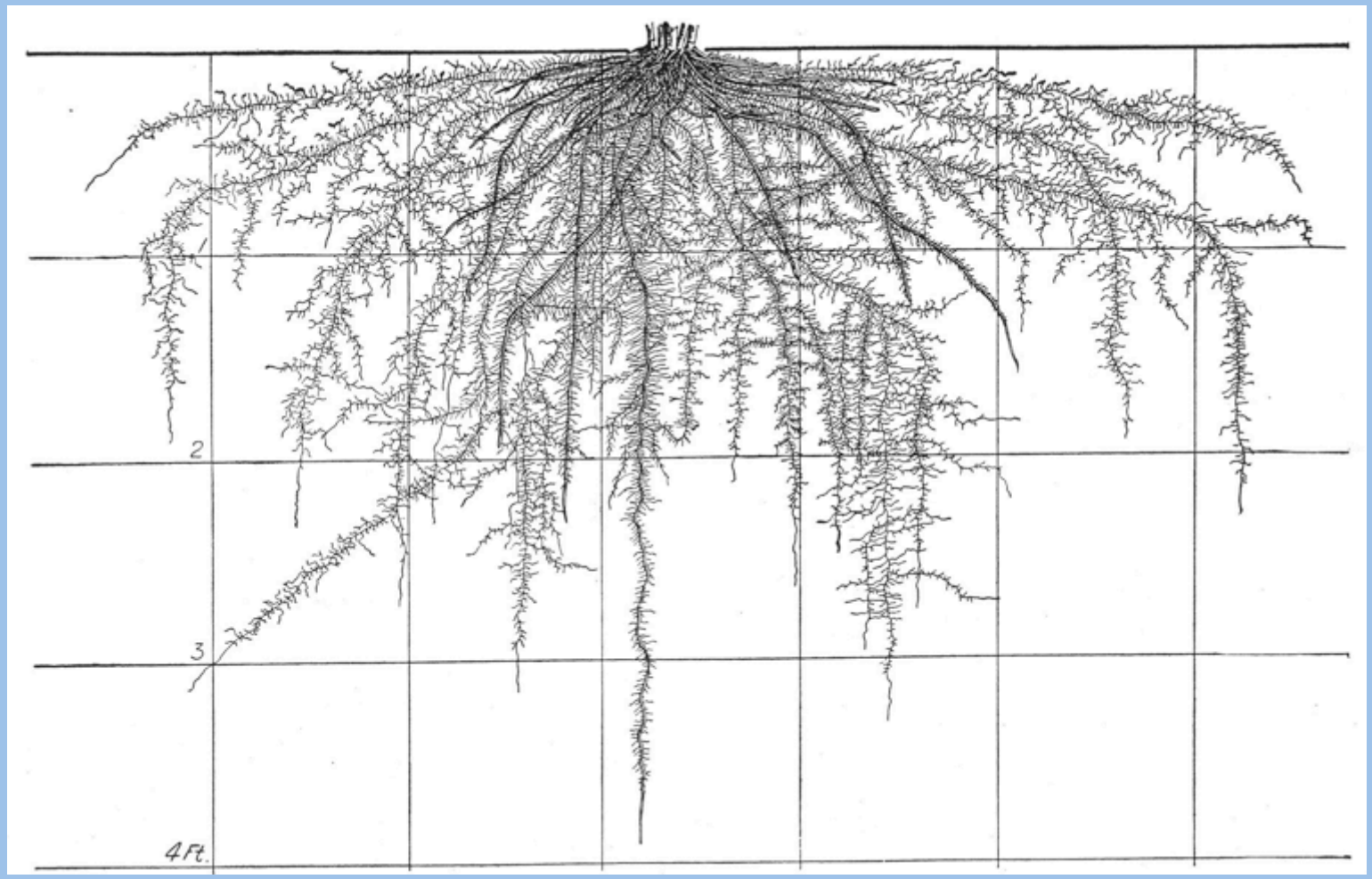
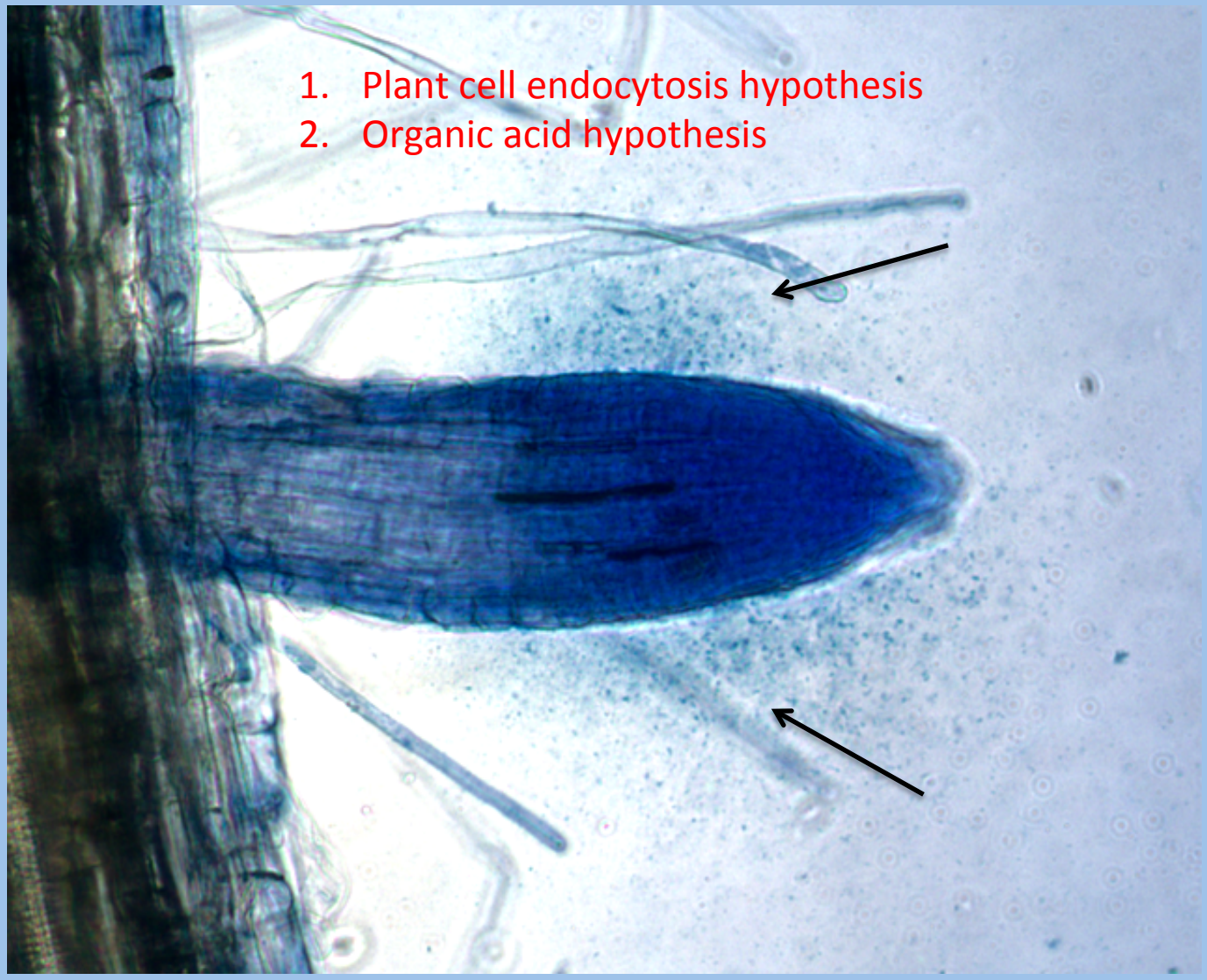
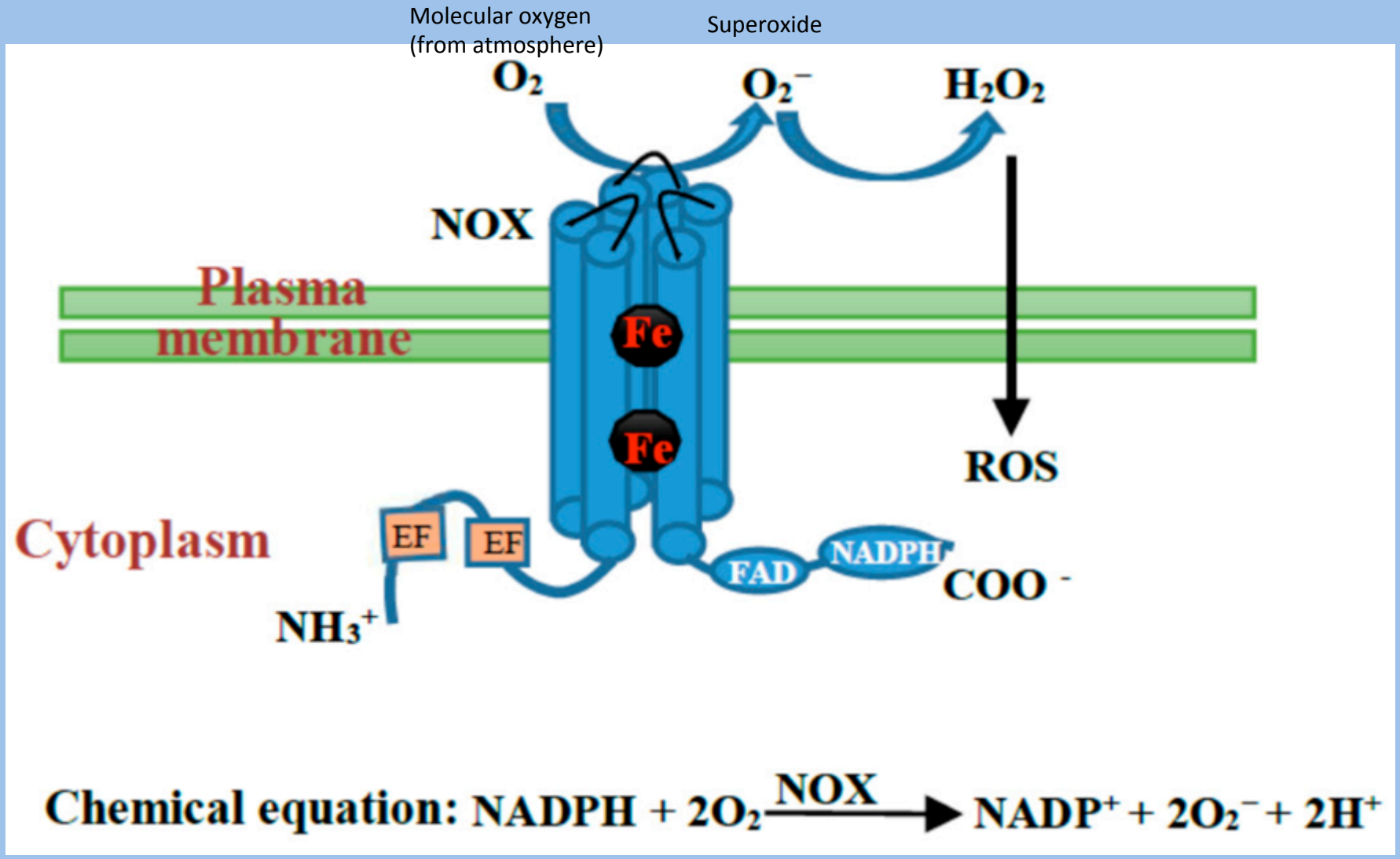


Illustration of a root system of corn (Illustration by Botanist John E. Weaver, 1927)

Bacteria entering root epidermal cells of Phragmites 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.

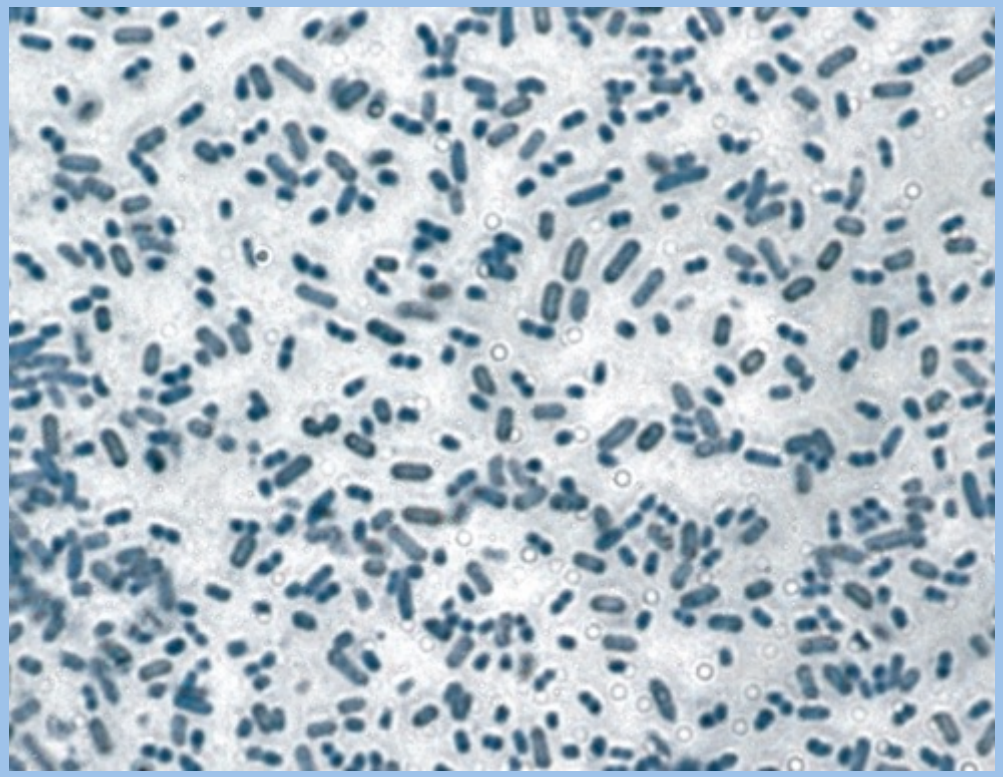


REACTIVE OXYGEN DEFENSE RESPONSE OF THE HOST CELL INVOLVES MEMBRANE-BOUND NADPH OXIDASES (NOX)



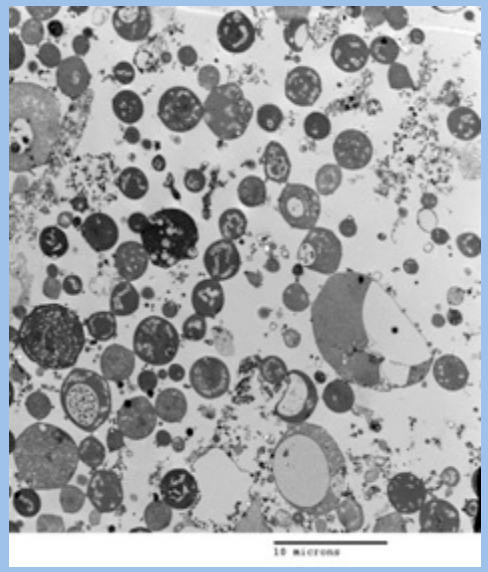
Bacterium *Bacillus subtilis*

Bacteria with cell walls (rods)



Reactive oxygen
(superoxide)

Spherical bacterial protoplasts
(no cell walls)



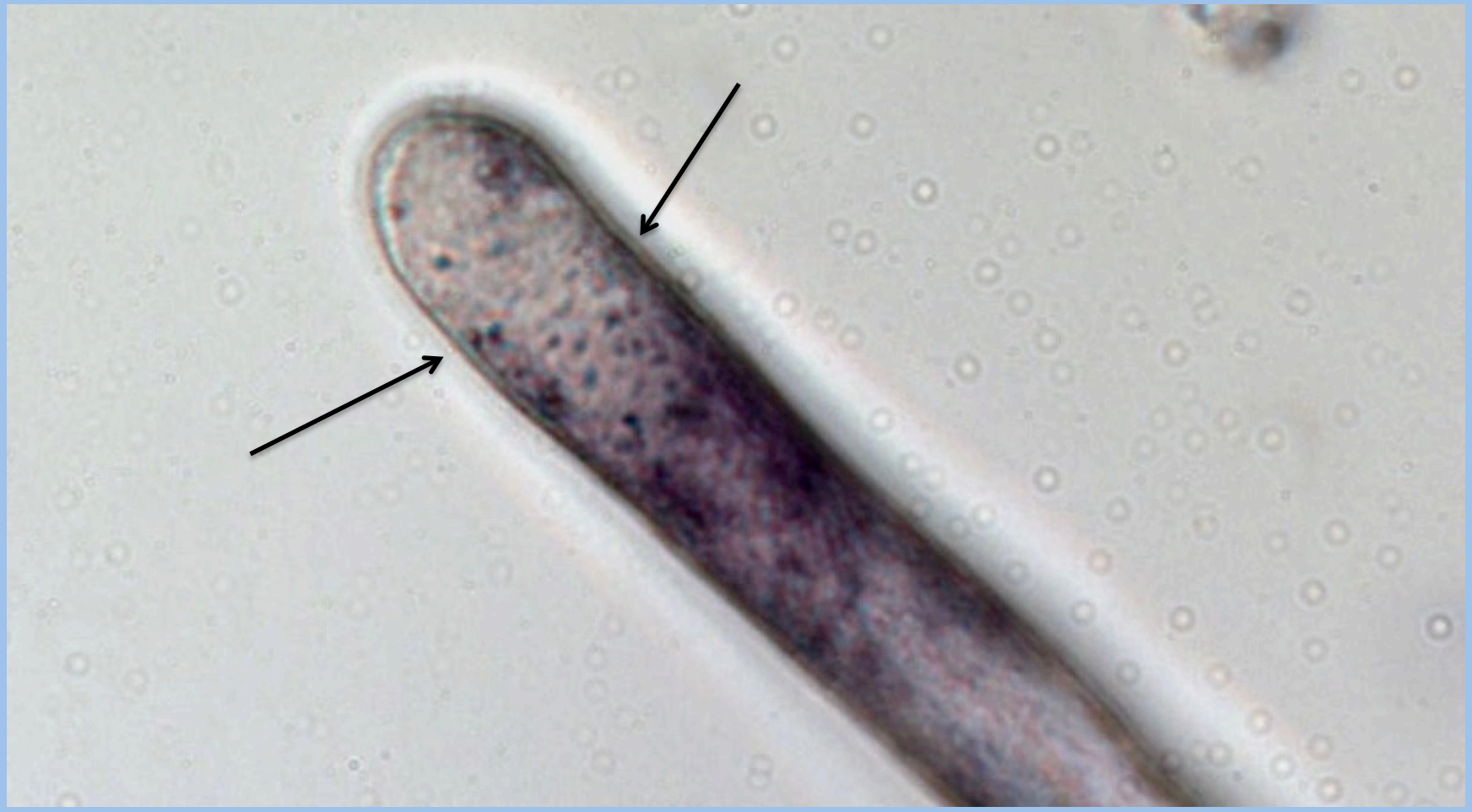
Bacterial protoplasts
are called L-forms.

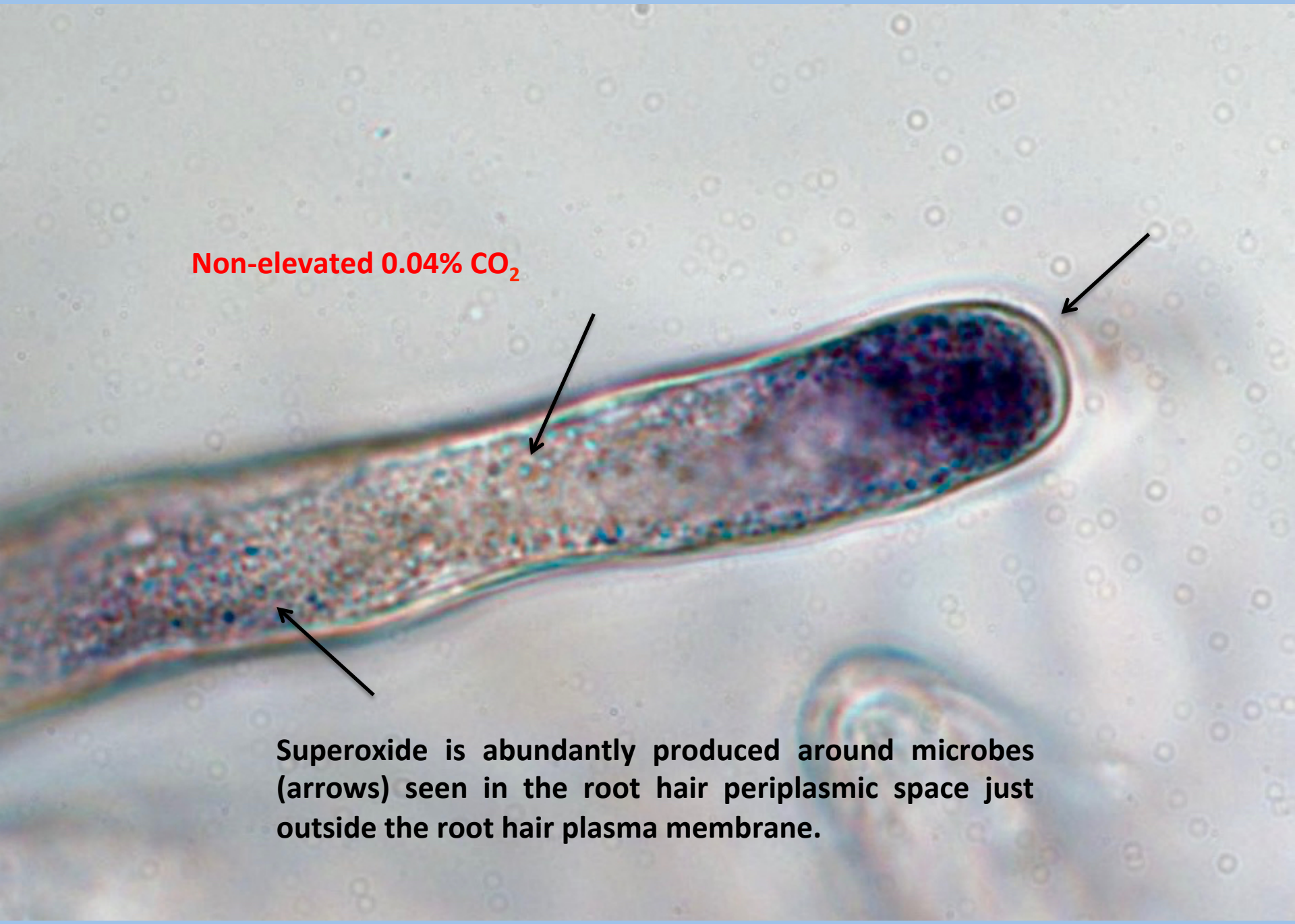
Inside root cells superoxide strips cell walls off of the microbes!

Root tip from the 0.04% CO_2 treatment of tall fescue showing superoxide (purple color; Nitro Blue Tetrazolium (NBT) staining) concentrated in root tip where microbes enter root cells.

NBT stained tall fescue seedling root showing superoxide at tips of root hairs (arrows) where microbe protoplasts accumulate.

NBT stained tall fescue root hair tip from the 0.04% CO_2 treatment showing purple stained superoxide around microbes.





Superoxide is abundantly produced around microbes (arrows) seen in the root hair periplasmic space just outside the root hair plasma membrane.

Reactive Oxygen Staining Technique

Assay for bacterial endophytes involves growth on agarose. Then staining with Diaminobenzidine tetrahydrochloride (DAB) overnight.

Reactive oxygen secretion is used by all eukaryotes to kill endoparasitic bacteria. It is part of the innate Defensive system of all Eukaryotes.

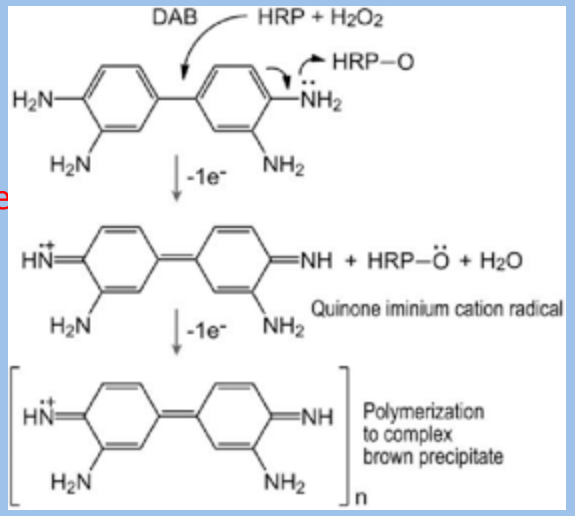
How DAB Works:

1) Plant cells secrete superoxide Onto intracellular bacteria to degrade them.

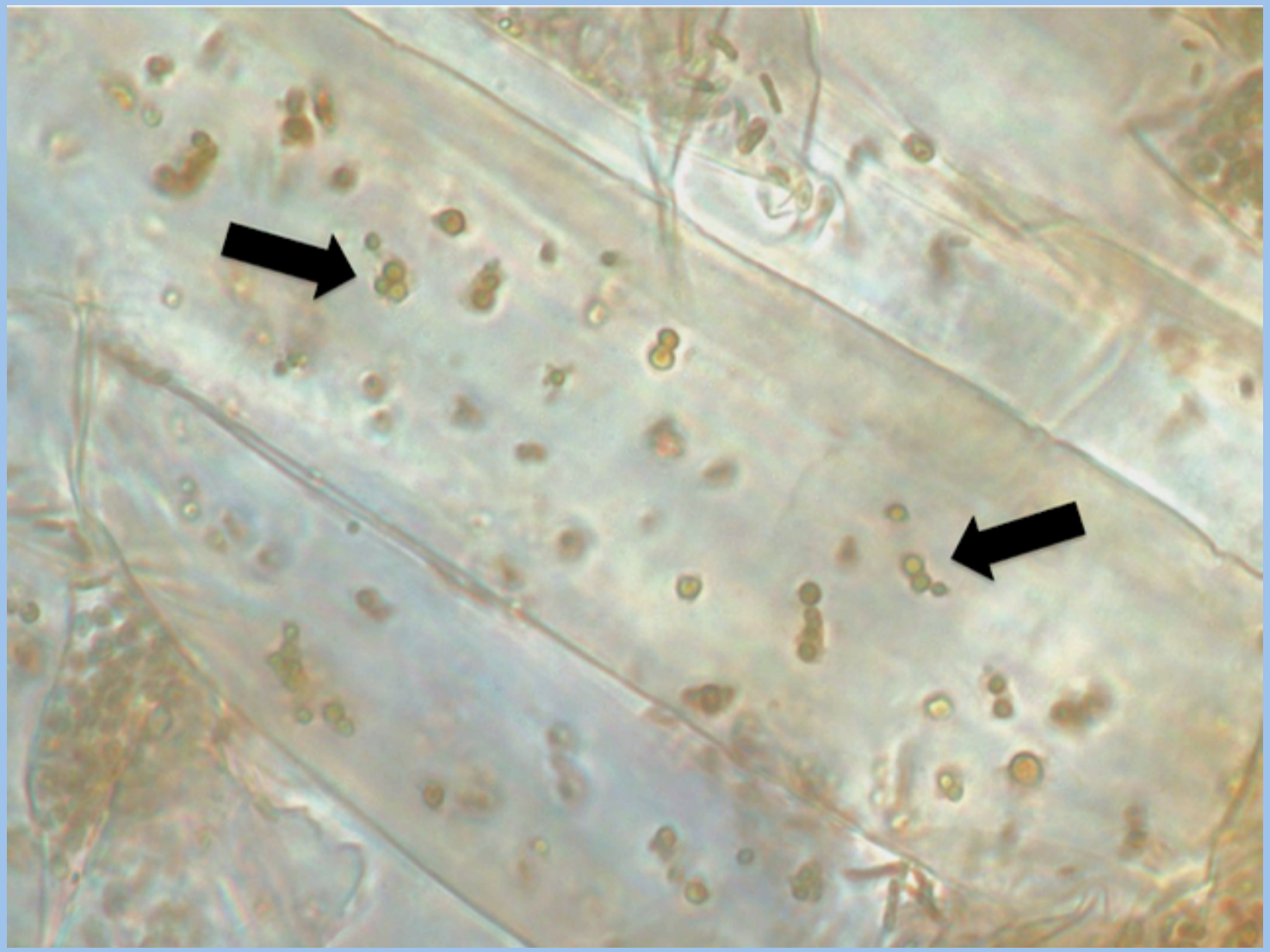
2) Plant uses superoxide dismutase to transform Superoxide to water and Hydrogen peroxide.

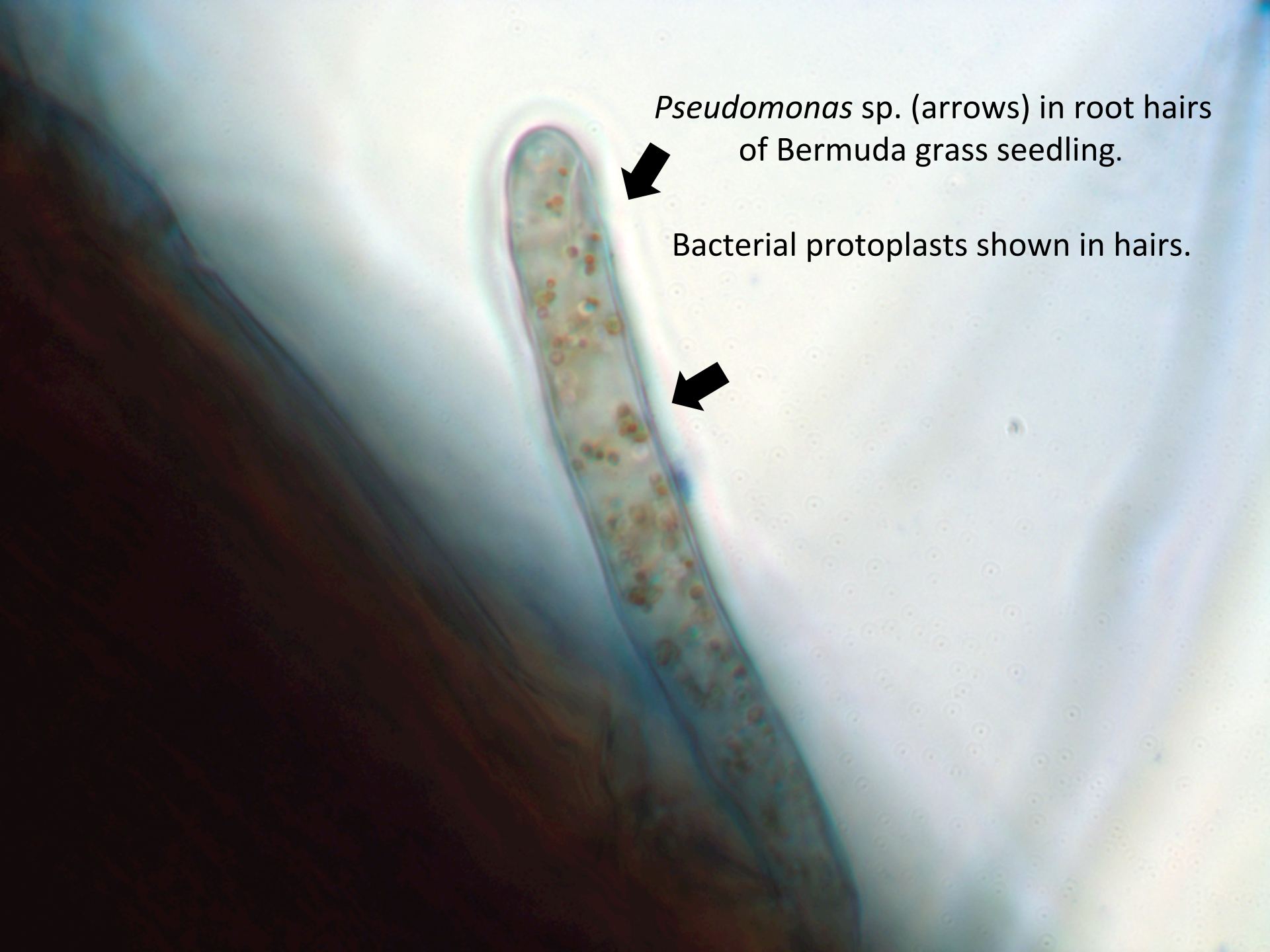
3) DAB reacts with Hydrogen peroxide to form Brown/red coloration

HRP is peroxidase



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*.




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

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.

Circulation may also increase the efficiency of nutrient exchange between root cells and microbe protoplasts by reducing nutrient gradients.

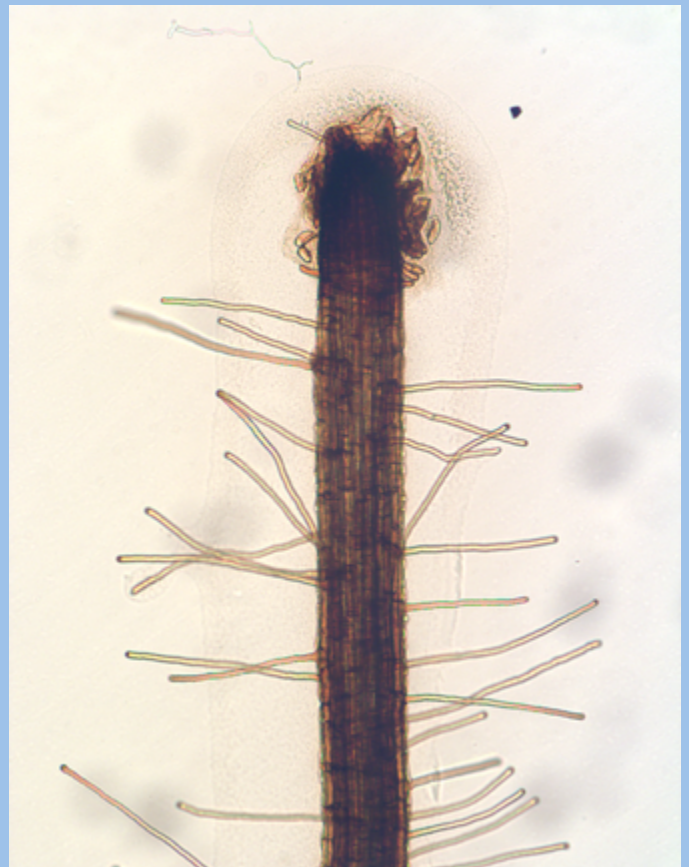
Qiang Chen

What is the function of root hairs?

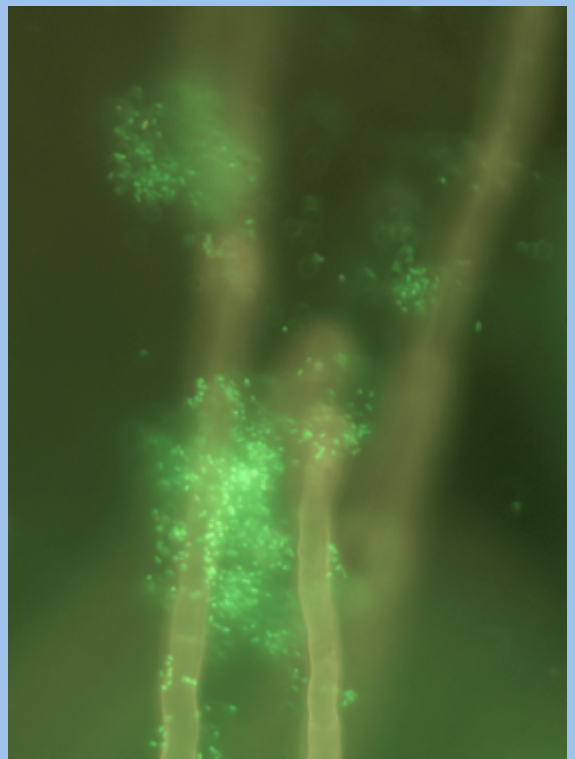
James White 11/5/2017
Rutgers University

Root hairs function to eject rhizophagy microbes out into the soil where they may acquire nutrients.

Root growing in agarose showing extension of root hairs beyond the rhizoplane and the bacterial biofilm on the rhizoplane.



Bacteria emerging from tips of elongating root hairs. Stained with nuclear stain Syto 13.



Bacteria emerging from root hair tip. Bacteria in hairs are present as wall-less L-forms. Bacteria reform their walls after exiting from the tip of the hair.

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. This may be due to potassium loading into the vacuole at the base of the root hair cell.

Mystery of the vanishing nutrients!!

As Carbon Dioxide Levels Rise, Major Crops Are Losing Nutrients

1) Wheat (C3 plant) showed declines in protein, magnesium, iron and zinc.
2) Soybeans and field peas (with rhizobia) showed declines in magnesium, iron and zinc.
3) Maize and sorghum (C4 plants) were less affected.

Myers, S. et al. 2014. Increasing CO_2 threatens human nutrition. Nature 510: 139-142.

Hypothesis

We hypothesize that elevated CO_2 inhibits the extraction of nutrients from microbes in the rhizophagy cycle through inhibition of superoxide formation in root cells.

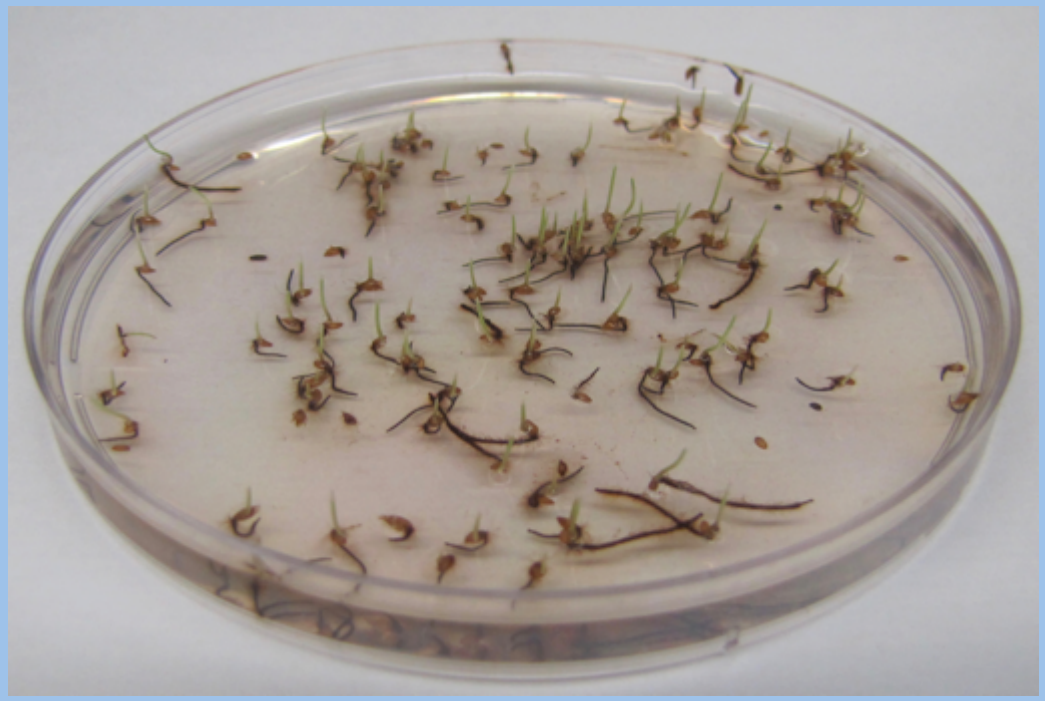
Carbon dioxide inhibits generation of superoxide that plants use to extract nutrients from microbes!

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.

Rajan Verma

Experiments with seedlings in elevated CO_2 atmospheres



Experiments were conducted where seedlings of plants were grown under two levels (non-elevated and elevated) of atmospheric carbon dioxide. Dry ice was used to elevate carbon dioxide in chambers.

Experiments with tall fescue seedlings

Non-Elevated 0.04% CO_2

Roots from seedlings in the non-elevated level of CO_2 (0.04%) show superoxide presence (blue color due to NBT staining) in cells around the root tip meristem. Roots also show formation of long root hairs just behind the root tip meristem. The long root hairs form because of the abundant presence of microbe protoplasts in root hairs.

Elevated 0.06% CO_2

Tall fescue seedling roots in the elevated CO_2 treatment (0.06%) do not show superoxide formation (blue color) in cells around the root tip meristem. Although, some blue color is observable in the interior of the meristem. In this treatment root hair development appears to be suppressed with no root hairs forming near the root tip.

Non-elevated 0.04% CO_2

Superoxide is visible in the tips of the root hairs where microbe protoplast accumulate. It is the presence of these microbes in root hairs that stimulates hairs to elongate. This may be the result of nitric oxide signaling by microbes in hair tips.

Elevated 0.06% CO_2

Tall fescue seedling roots in the elevated CO_2 treatment (0.06%) do not show superoxide formation (blue color) in root hairs. In this treatment root hair development appears to be suppressed.

Wheat elevated CO_2 experiment in protein-agarose

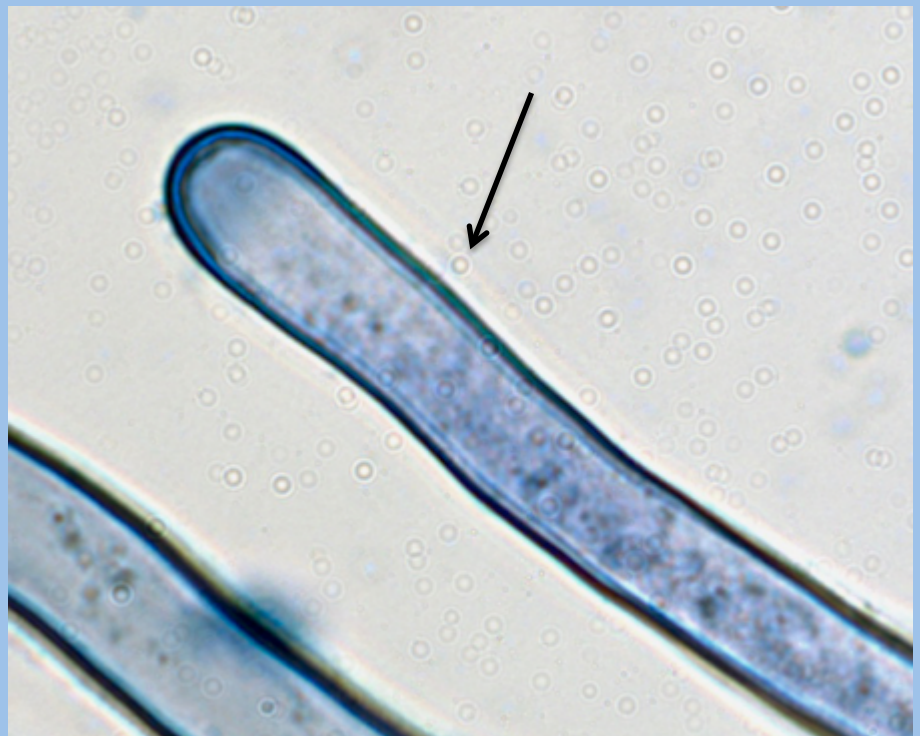
- Winter wheat seeds surface disinfected (45 min 4% NaOCl) to reduce surface microbes.
- Microbes vectoring within seeds were not affected.
- Seeds placed onto 0.1% protein (denatured lipase) agarose (protein stimulates root hair elongation in absence of microbes).
- Seedlings germinated and grown for 3 days in lab ambient air (approx. 0.04% CO_2).
- Seedlings placed in gas chambers with 0.04% CO_2 (current atmosphere concentration) or 0.06% CO_2 (elevated level) and incubated 4 days at room temperature.
- Seedlings removed from chambers and stained for 15 hours by flooding plates with diaminobenzidine tetrachloride stain to visualize H_2O_2.
- Seedlings from both treatments examined for microscopic evidence of rhizophagy cycle activity in roots.

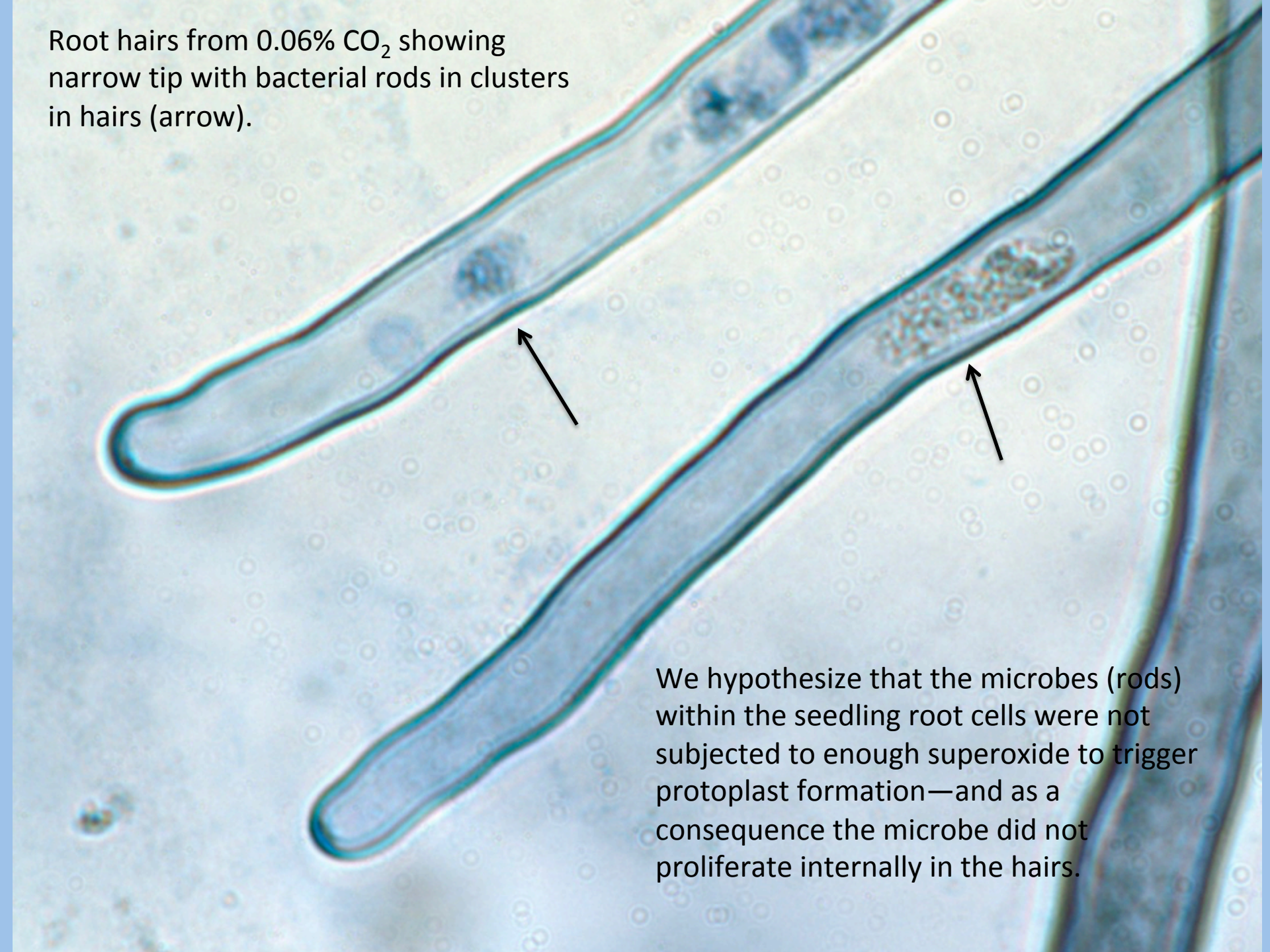
Wheat root from 0.04% CO_2 showing violet NBT staining in outer cells of the root tip due to presence of superoxide around microbes in cells.

Wheat root from 0.06% CO_2 showing absence of violet NBT staining due to suppression of superoxide in outer layers of the root tip by elevated CO_2 .

Root hair from 0.06% CO_2 showing narrow tip without internal bacteria (arrow).

Root hairs from 0.04% CO_2 showing swollen tips with masses of bacteria evident internally at tip (arrows; DAB stained).

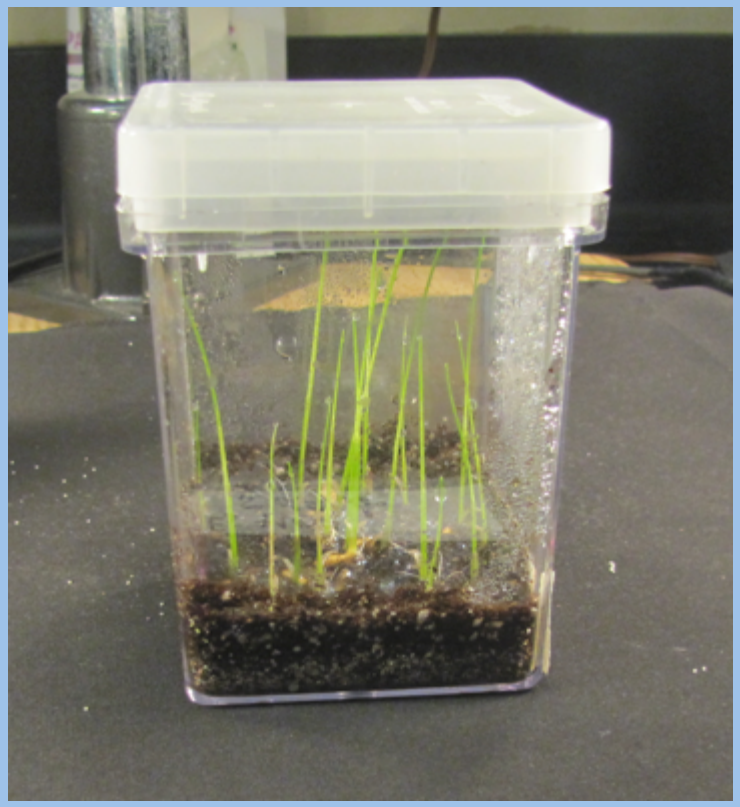



Root hairs from 0.06% CO_2 showing narrow tip with bacterial rods in clusters in hairs (arrow).
We hypothesize that the microbes (rods) within the seedling root cells were not subjected to enough superoxide to trigger protoplast formation—and as a consequence the microbe did not proliferate internally in the hairs.

Examination of effects of elevated CO_2 on rhizophagy cycle and root hair formation in winter wheat in potting mix

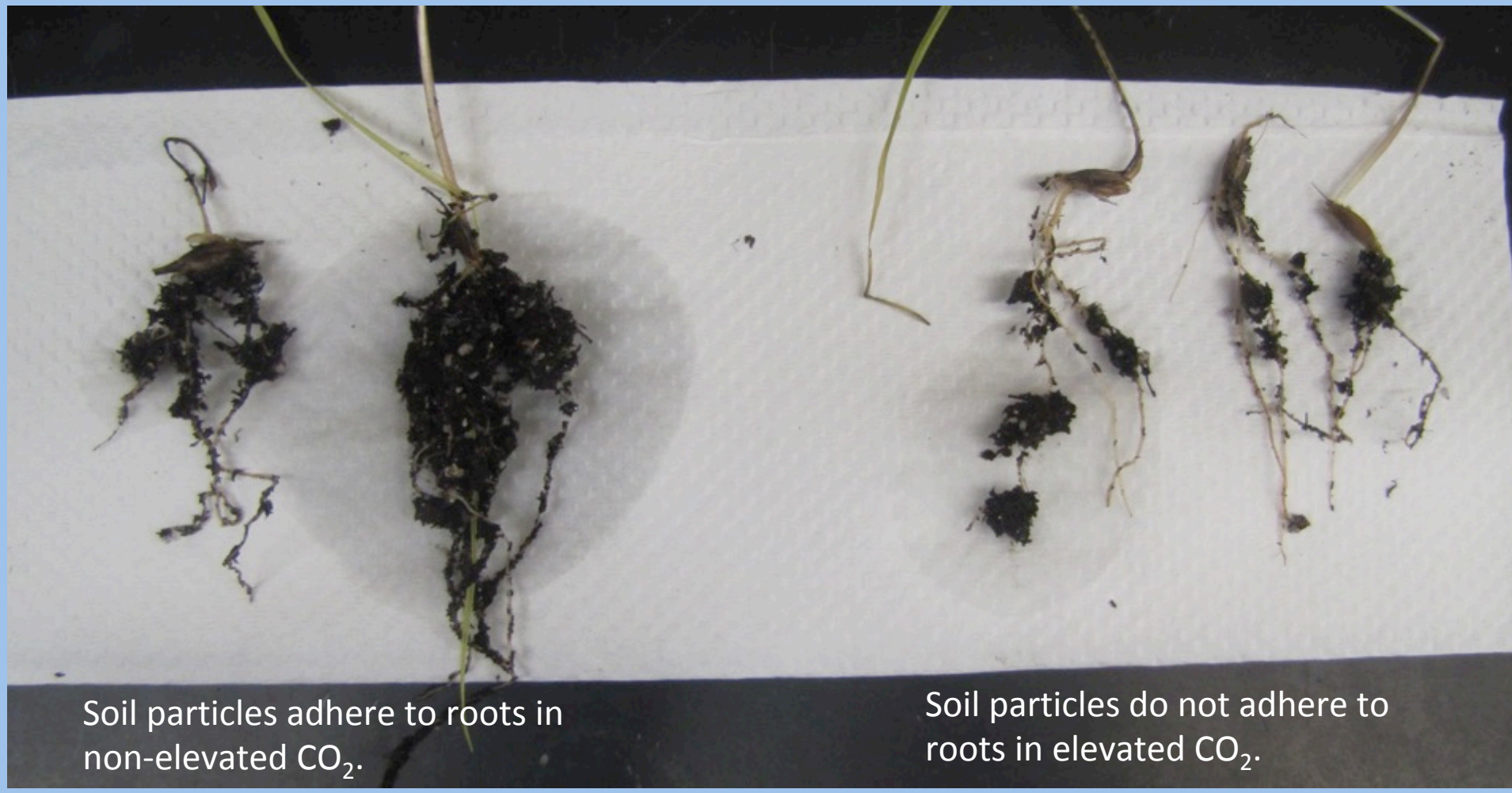
- Wheat seeds were germinated in potting mix and grown in chambers with air at 0.04% CO_2 (non-elevated) or air with elevated CO_2 (approx. 0.06%).
- Incubated for 21 days in the laboratory.
- Chambers were opened and plants pulled from potting mix to examine roots.
- Seedling roots were then examined to determine differences in root development between the two treatments.

Elevated Atmospheric CO_2 Experiments



Plants in non-sterile potting mix

Plants placed in gas chambers

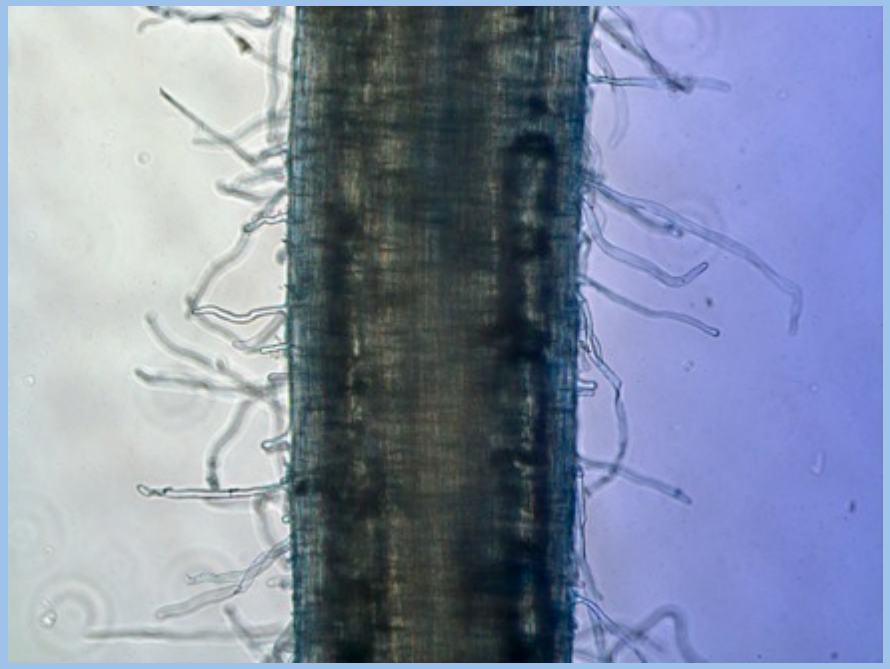


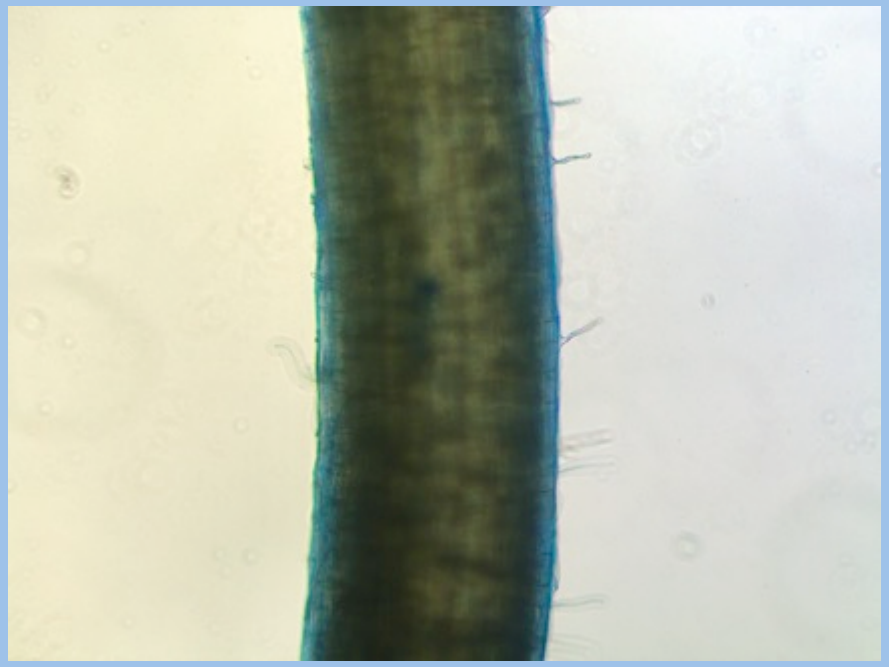
Non-Elevated CO_2 (0.04%)

Elevated CO_2 (0.06%)

Reduced soil adherence to roots in elevated CO_2 may be the result of reduced secretion of root exudates, reduced microbial activity in soil, and reduced root hair elongation.

Elevated CO2 suppresses root hair formation in winter wheat. Where more microbes are present in root cells, root hairs are longer; absence of microbes in root cells results in failure of hairs to form. Root hair elongation may be the result of nitric oxide signaling by the microbes accumulating in the tip of the root hair.





Non-Elevated CO_2 (0.04%)

Elevated CO_2 (0.06%)

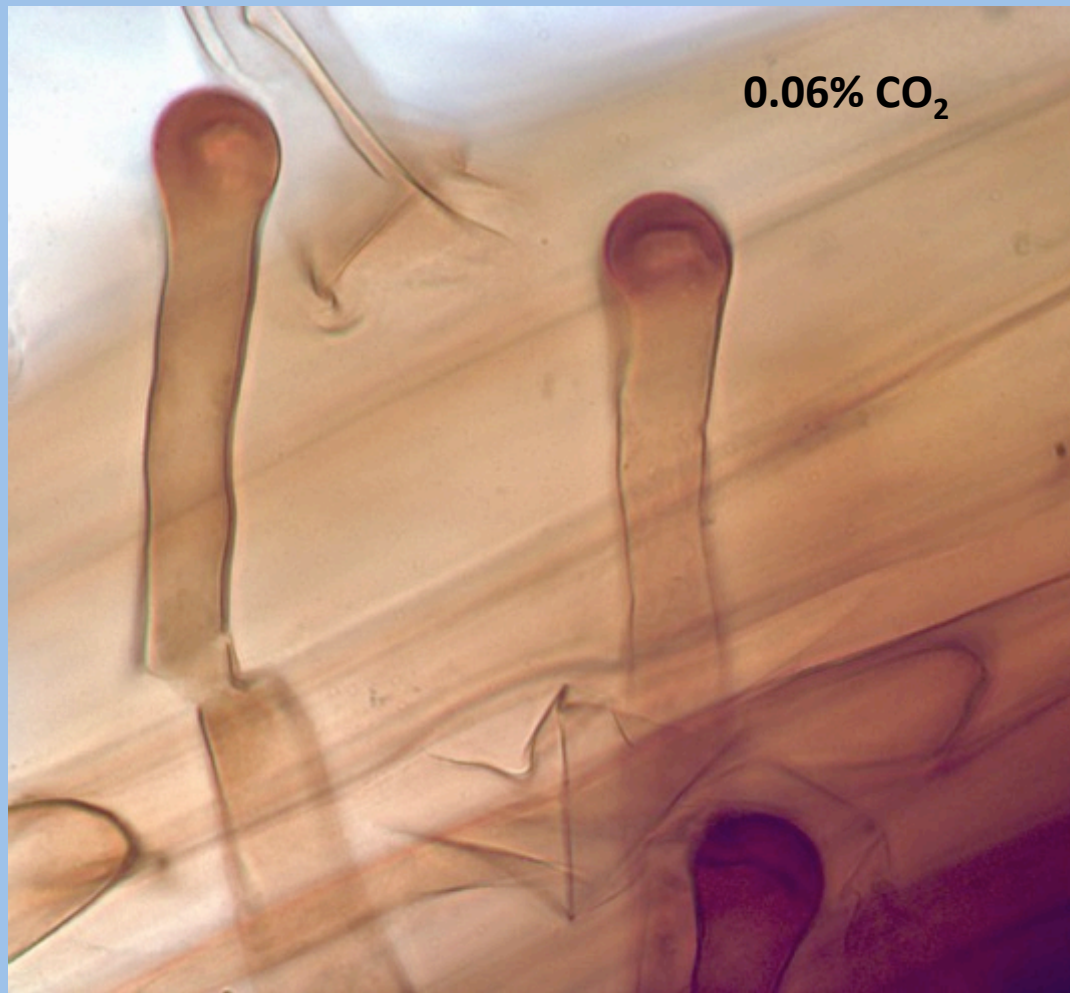
Nkolika Obi

Tomato Seedlings: Elevated CO_2 Experiment

- Tomato seeds surface disinfected (5 min 4% NaOCl) to reduce surface microbes.
- Seeds placed into potting mix.
- Seeds placed in gas chambers with 0.04% CO_2 (current atmosphere concentration) or 0.06% CO_2 and incubated 21 days at room temperature.
- Seedlings removed from chambers and stained for 15 hours by flooding plates with diaminobenzidine tetrachloride stain to visualize H_2O_2.
- Seedlings from both treatments examined for microscopic evidenc of rhizophagy cycle activity in roots.
- Seedling shoots analyzed for nutrient content at Penn State Plant Analysis Lab.

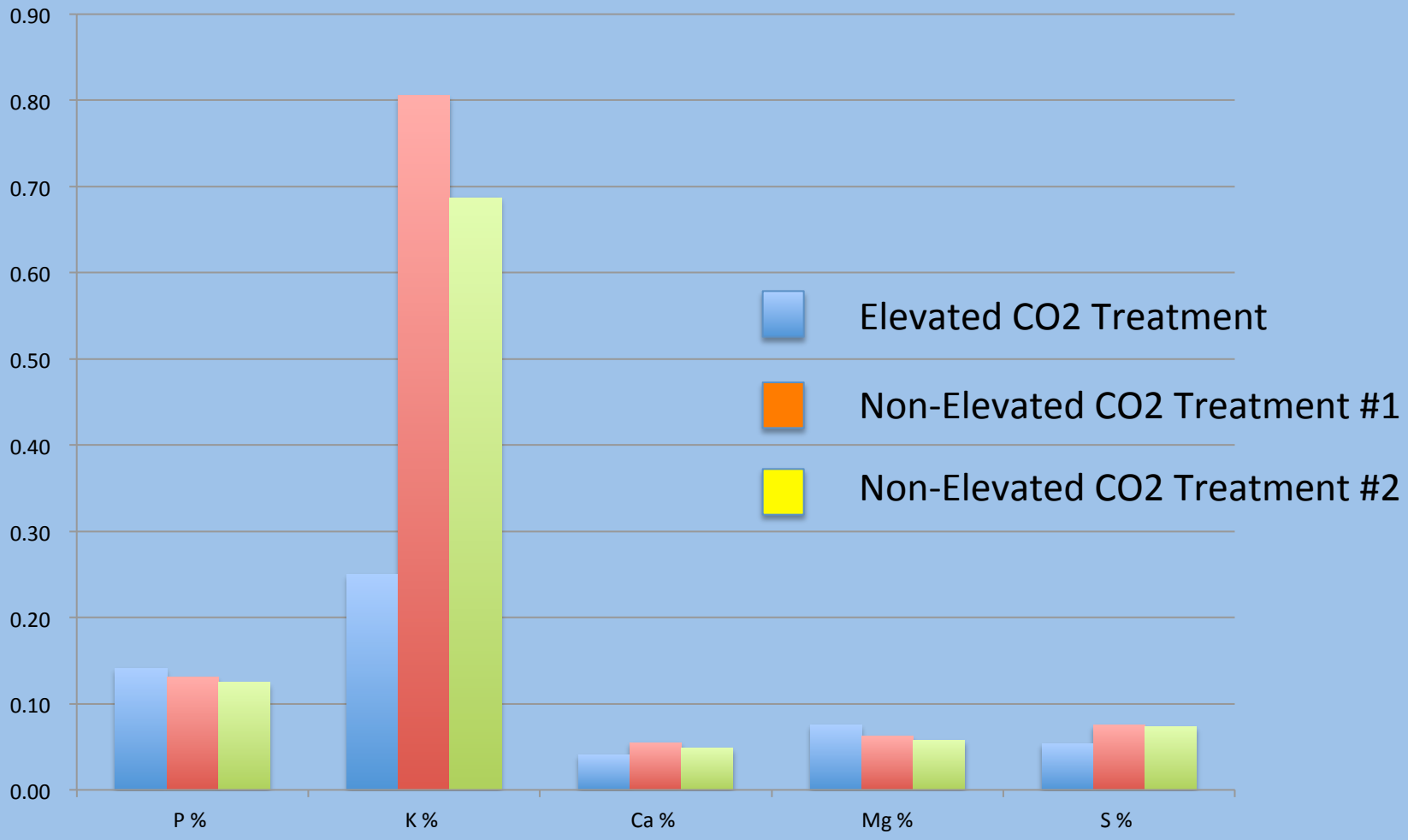
Microbe protoplasts (arrows) may be seen within root hairs of the 0.04% CO_2 air; but were not visible in hairs of the elevated CO_2 treatment (DAB stained).

Microbe protoplasts (arrows) may be seen within root hairs of the 0.04% CO_2 air; but were not visible in hairs of the elevated CO_2 treatment (DAB stained).



Microbe protoplasts (arrows) may be seen within root epidermal cells of the 0.04% CO_2 air; but were not visible in cells of the elevated CO_2 treatment (DAB stained).

Tomato seedling nutrient absorption experiment (macronutrients)



What nutrients does the rhizophagy cycle provide?

1. Macronutrients → nitrogen, potassium, calcium (?), sulfur (?)

Hill et al. (2013) reported that absorption of N via direct degradation of bacteria was 10 to 100X slower than absorption of mineralized N.

2. Micronutrients → iron, zinc, manganese ?????

Microbes contain metals in order of concentration: Mg > Ca > Fe > Zn etc.. (Monowar et al., 2019).

High-affinity zinc and iron binding proteins are common in soil microbes (Hantke, 2005).

Hill, P. W., Marsden, K. A., & Jones, D. L. (2013). How significant to plant N nutrition is the direct consumption of soil microbes by roots? *The New Phytologist*, *199*(4), 948–955. http://doi.org/10.1111/nph.12320

Hantke K. (2005) Bacterial zinc uptake and regulators. Current Opinion Microbiol. 8: 196-202.

Tahmina Monowar, Md. Sayedur Rahman, Subhash J. Bhore, Gunasunderi Raju, and Kathiresan V. Sathasivam (2019). "Secondary Metabolites Profiling of Acinetobacter baumannii Associated with Chili (Capsicum annuum L.) Leaves and Concentration Dependent Antioxidant and Prooxidant Properties," BioMed Research International, vol. 2019, Article ID 6951927, 13 pages, 2019. https://doi.org/10.1155/2019/6951927.

Conclusions

- Elevated CO_2 inhibits superoxide formation in plant root cells.
- Without superoxide, microbes do not convert to protoplasts within root cells.
- Without protoplast phases in root cells, root hairs do not elongate.
- In elevated CO_2 , the rhizophagy cycle in roots is suppressed and nutrient absorption into roots is reduced.







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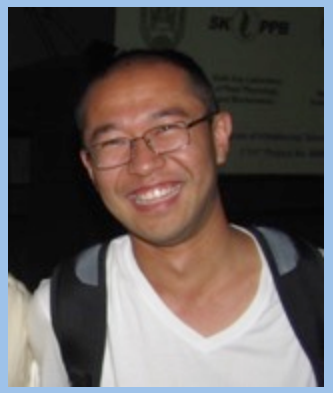






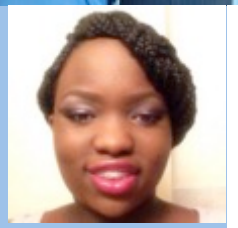

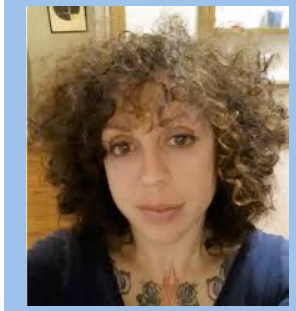

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New Jersey Agric. Exp. Sta.;
USDA NIFA Multistate 3147;
Rutgers Center for Turfgrass Science;
USGS-Rutgers U (CESU Study Agreement)