Elevated Atmospheric CO_2 Inhibits the Rhizophagy Cycle in Tomato Seedling Roots

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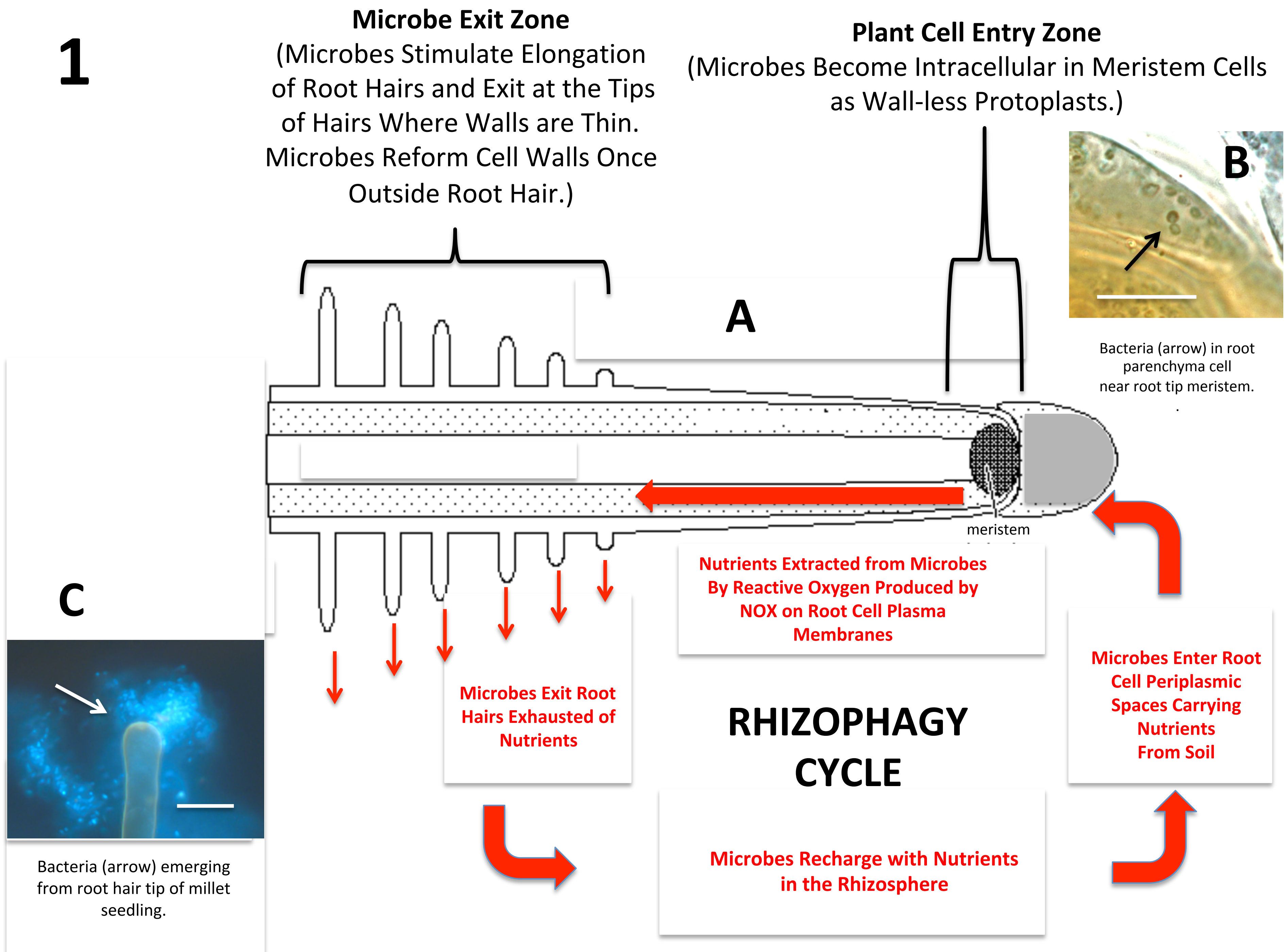
Data File: March 4, 2019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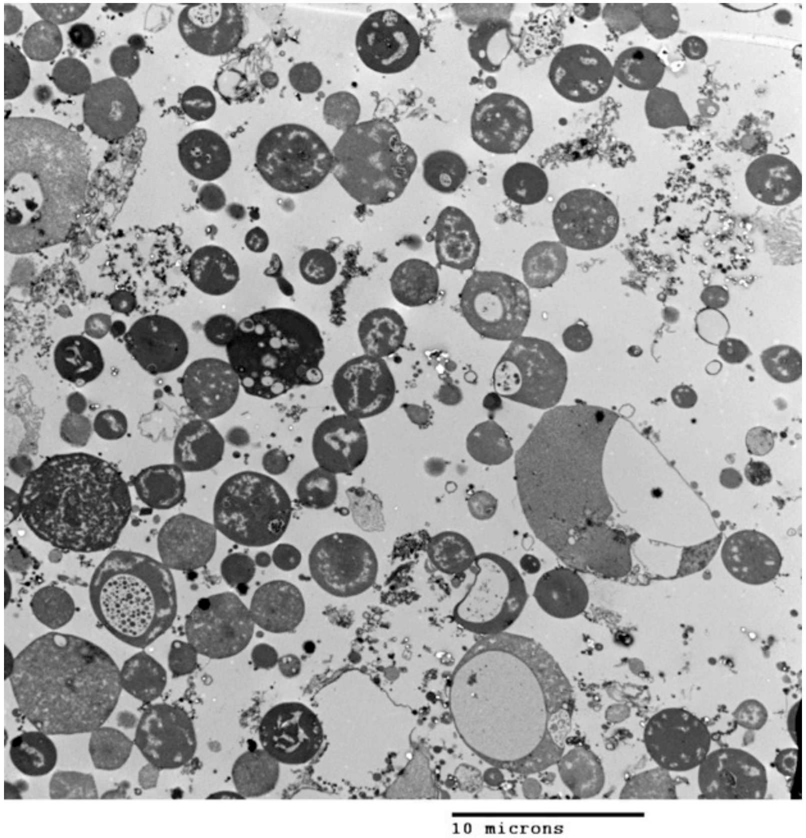
Microbe Exit Zone
(Microbes Stimulate Elongation of Root Hairs and Exit at the Tips of Hairs Where Walls are Thin. Microbes Reform Cell Walls Once Outside Root Hair.)
Plant Cell Entry Zone
(Microbes Become Intracellular in Meristem Cells as Wall-less Protoplasts.)
A
B
Bacteria (arrow) in root parenchyma cell near root tip meristem.
meristem
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 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

TEM of *Bacillus subtilis* L-forms

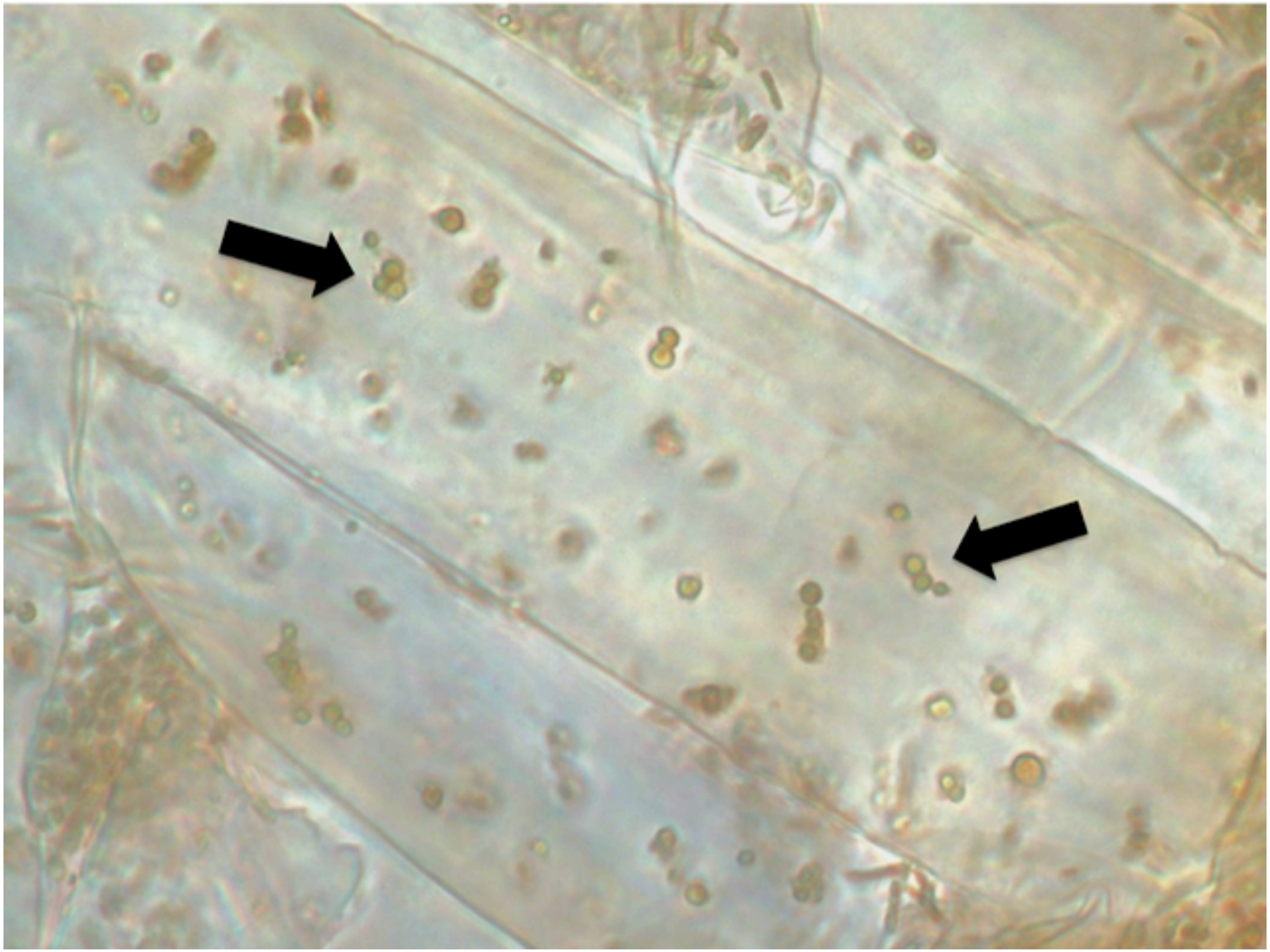
Photo by Mark Leaver, New Castle University, UK

L-forms = Protoplasts



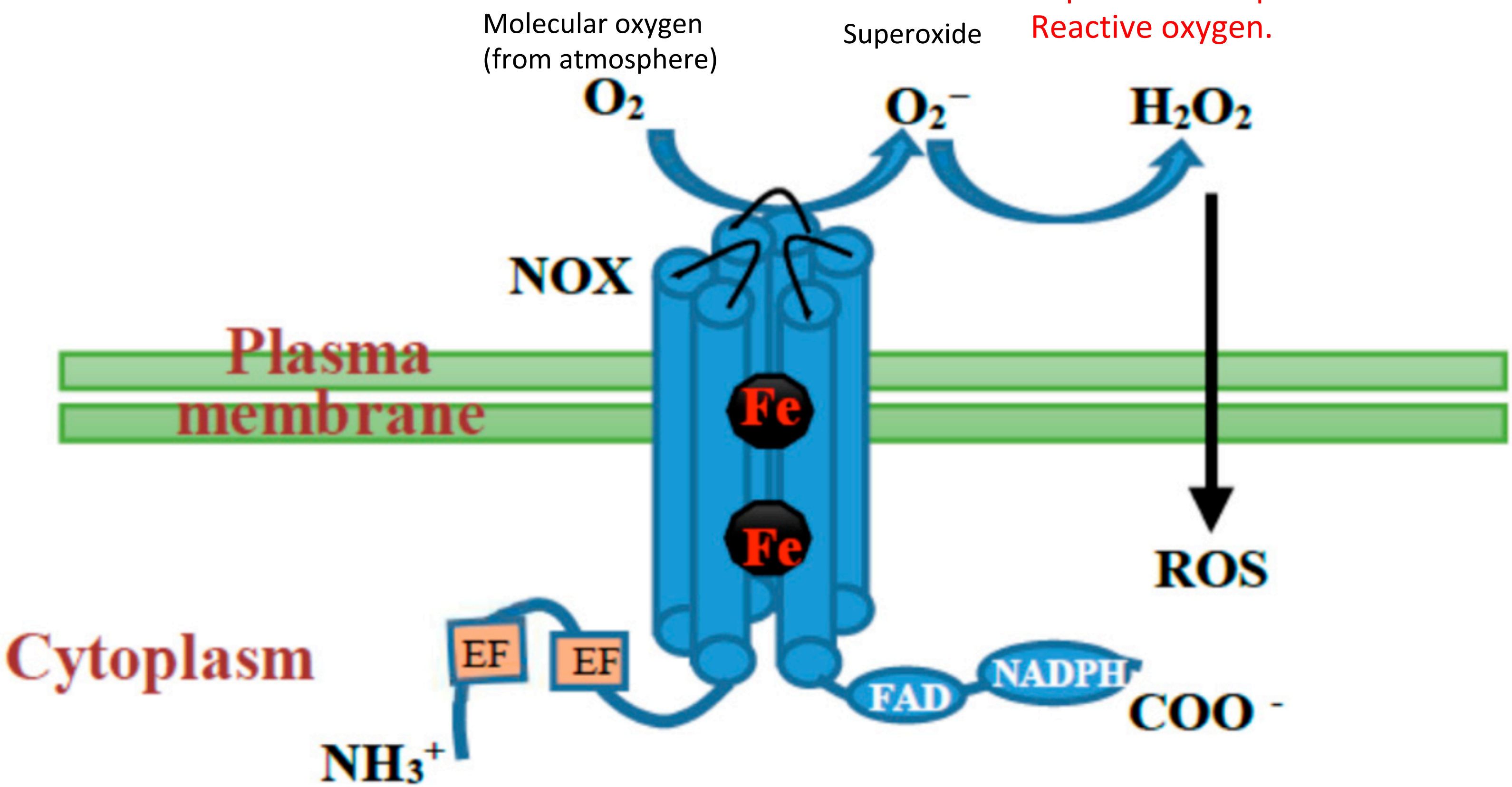
L-forms are bacterial cells that do not form cell walls (also called ‘cell wall deficient bacteria’). L-forms typically are seen inside eukaryotic cells. They are thought to be a mechanism to evade host defense response. L-form bacteria are typically variable in size.

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 causes bacteria to become protoplasts and extracts nutrients from the bacteria (mostly pseudomonads) that are symbiotic with *Phragmites*.



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

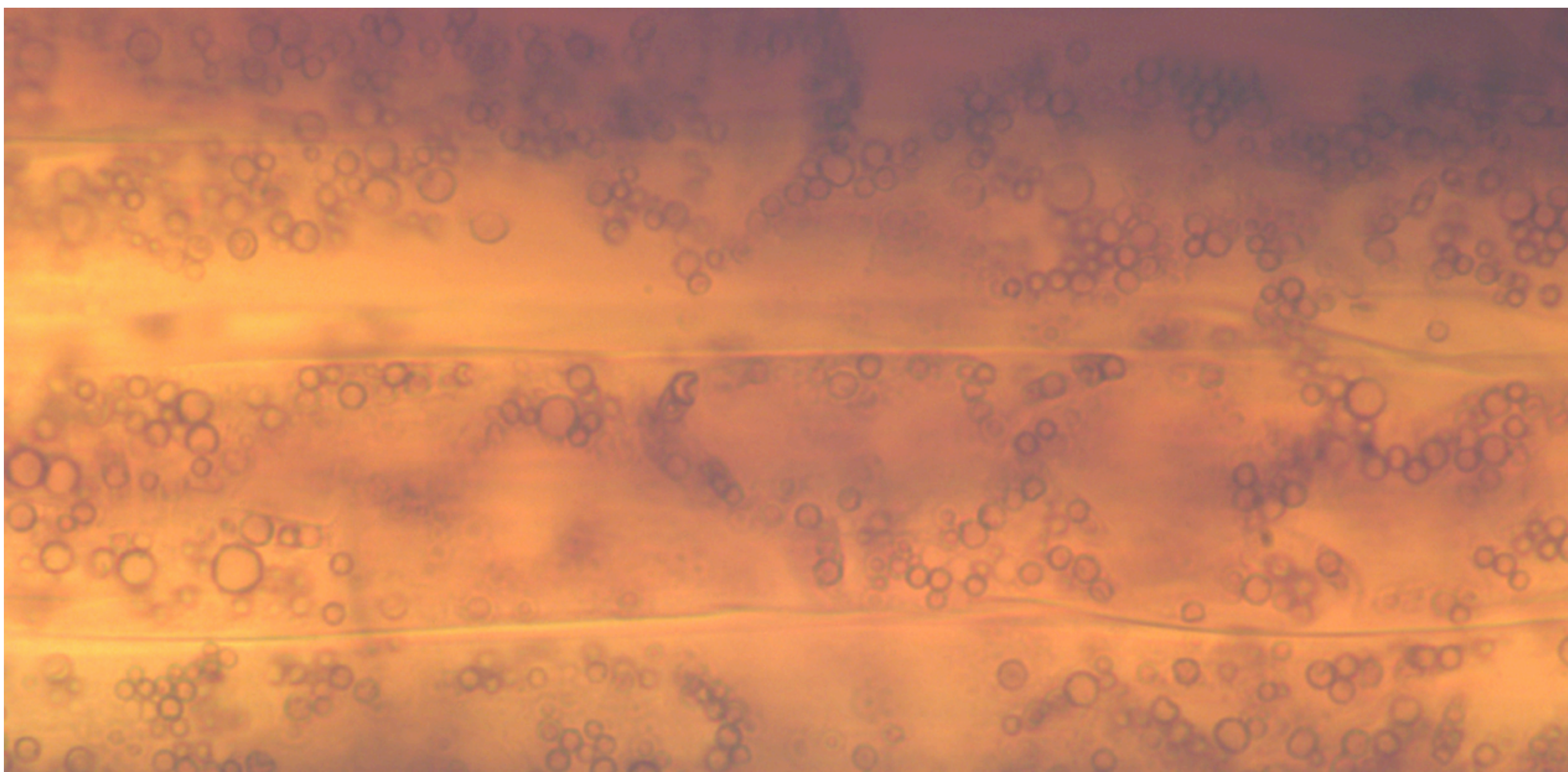
Superoxide is a potent form of Reactive oxygen.

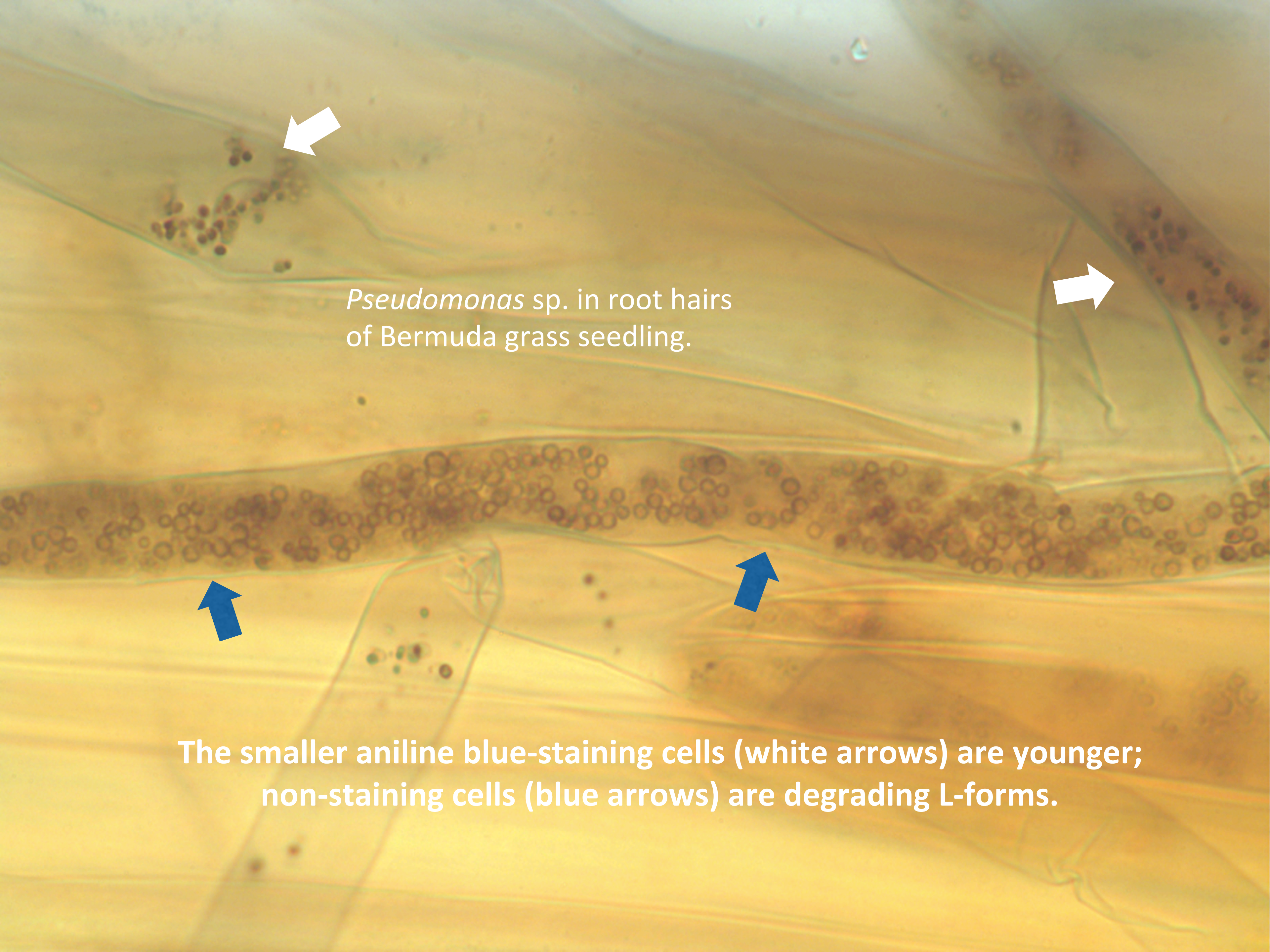


Chemical equation: $NADPH + 2O_2 \xrightarrow{NOX} NADP^+ + 2O_2^- + 2H^+$

How reactive oxygen affects intracellular microbes

1. NADPH oxidase on plant cell plasma membrane converts O_2 to superoxide $O_2^{\cdot-}$.
2. Superoxide breaks down the microbial cell walls.
3. Superoxide damages proteins in the microbe plasma membrane.
4. Superoxide causes leakage in the microbe plasma membrane.
5. Superoxide enters microbe cells and damages proteins and nucleic acids.
6. Microbe protoplasts swell and lose internal proteins as contents are oxidized.



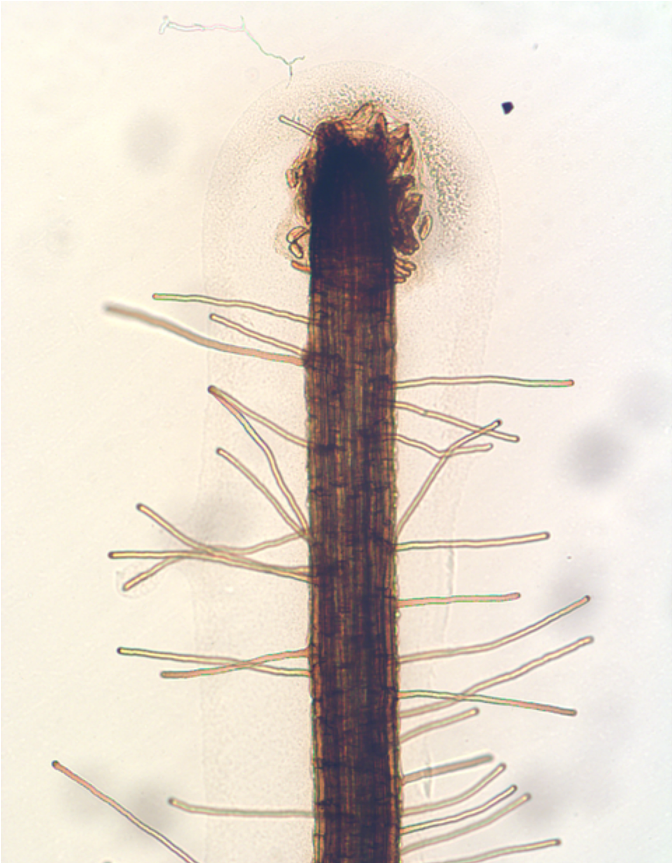

Pseudomonas sp. in root hairs
of Bermuda grass seedling.
The smaller aniline blue-staining cells (white arrows) are younger;
non-staining cells (blue arrows) are degrading L-forms.

What is the function of root hairs?

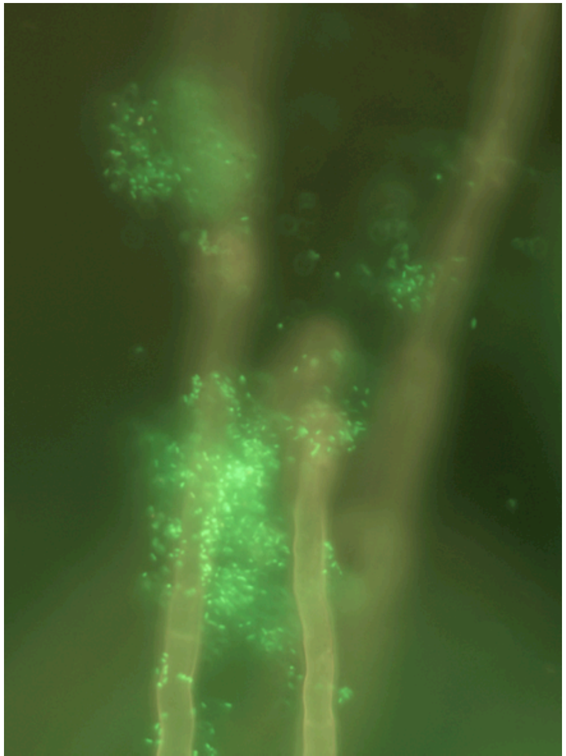
James White 11/5/2017
Rutgers University

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

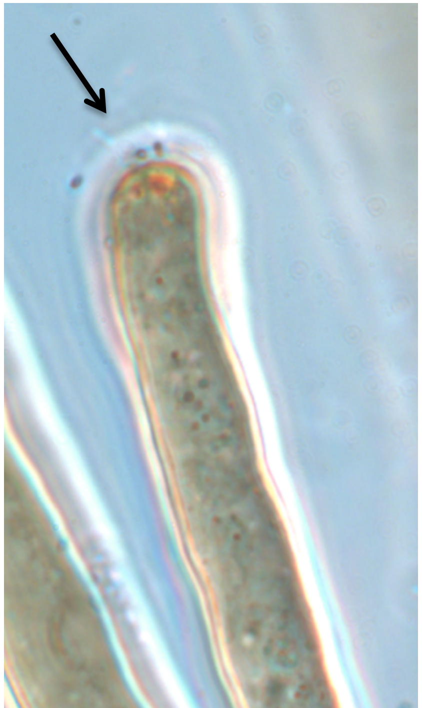
Root growing in agarose showing extension of root hairs beyond the rhizoplane and the bacterial biofilm on the rhizoplane.



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

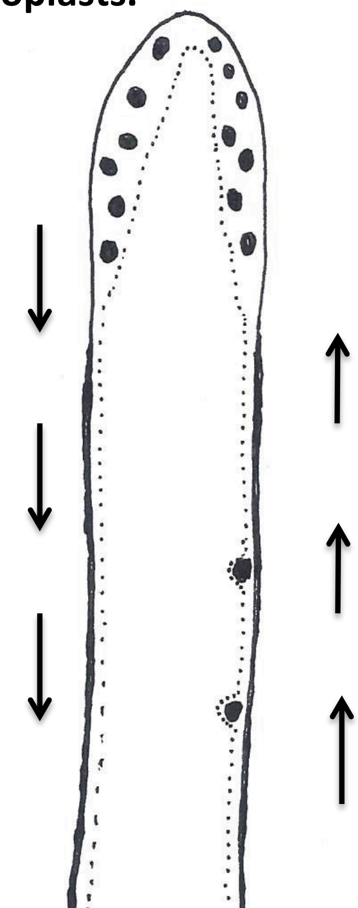


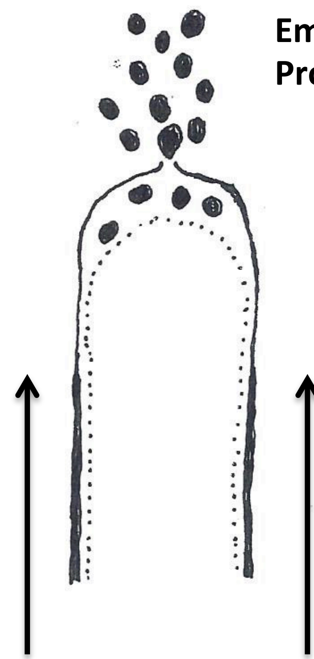
Bacteria emerging from root hair tip. Bacteria in hairs are present as wall-less L-forms. Bacteria reform their walls after exiting from the tip of the hair.



Cyclosis/Expansion Wave Mechanism for Microbe Expulsion from Root Hairs in Rhizophagy Cycle

1. Cyclosis moves microbes to tip and facilitates replication of microbe protoplasts.



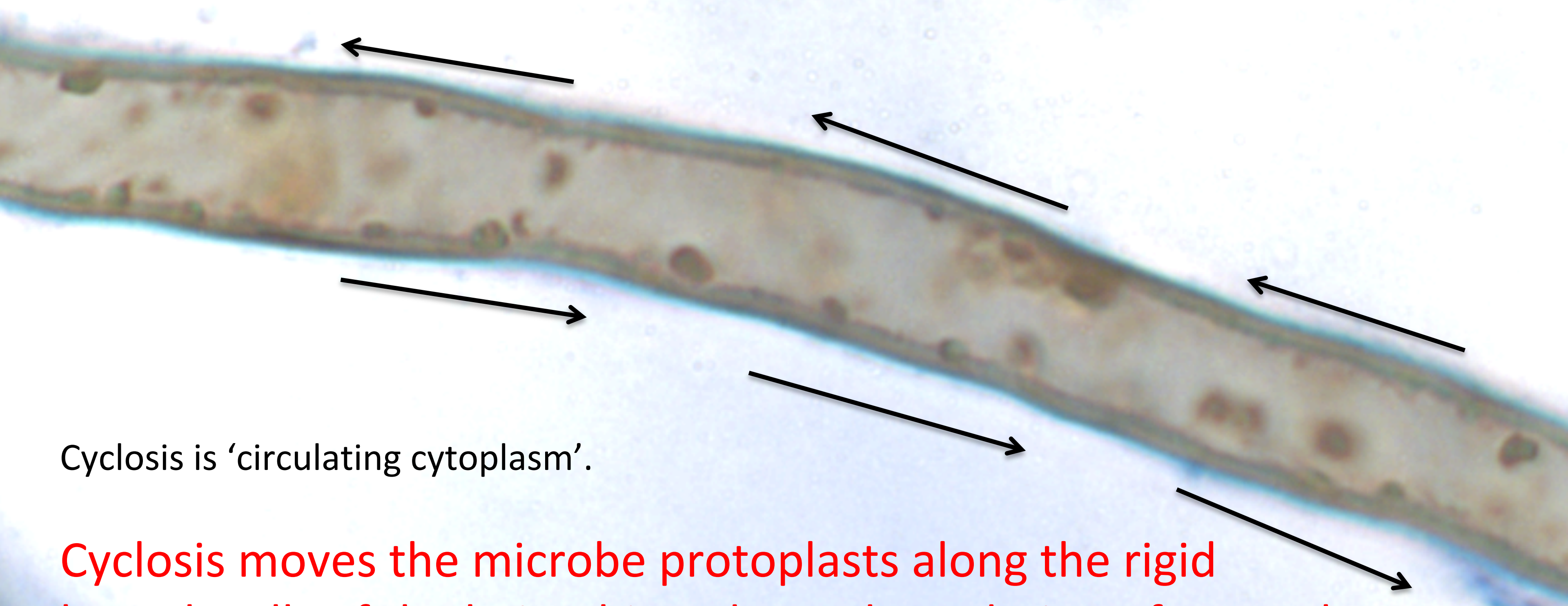


Expansion Wave

2. A wave of expansion in the hair protoplast begins in the base of the hair and progresses to the tip of the hair. This expansion wave forces microbe protoplasts through pores that form in the thin wall at the hair tip.

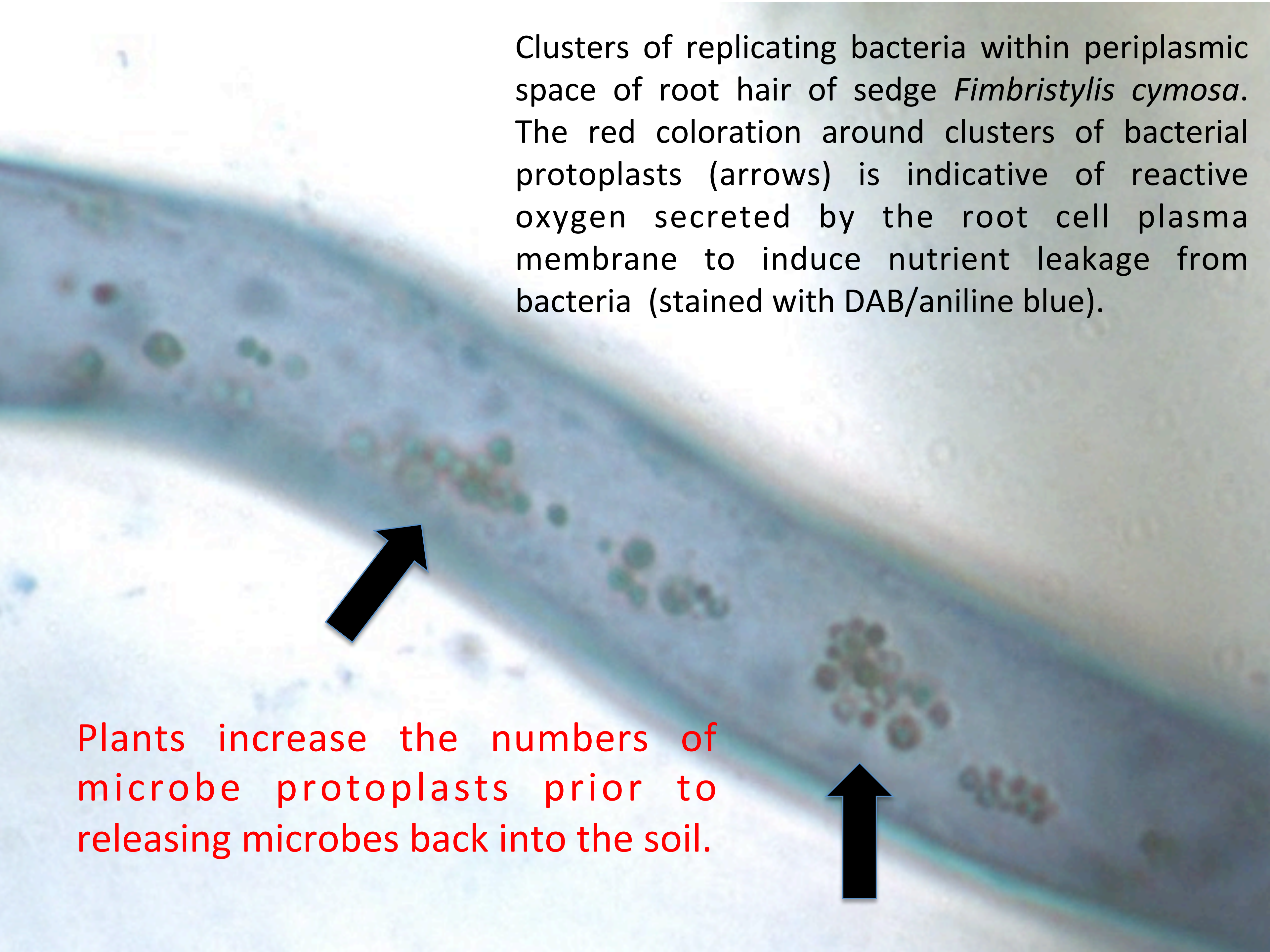
James White
Rutgers University
Feb. 23, 2019

Root hair of *Fimbristylis cymosa* showing bacterial protoplasts in periplasmic space. Bacteria are seen to be surrounded by red-staining reactive oxygen. Bacteria are transported to the tip of the hair and deposited by the action of unidirectional cyclosis in the root hair cell.



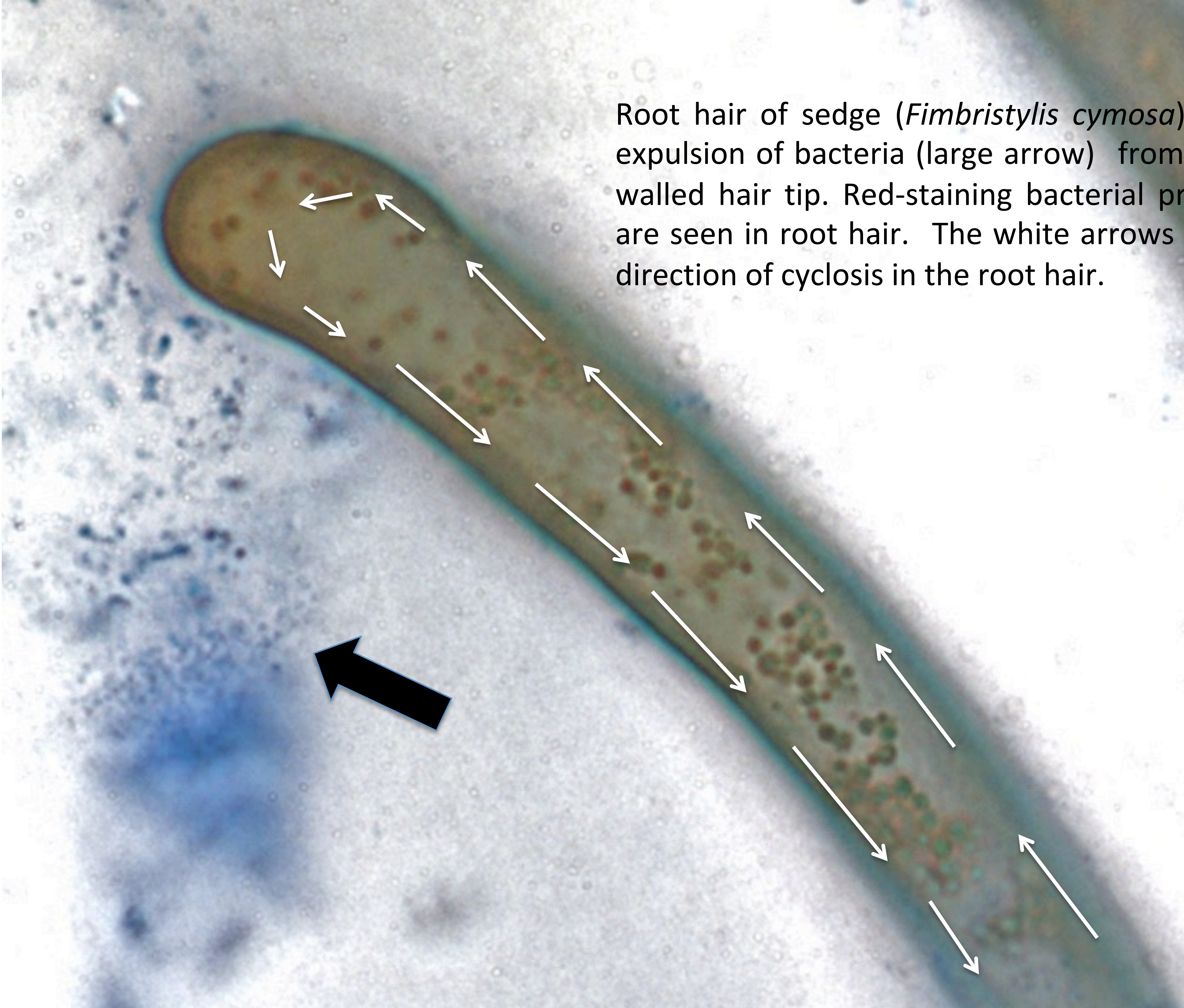
Cyclosis is ‘circulating cytoplasm’.

Cyclosis moves the microbe protoplasts along the rigid lateral walls of the hair. This reduces degradation of protoplasts and increases their recovery and replication.



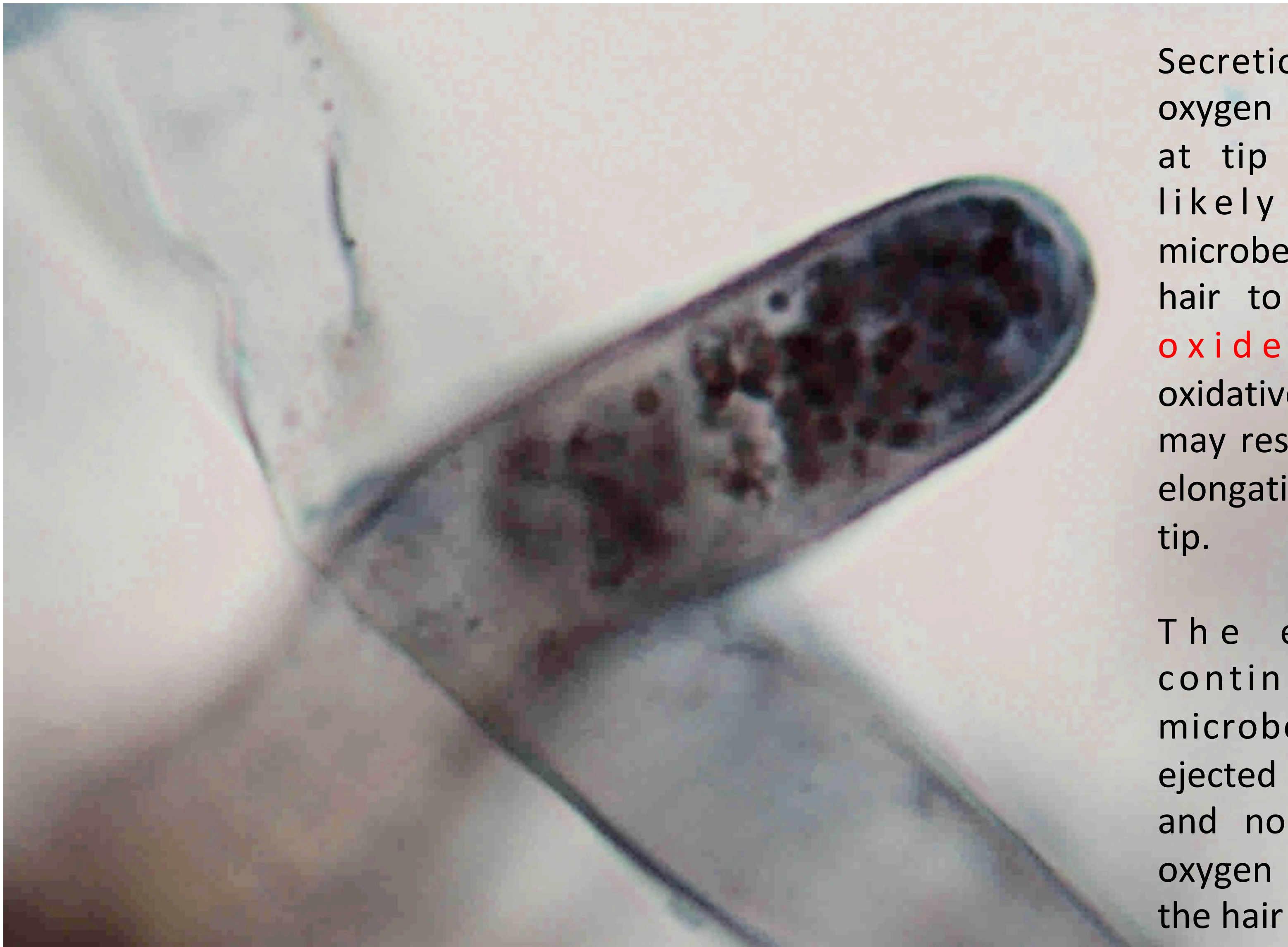
Clusters of replicating bacteria within periplasmic space of root hair of sedge *Fimbristylis cymosa*. The red coloration around clusters of bacterial protoplasts (arrows) is indicative of reactive oxygen secreted by the root cell plasma membrane to induce nutrient leakage from bacteria (stained with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

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



Root hair of sedge (*Fimbristylis cymosa*) showing expulsion of bacteria (large arrow) from the soft-walled hair tip. Red-staining bacterial protoplasts are seen in root hair. The white arrows show the direction of cyclosis in the root hair.

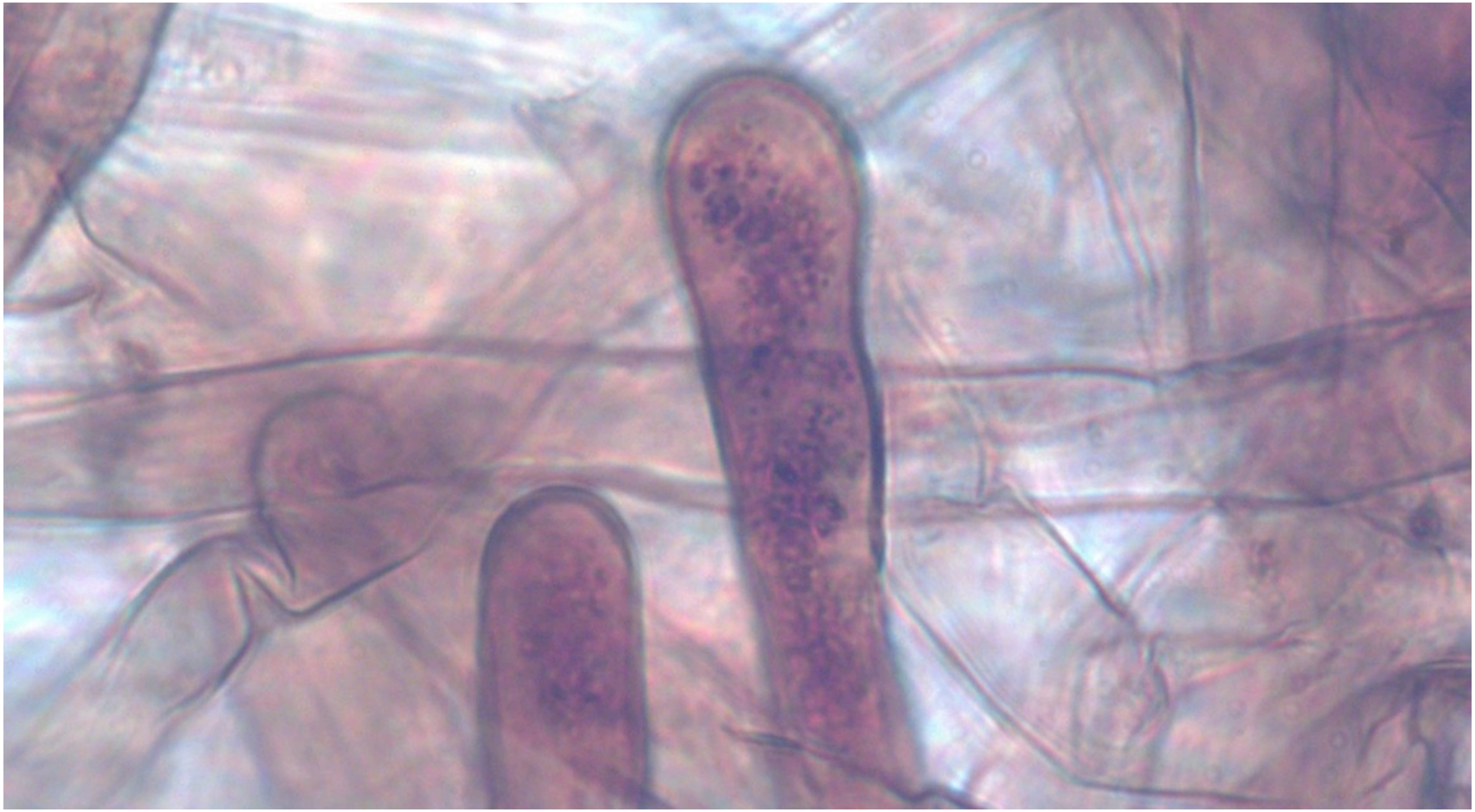
Bacterial concentration in root hairs appears to increase as hairs elongate. This image is of a tomato seedling root hair (stained with DAB/aniline blue).



Secretion of reactive oxygen around bacteria at tip (brown color) likely causes the microbe and the root hair to produce nitric oxide to reduce oxidative damage. This may result in continued elongation at the hair tip.

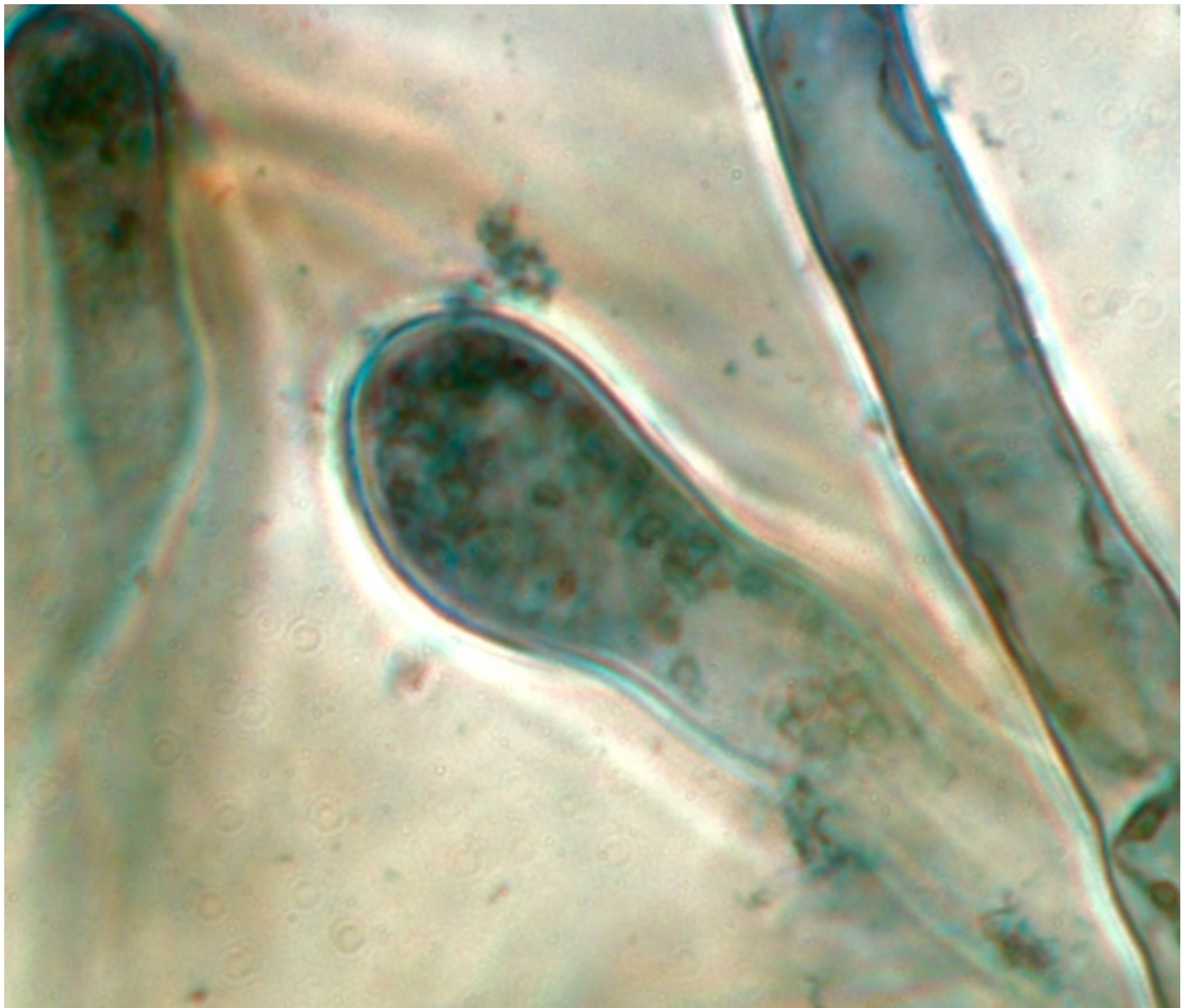
The elongation continues until all microbes have been ejected from the hair and no more reactive oxygen is produced at the hair tip.

Tomato seedling root hairs containing bacteria showing intense hydrogen peroxide staining (brown) around bacteria. Often root hair tips with internal bacteria appear swollen. This shows the elasticity of the wall at the hair tip. Microbes continue to replicate in the hair.

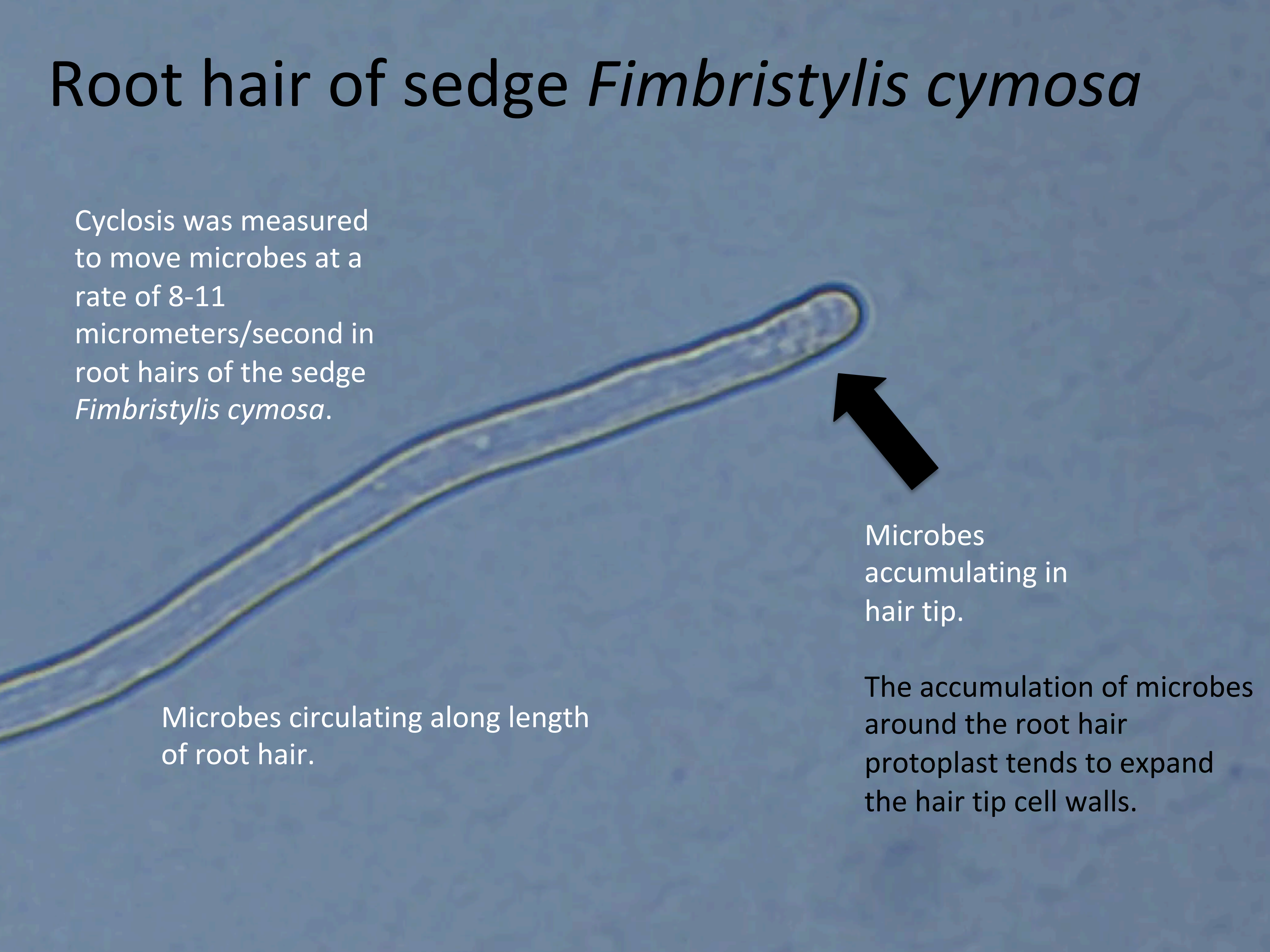


Tomato seedling root hair showing swollen tip containing microbes.

Tomato root hair showing microbes being ejected from the hair tip.



Root hair of sedge *Fimbristylis cymosa*



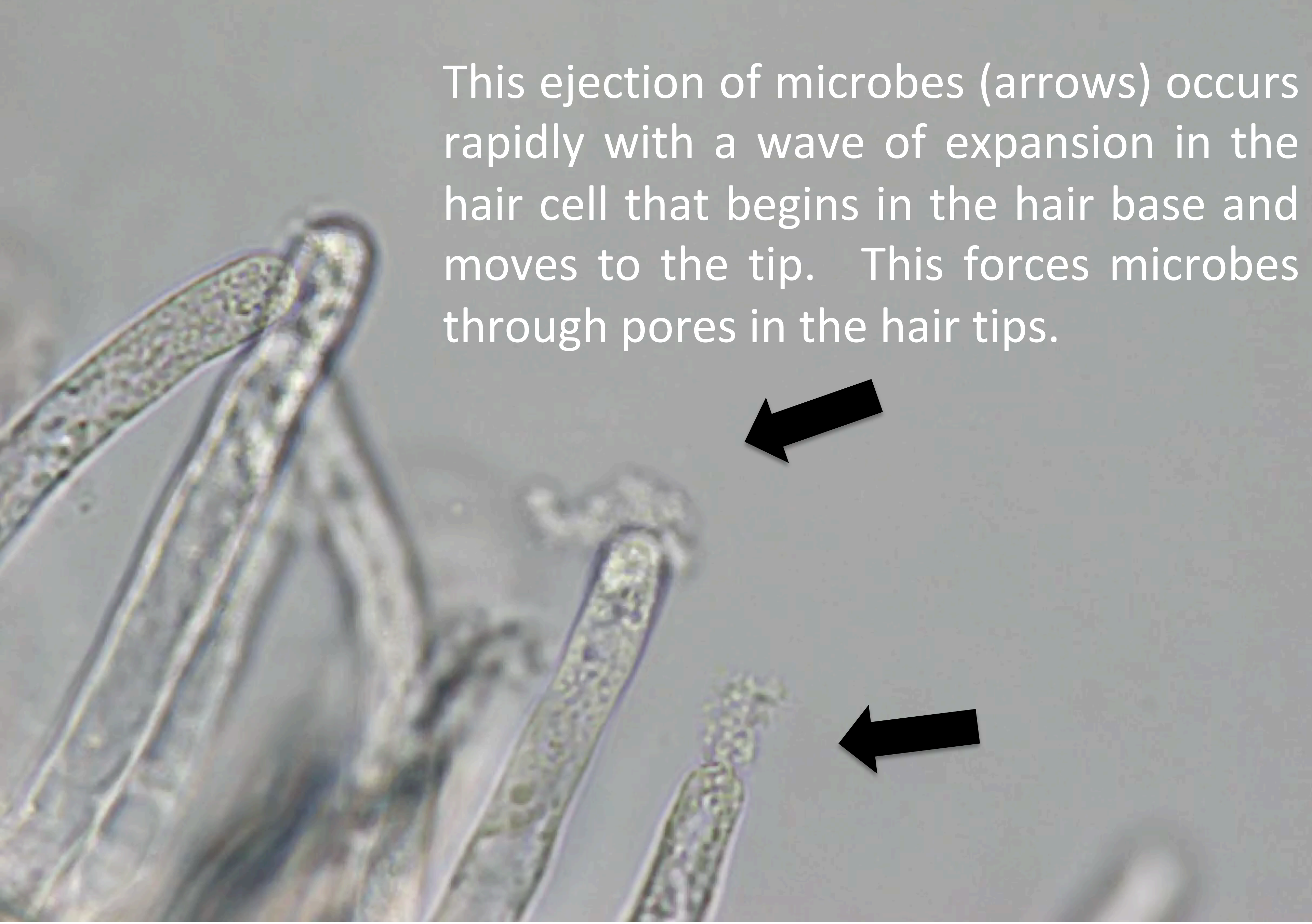
Cyclosis was measured to move microbes at a rate of 8-11 micrometers/second in root hairs of the sedge *Fimbristylis cymosa*.

Microbes circulating along length of root hair.

Microbes accumulating in hair tip.

The accumulation of microbes around the root hair protoplast tends to expand the hair tip cell walls.

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.



What nutrients does the rhizophagy cycle provide?

1. Proteins → nitrogen

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).

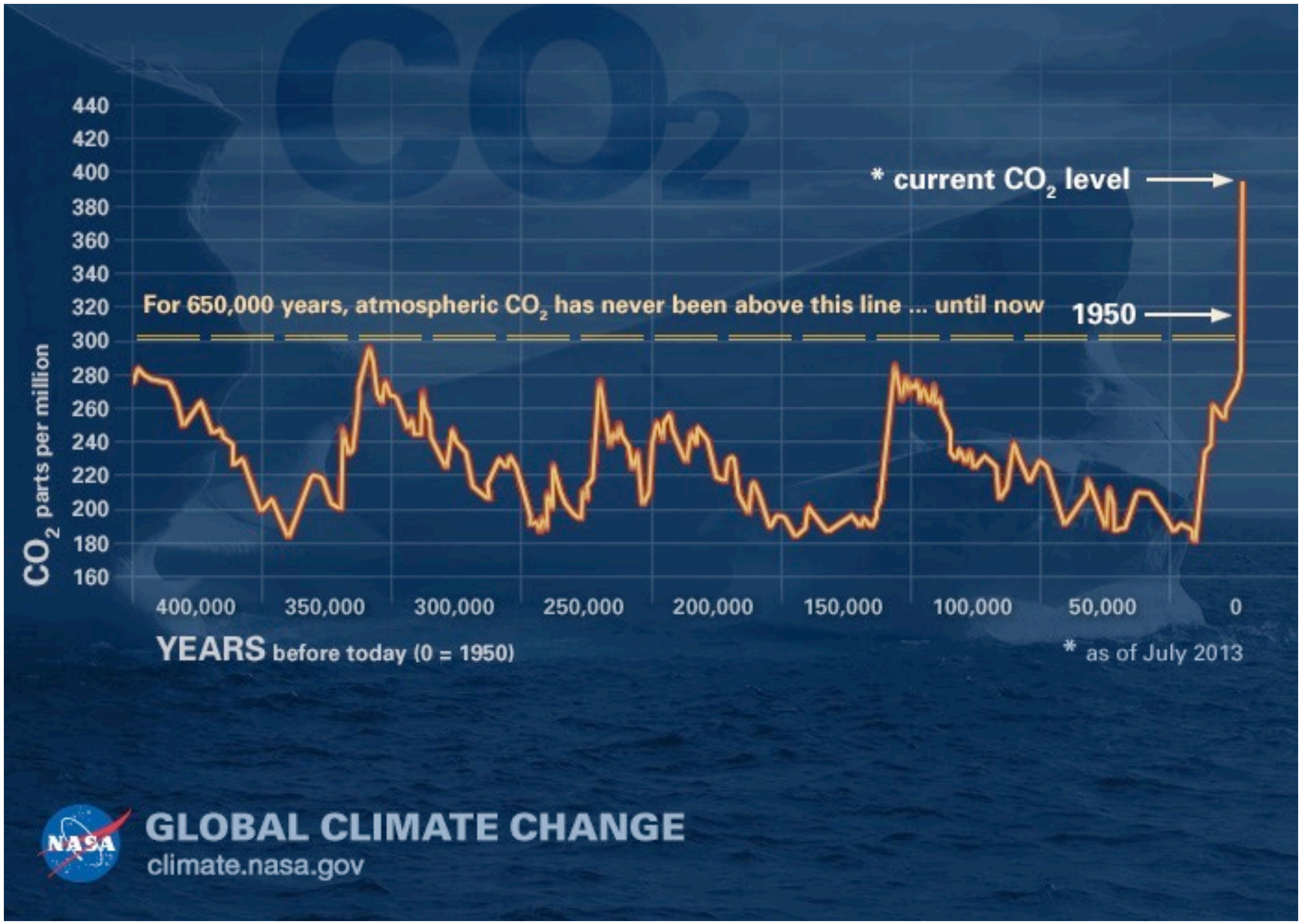
High-affinity zinc and iron binding proteins are common in soil microbes (Hantke, 2005).

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.

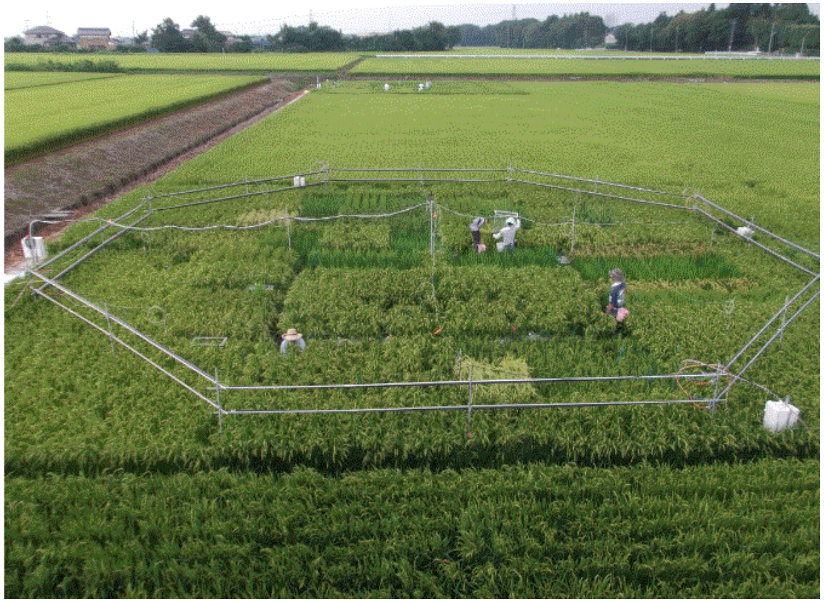
Increasing levels of CO_2 in atmosphere due to heavy use of fossil fuels



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.

The rhizophagy cycle may be affected by atmospheric CO_2 levels!

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.

Tomato Seedlings: Elevated CO_2 Experiment

- Tomato seeds surface disinfected (5 min 4% NaOCl) to reduce surface microbes.
- Seeds placed onto water agarose.
- Seeds placed in gas chambers with 410 ppm CO_2 (current atmosphere concentration) or 560 ppm CO_2 (projected 100 year concentration) and incubated 7 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.
- Root lengths and root hair lengths measured.

Elevated CO_2 suppresses root hair length growth but does not impact overall root growth.

Lower root hair growth may be due to reduced numbers of microbes in root root hairs.

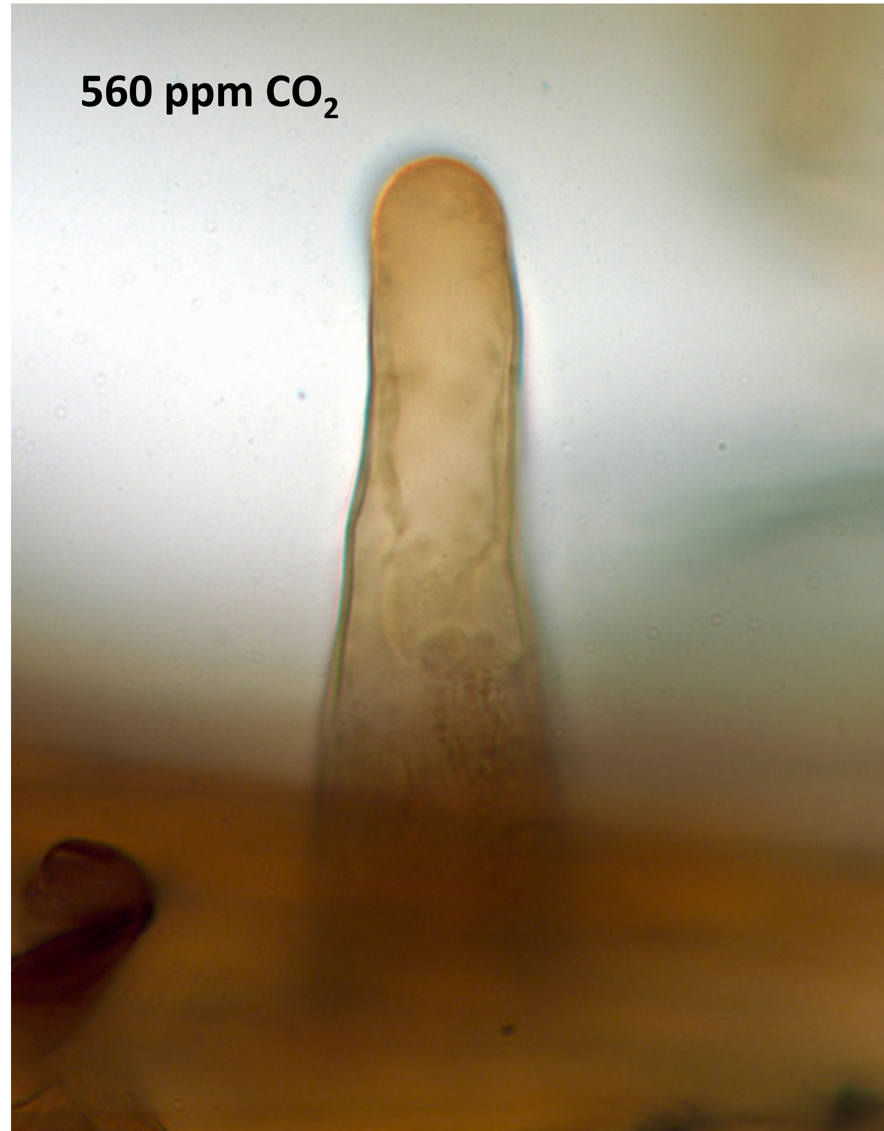
Tomato seedling root hair lengths in air with two levels of CO_2 after 7 days

410 ppm CO_2	**560 ppm CO_2**
(1)[1] 666.85 ± 225.23 μm (N=16)[2]	(1) 258.46 ± 71.04 μm (N=14)
(2) 673.03 ± 152.23 μm (N=16)	(2) 148.43 ± 77.86 μm (N=13)
(3) 733.52 ± 148.26 μm (N=16)	(3) 443.45 ± 165.27 μm (N=16)
(4) 785.71 ± 141.60 μm (N=16)	(4) 318.75 ± 90.14 μm (N=16)
(5) 650.54 ± 182.32 μm (N=16)	(5) 335.85 ± 94.29 μm (N=16)
(6) 650.14 ± 155.97 μm (N=16)	(6) 142.54 ± 63.37 μm (N=13)
(7) 714.06 ± 159.99 μm (N=16)	(7) 183.21 ± 83.93 μm (N=16)
(8) 657.33 ± 185.26 μm (N=16)	(8) 228.43 ± 66.10 μm (N=15)
Means = 691.40 ± 48.80 μm (N=8)	**257.39 ± 104.16 μm (N=8)**

[1] Seedling number;
[2] Data given as mean ± standard deviation, N = number of observations.

Overall root growth was not suppressed. In 410 ppm root length = 18.25 ± 8.48 mm (N=12); in 560 ppm root length was 19.41 ± 8.55 mm (N=12).

Microbe protoplasts (arrows) may be seen within root hairs of the 410 ppm CO_2 air; but were not visible in hairs of the elevated CO_2 treatment.



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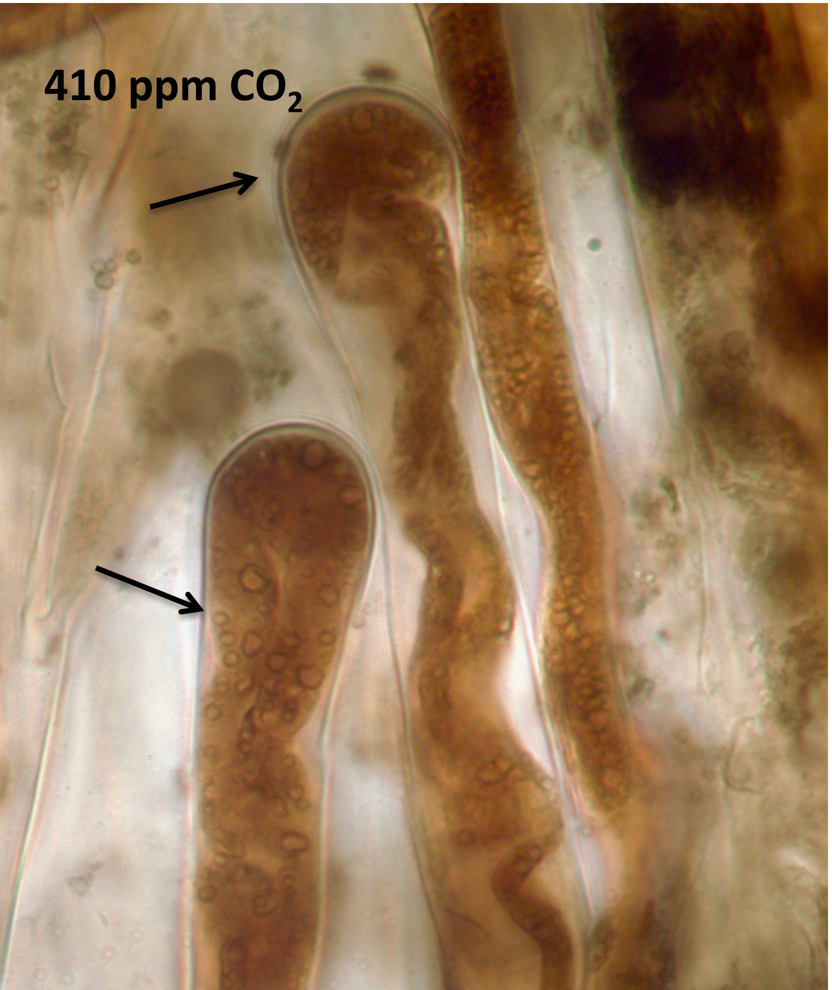
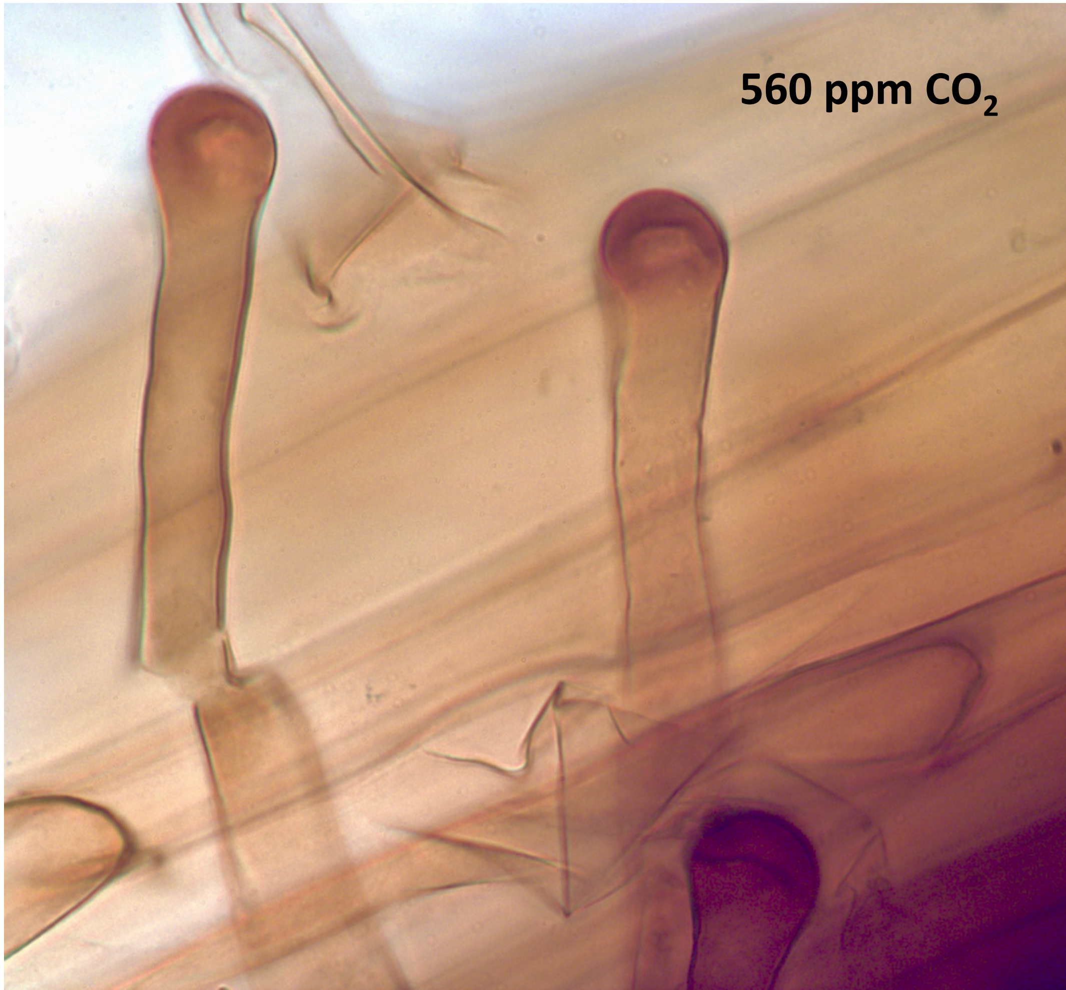
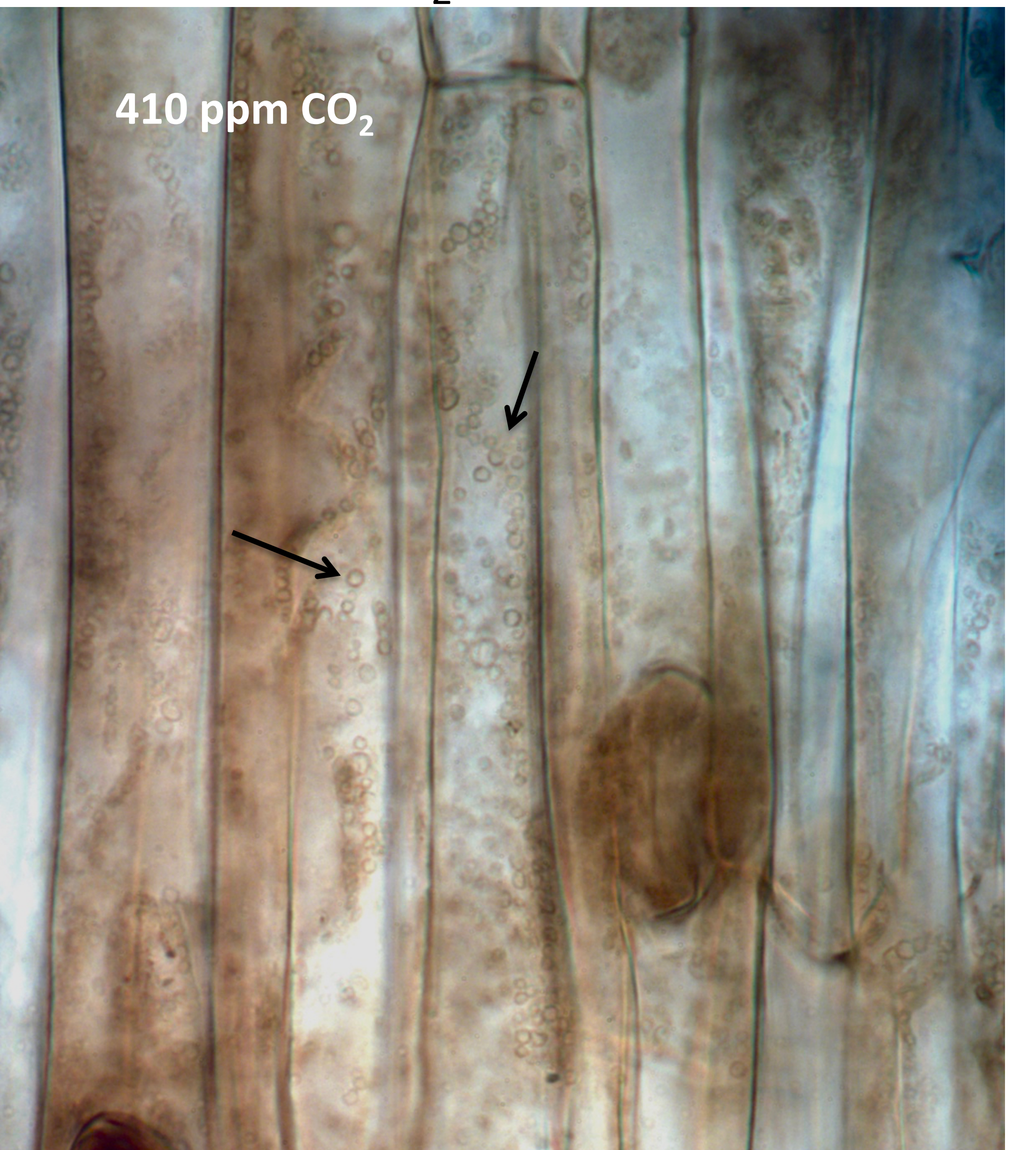




Microbe protoplasts (arrows) may be seen within root epidermal cells of the 410 ppm CO_2 air; but were not visible in cells of the elevated CO_2 treatment.



Conclusions

- Elevated CO_2 reduced superoxide (and H_2O_2) formation around microbes in seedling roots.
- Reduced reactive oxygen may result in fewer microbes converting to protoplasts and being replicated within outer layers of roots.
- Elevated CO_2 resulted in fewer microbes being present and oxidized within root epidermal cells and root hairs.
- Fewer microbes in roots could explain why elevated CO_2 suppresses root hair elongation.
- More work is needed to show reduced nutrient absorption from microbes in elevated CO_2 environments.