

Laboratory Tests Using Ensoil Algal product in Creeping Bluegrass (*Poa reptans*), Clover (*Trifolium repens*) and Soybean (*Glycine max*)

James White and Xiaoqian (Ivy) Chang

Department of Plant Biology

Rutgers University, New Brunswick, NJ 08901

jwhite3728@gmail.com

848-932-6286

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Methodology

- Seeds of *Poa reptans* were subjected to surface disinfection by shaking in a 3% sodium hypochlorite solution for 1 hr.
- Seeds of Clover (*Trifolium repens*) were surface disinfected for 1 hr by shaking in 3% sodium hypochlorite.
- Seeds of soybean were surface disinfected by sequentially treating with 70% ethanol for 2 minutes, followed by a 4% sodium hypochlorite solution for 2 minutes, and soaking in a 200 ug/mL streptomycin solution for 10 minutes.
- Seeds were subsequently placed onto agarose culture medium supplemented with varying concentrations of Algal product (0.01, 0.1, 1 and 5%). We used 3 or 4 replicates per treatment.
- Seeds on agarose were germinated and grown in lab ambient conditions for 8-10 days depending on the plant being tested.
- Nitro blue tetrazolium (for superoxide) and potassium permanganate (for ethylene) staining was done to visualize endophytic bacteria in plant cells.

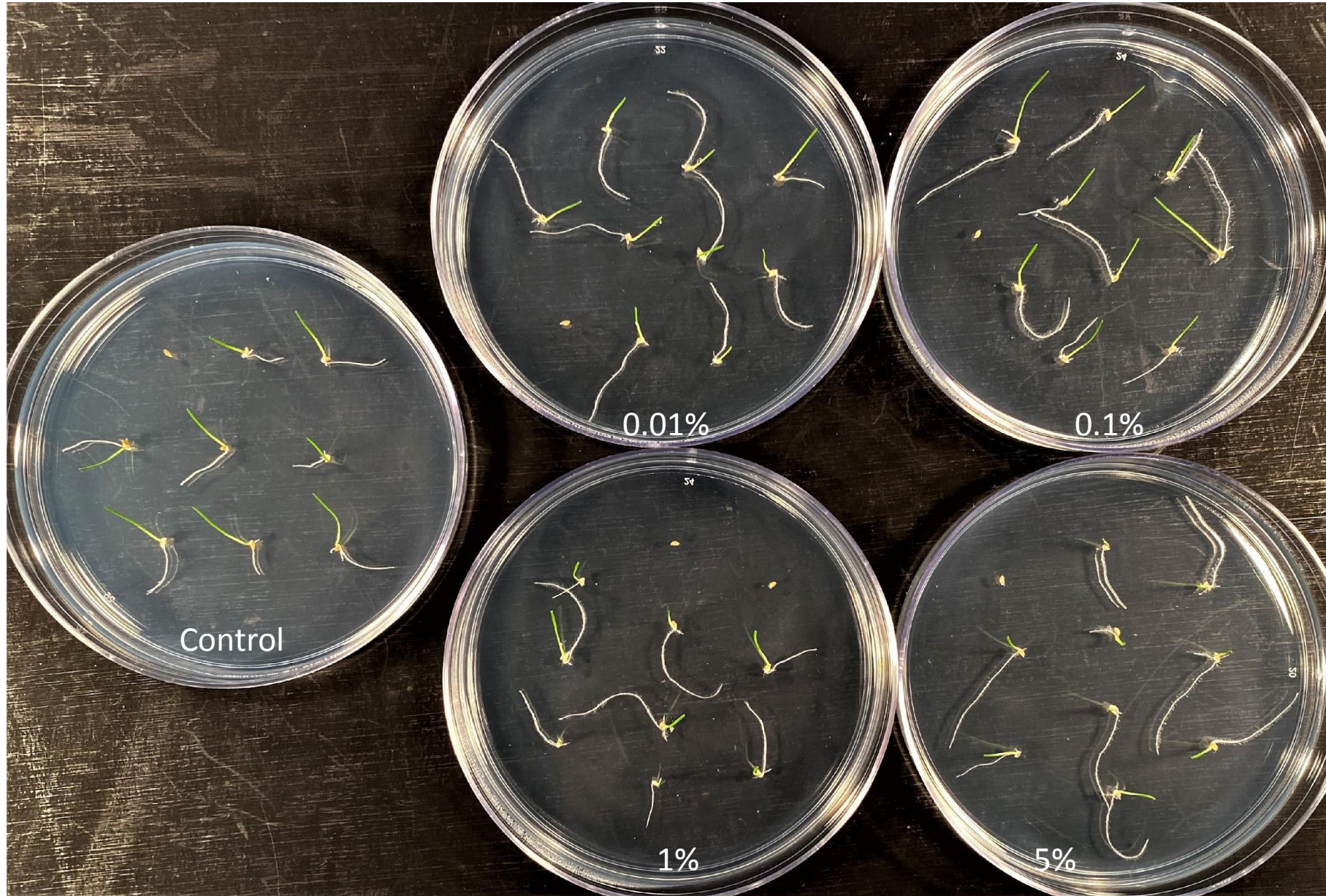
Table 1. Stimulation of Root and Root Hair Growth in *Poa reptans* Through Application of Ensoil Algal Product at Various Concentrations in agarose-based 8-day seedling assays

	Germination Rate	Gravitropic Response	Lateral Root Number per plant	Root Length	Root hairs	Root Exudates
Control	80%	2	0.77	+	-	No
0.01%	90%	5	0.77	++	+	No
0.1%	87%	5	0.90	++	++	No
1%	80%	9	1.00	++	++	Yes
5%	90%	5	0.90	+++	++	Yes

Results:

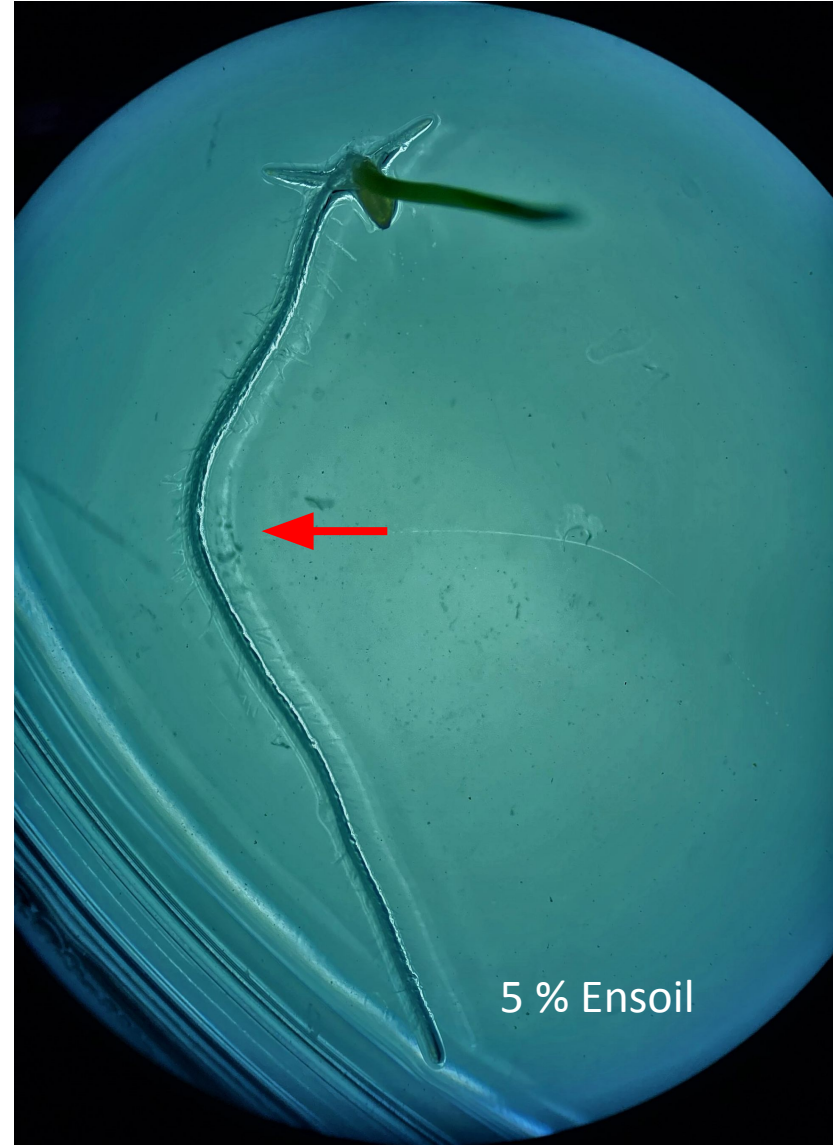
- Ensoil algal product application promoted seedling growth, exhibiting 6 positive growth promotional characters – germination, lateral root number, root length, root hair growth, root exudates and gravitropic response.

Stimulation of Root and Root Hair Growth in *Poa* Seedlings Through Application of Ensoil Algal Product at Various Concentrations

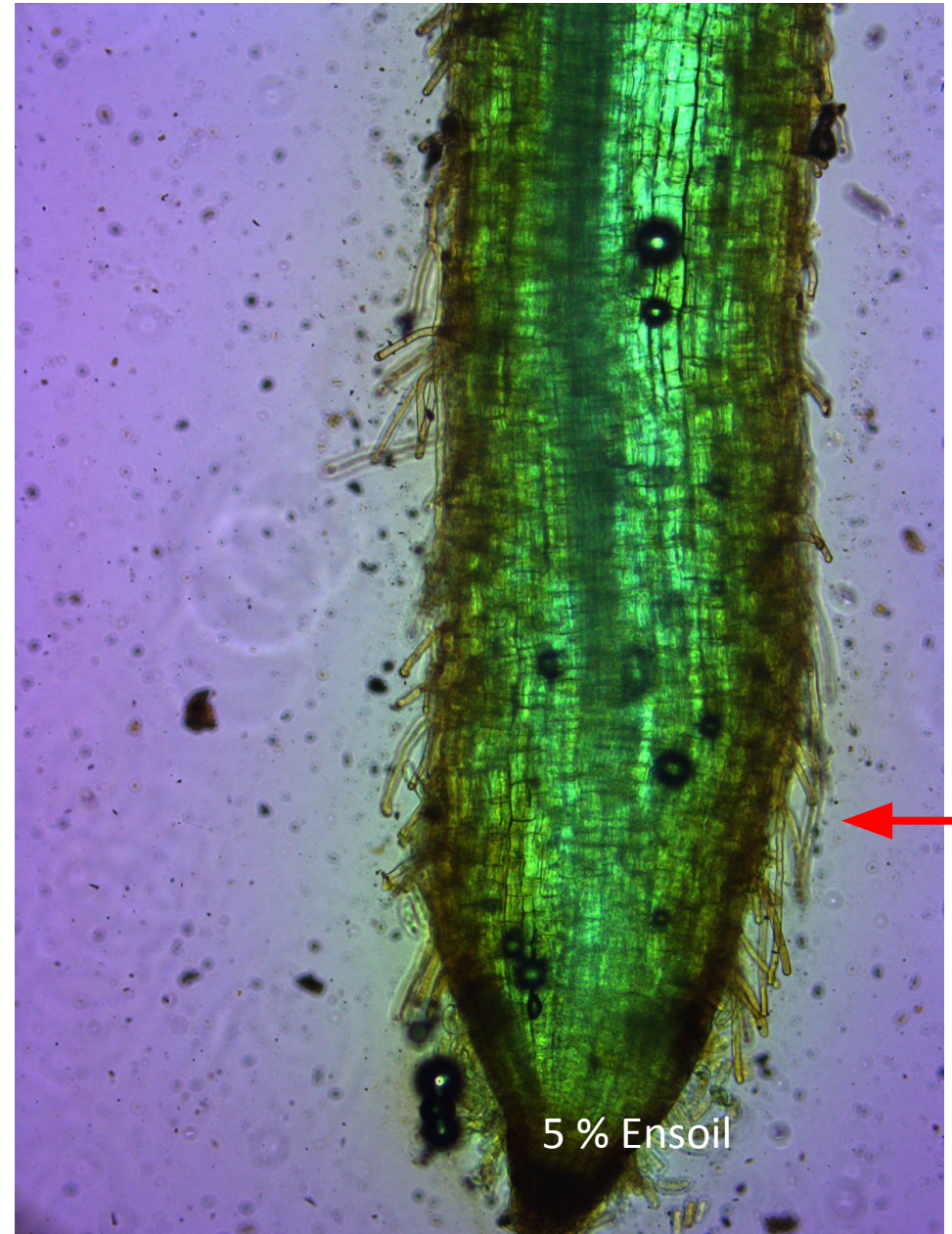


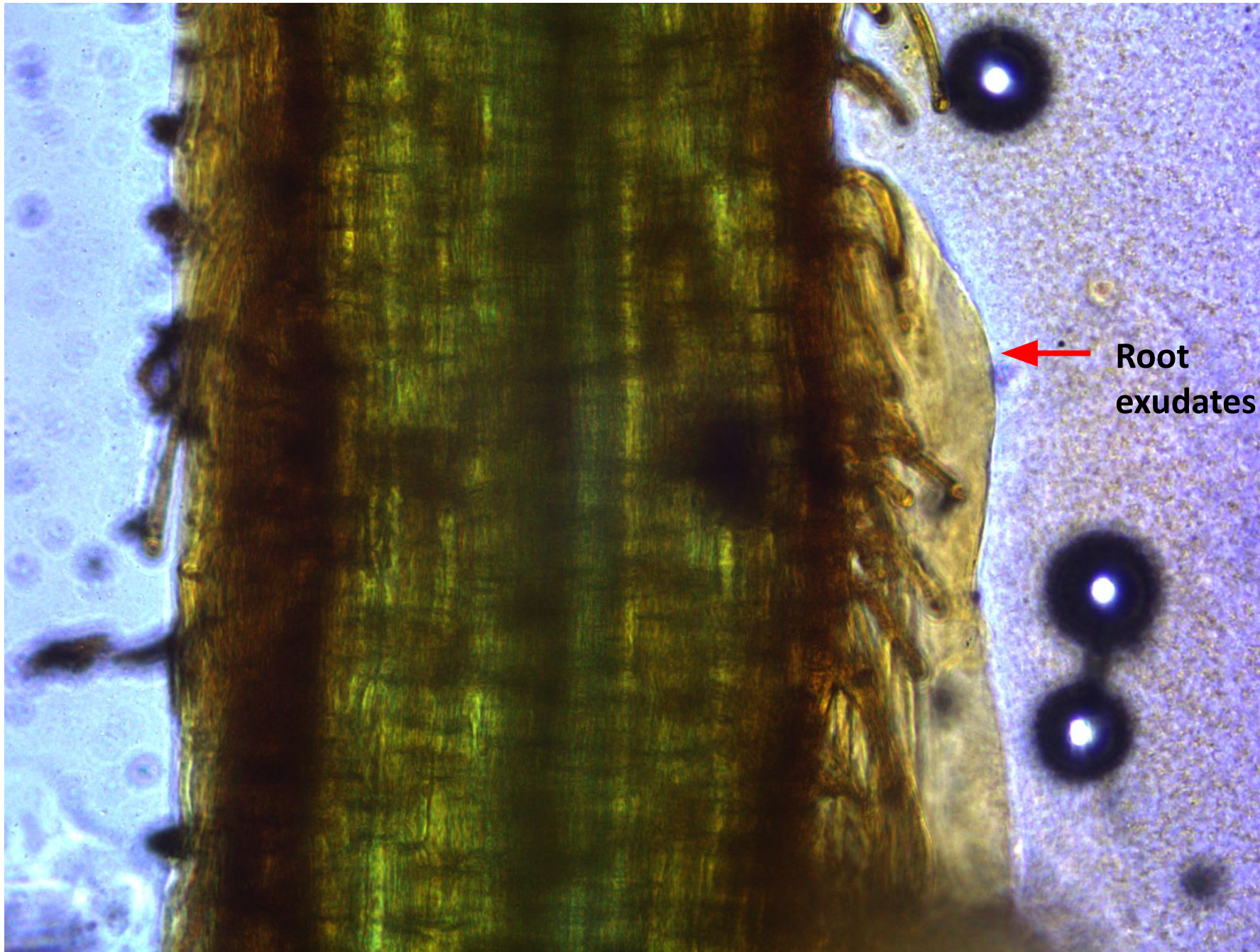
Stimulation of Root and Root Hair Growth in Poa Seedlings Through Application of 5% Ensoil Algal Product





Algal application promoted the growth of root hairs.





Algal application
stimulated the secretion
of exudates



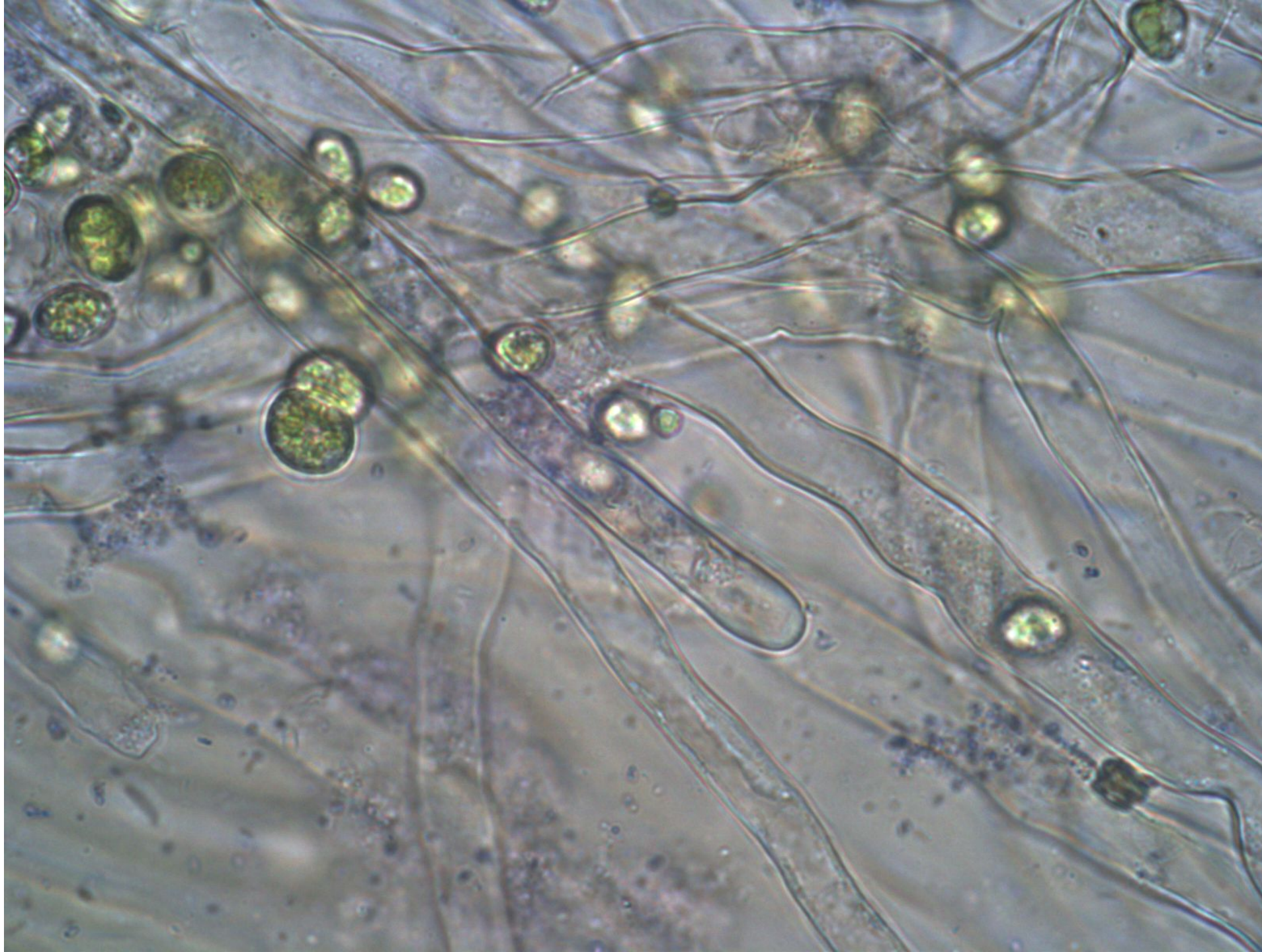
Algae surrounded by
bacteria

Bacteria (arrows) emerging from Ensoil alga.



Bacterial rods (arrows) within capsule of algal cells.

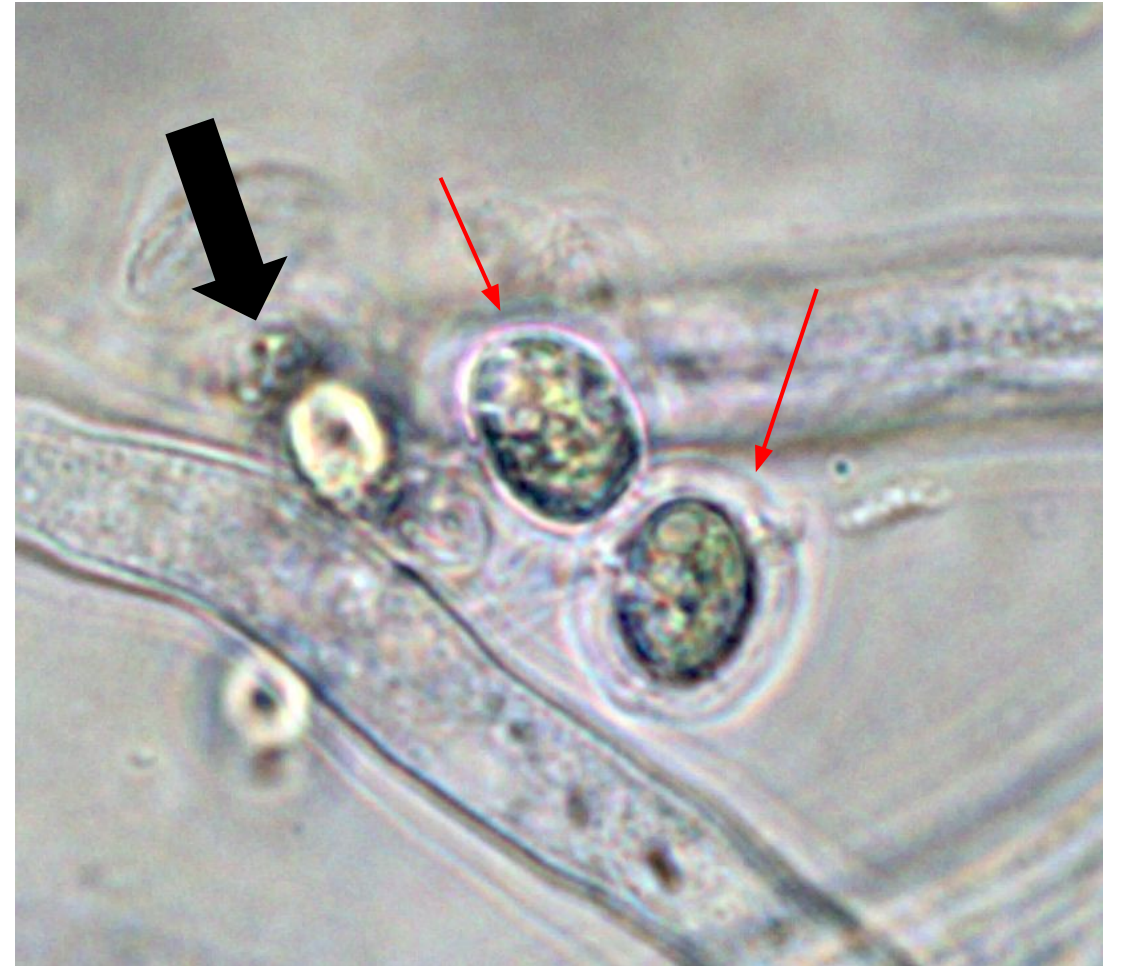




Algae enter root cells
and stick to root hairs

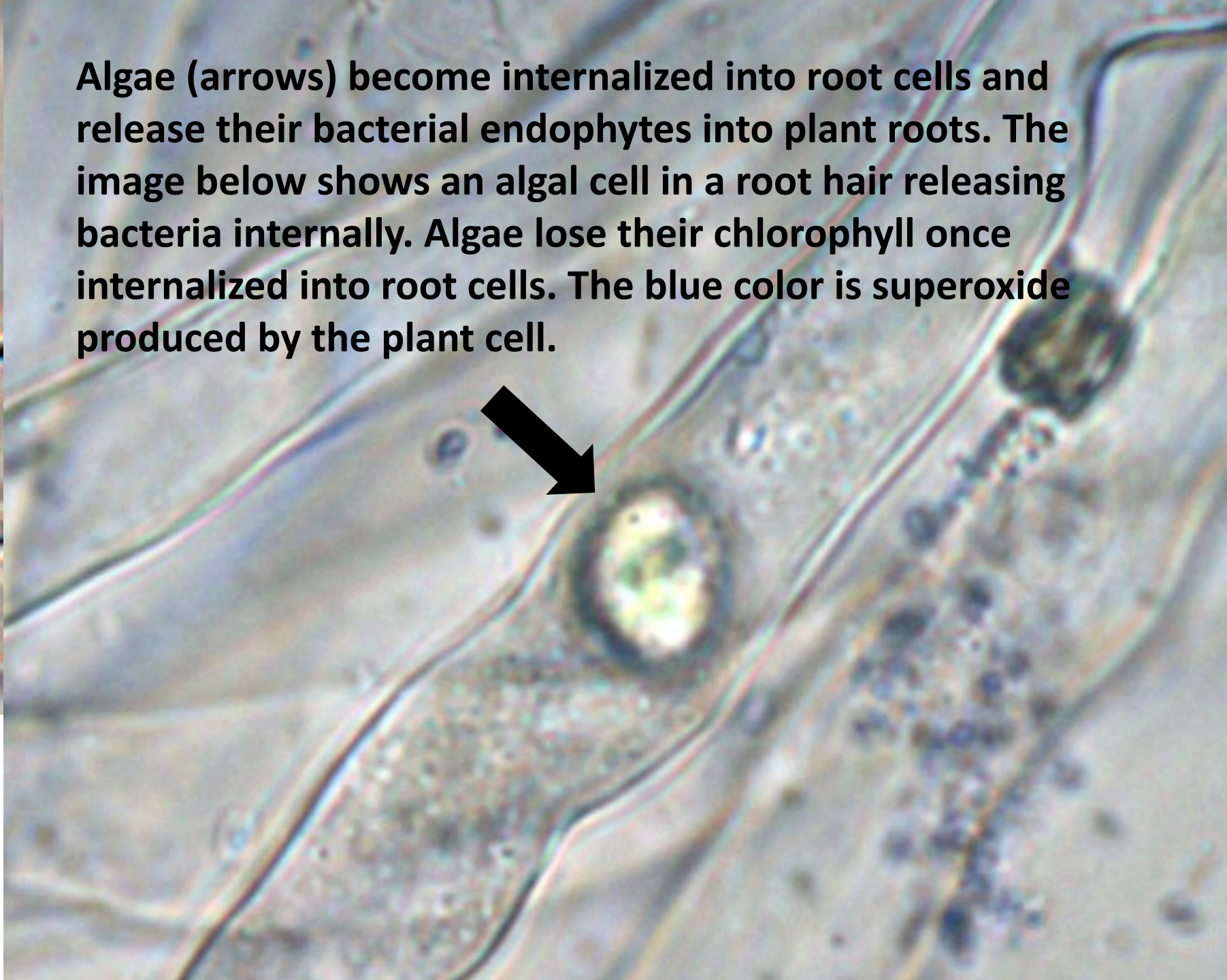
Algae contain bacterial endophytes that are delivered to plant roots.

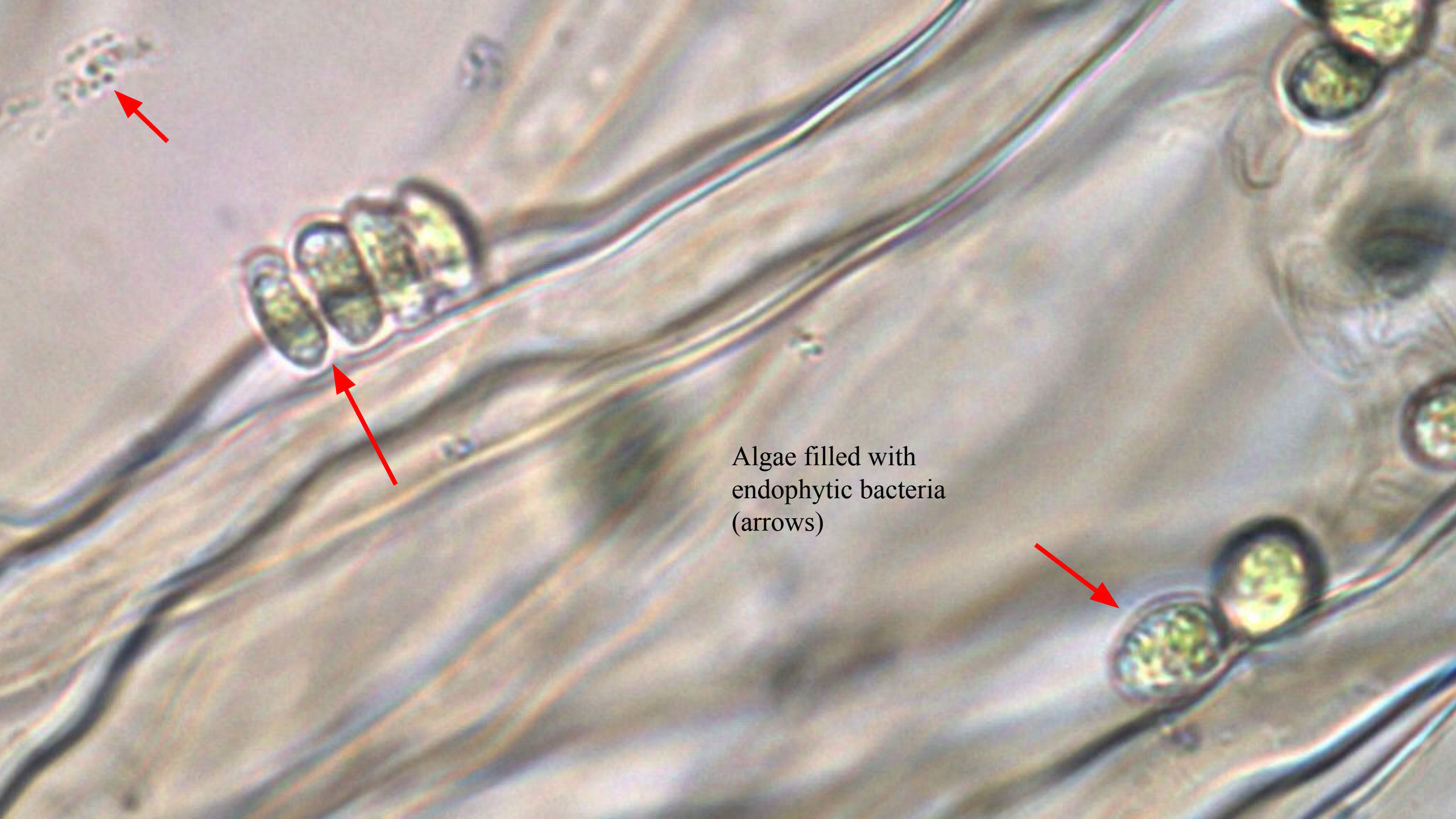
Black arrows show bacteria emerging from algae; red arrows show intact algae.





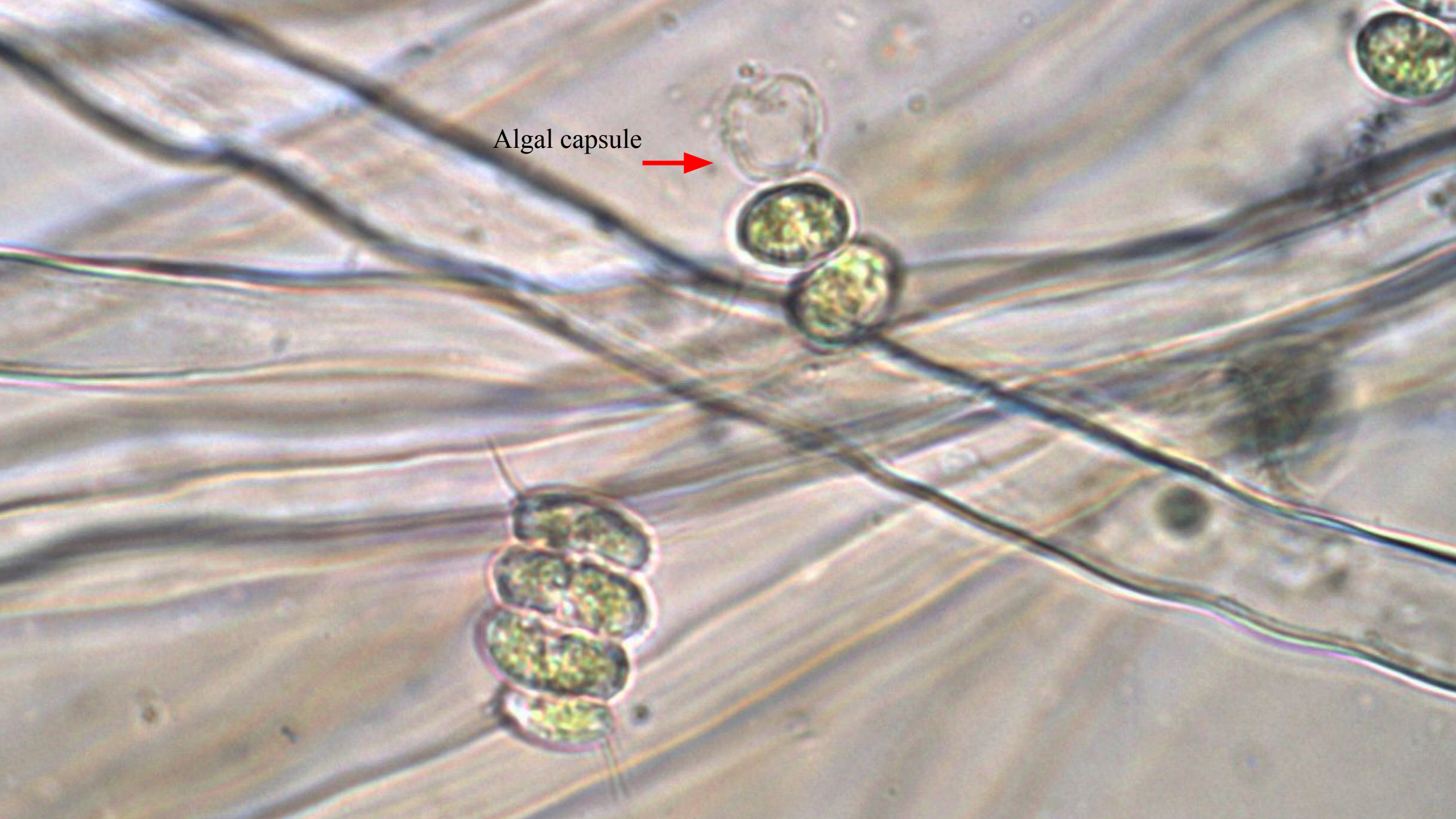
Algae (arrows) become internalized into root cells and release their bacterial endophytes into plant roots. The image below shows an algal cell in a root hair releasing bacteria internally. Algae lose their chlorophyll once internalized into root cells. The blue color is superoxide produced by the plant cell.

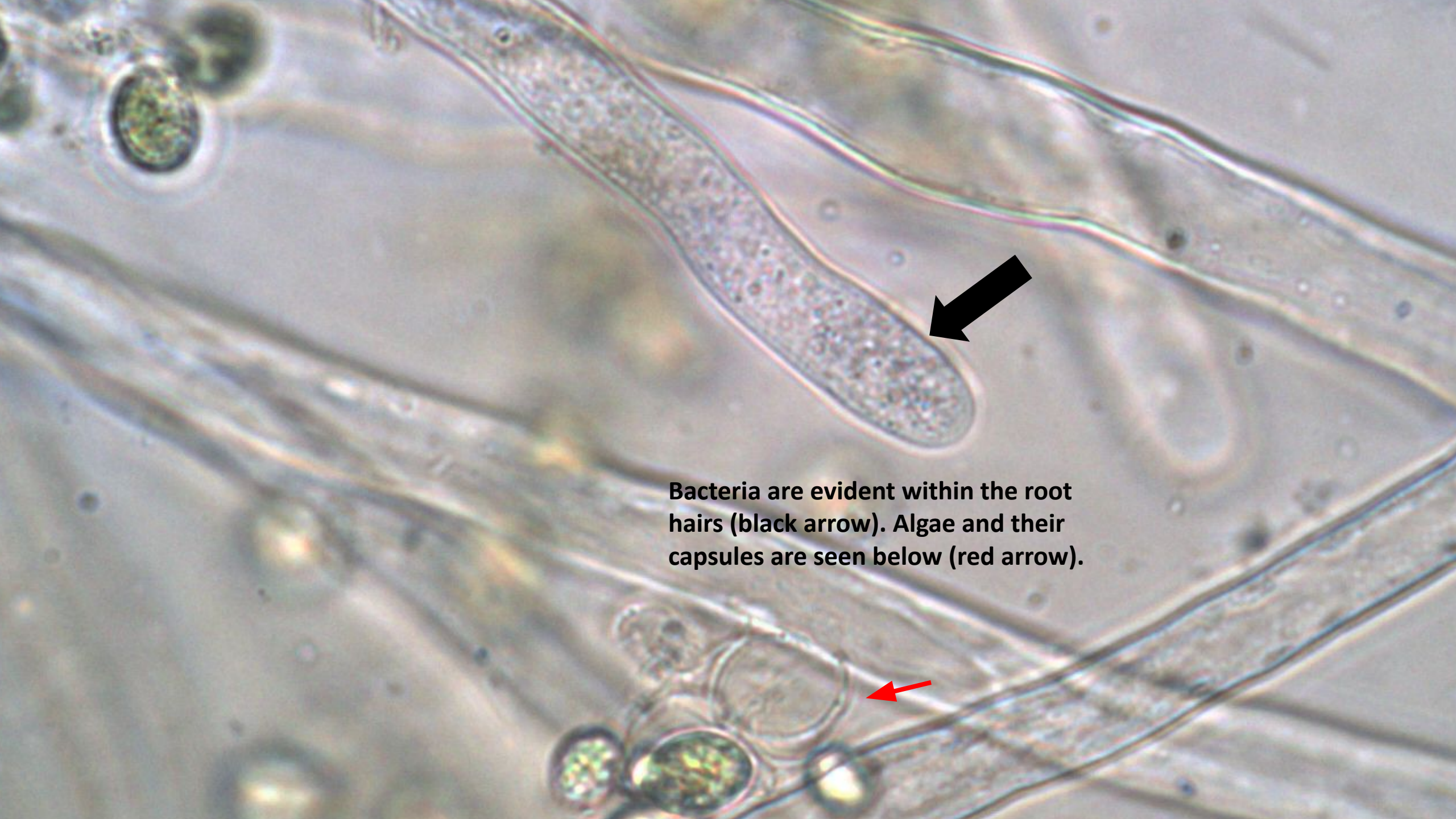




Algae filled with
endophytic bacteria
(arrows)

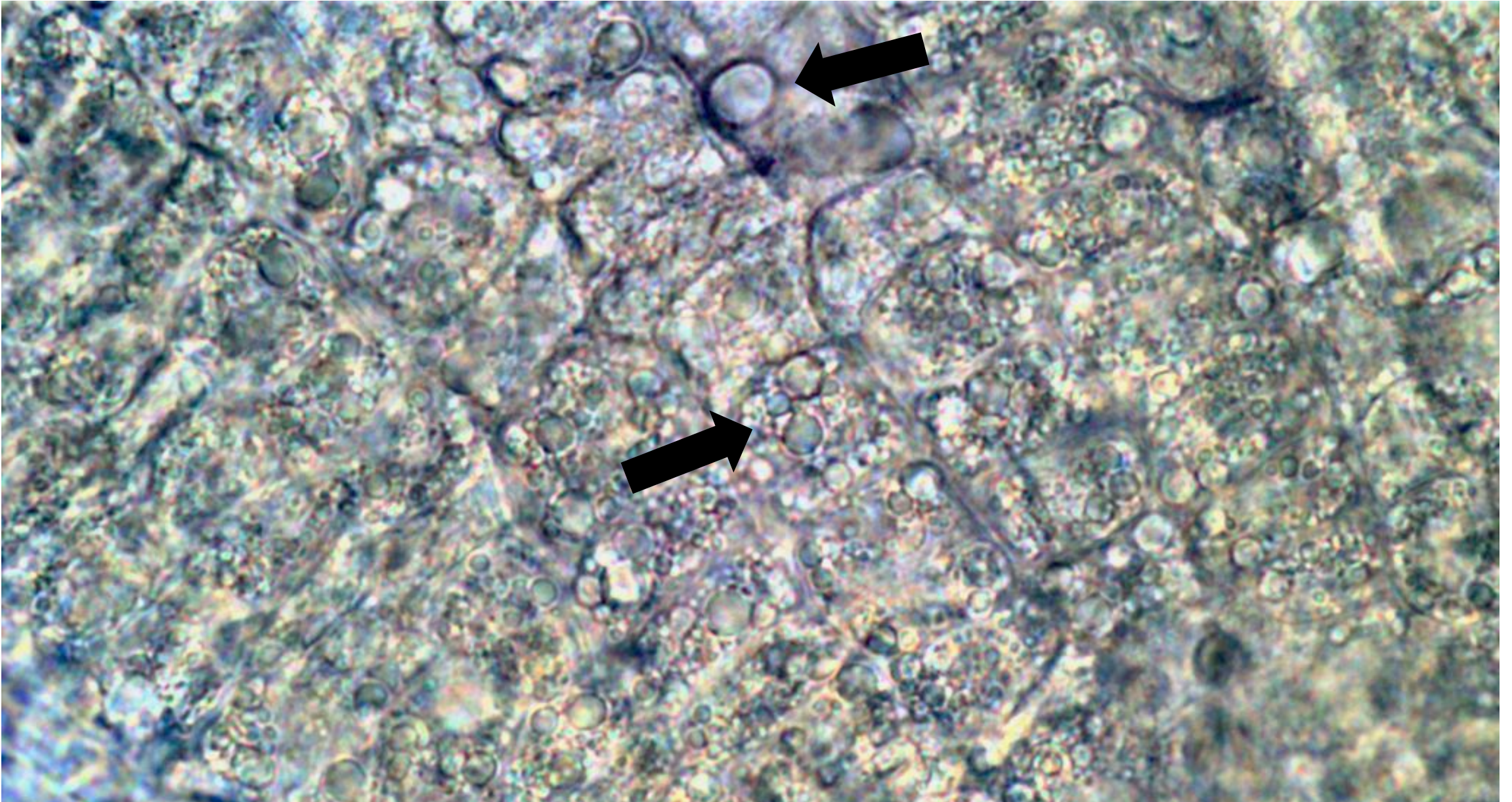
Algal capsule





Bacteria are evident within the root hairs (black arrow). Algae and their capsules are seen below (red arrow).

Image shows spherical structures (arrows) associated with the root tip meristem cells. These spherical structures may be algae releasing their bacteria into root tip cells. Note how green this tissue appears. The green color could be chlorophylls released into plant tissues.





Some spherical structures (red arrow) within root cap cells. These may be remnants of algal cells.

Remnants of algal capsules (arrows) may be evident within root hairs.







Some spherical structures (algal capsules) within and outside root hairs cells.



Some spherical structures (algal capsules) within and outside root hairs cells (red arrows). Bacteria emerging from root hair tips (black arrow).

5 %



Endophytic bacteria
within root hairs cells

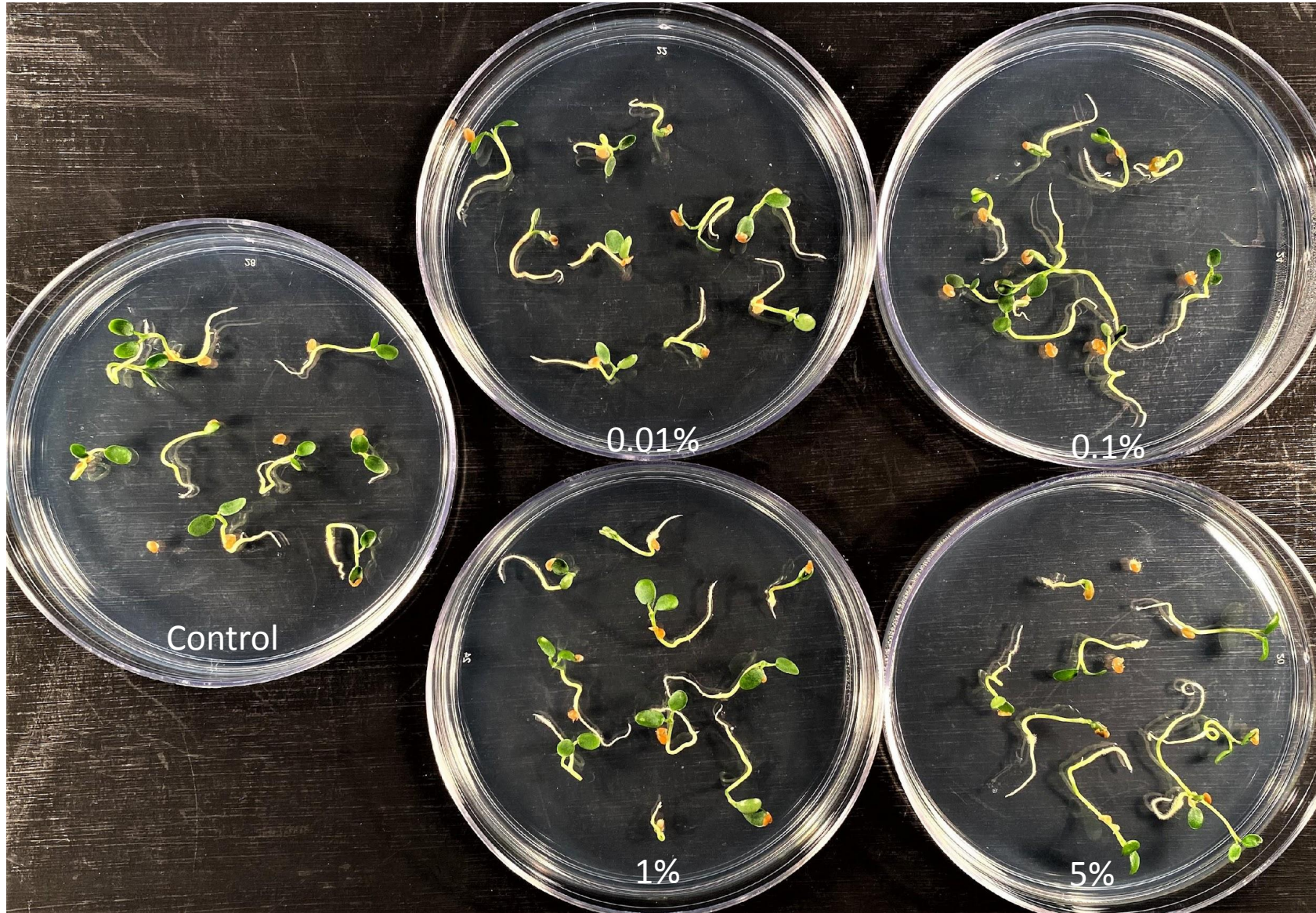
Table 2. Stimulation of Root and Root Hair Growth in Clover (*Trifolium repens*) Through Application of Ensoil Algae Product at Various Concentrations in agarose-based 8-day seedling assays

	Germination Rate	Gravitropic Response	Lateral Root Number per plant	Root Length	Root hairs
Control	83%	1	0.07	+	-
0.01%	90%	1	0.10	+	+
0.1%	90%	1	0.04	++	+
1%	87%	1	0.20	++	+
5%	97%	3	0.27	++	++

Results:

- Ensoil algal product application promoted seedling growth, exhibiting 5 positive growth promotional characters – germination, lateral root number, root length, root hair growth and Gravitropic Response.
- 5% product application performed best in seedling growth promotion.

Stimulation of Root and Root Hair Growth in Clover Seedlings Through Application of Ensoil Algal Product at Various Concentrations



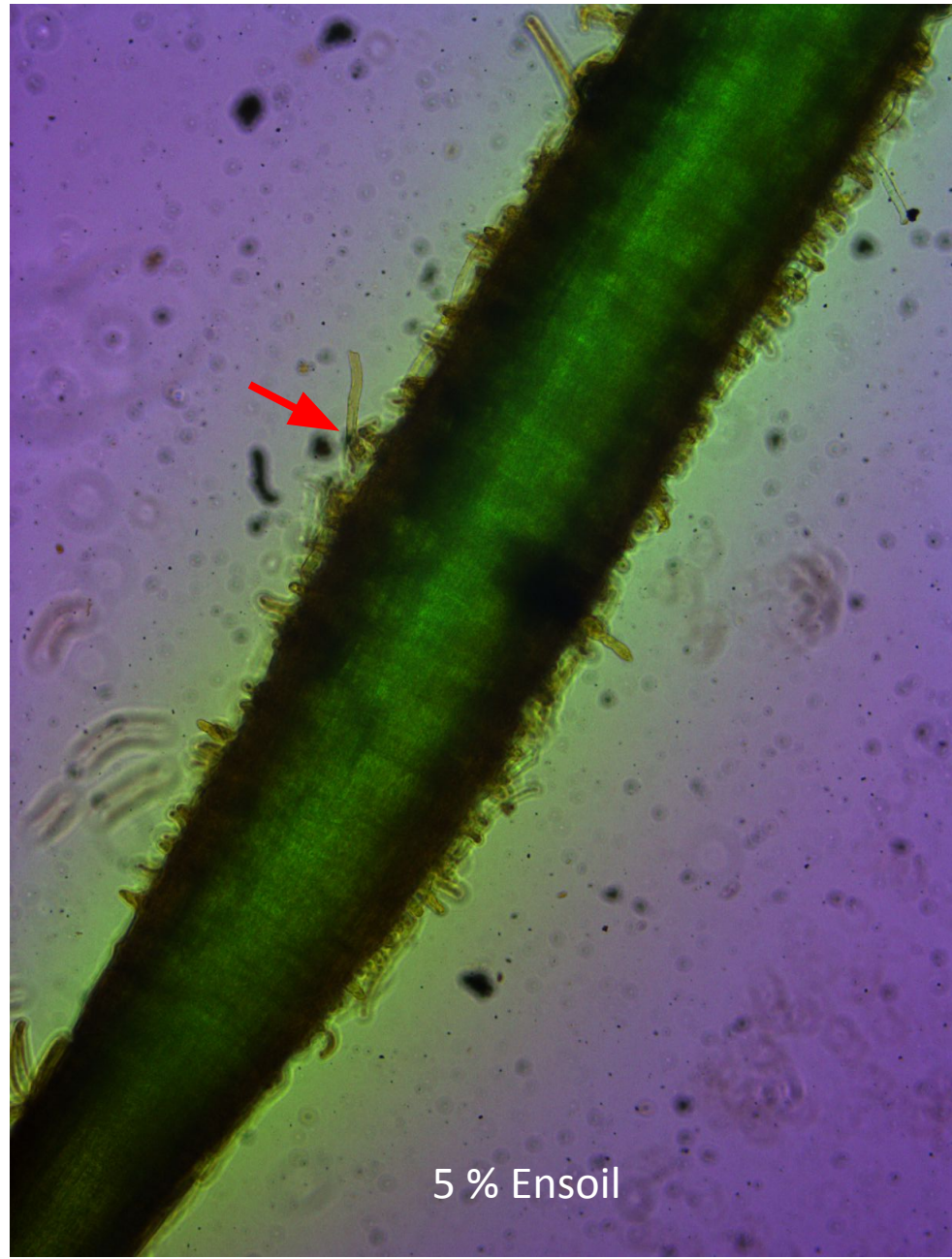
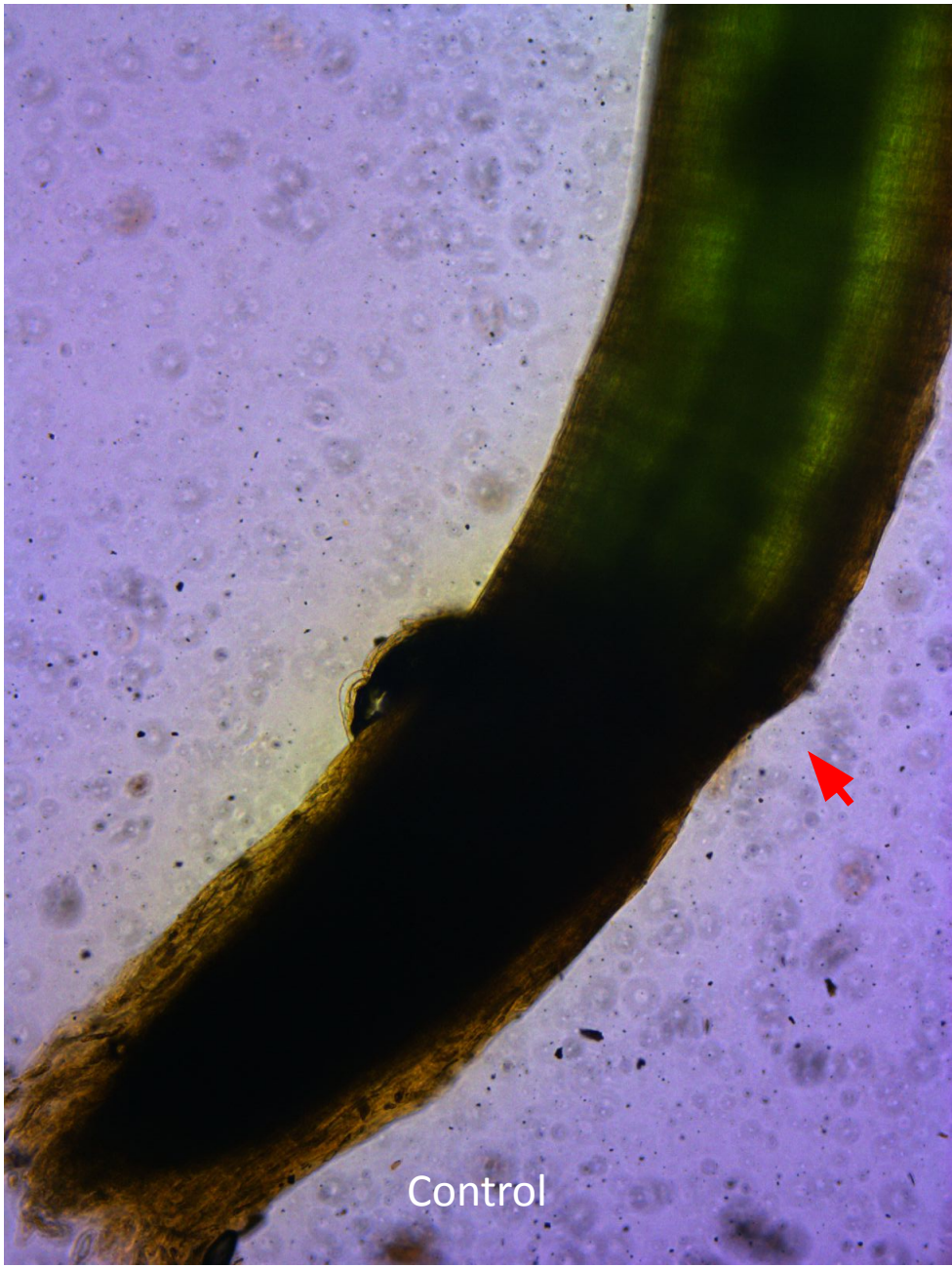
Stimulation of Root and Root Hair Growth in Clover Seedlings Through Application of 5% Ensoil Algal Product



Control

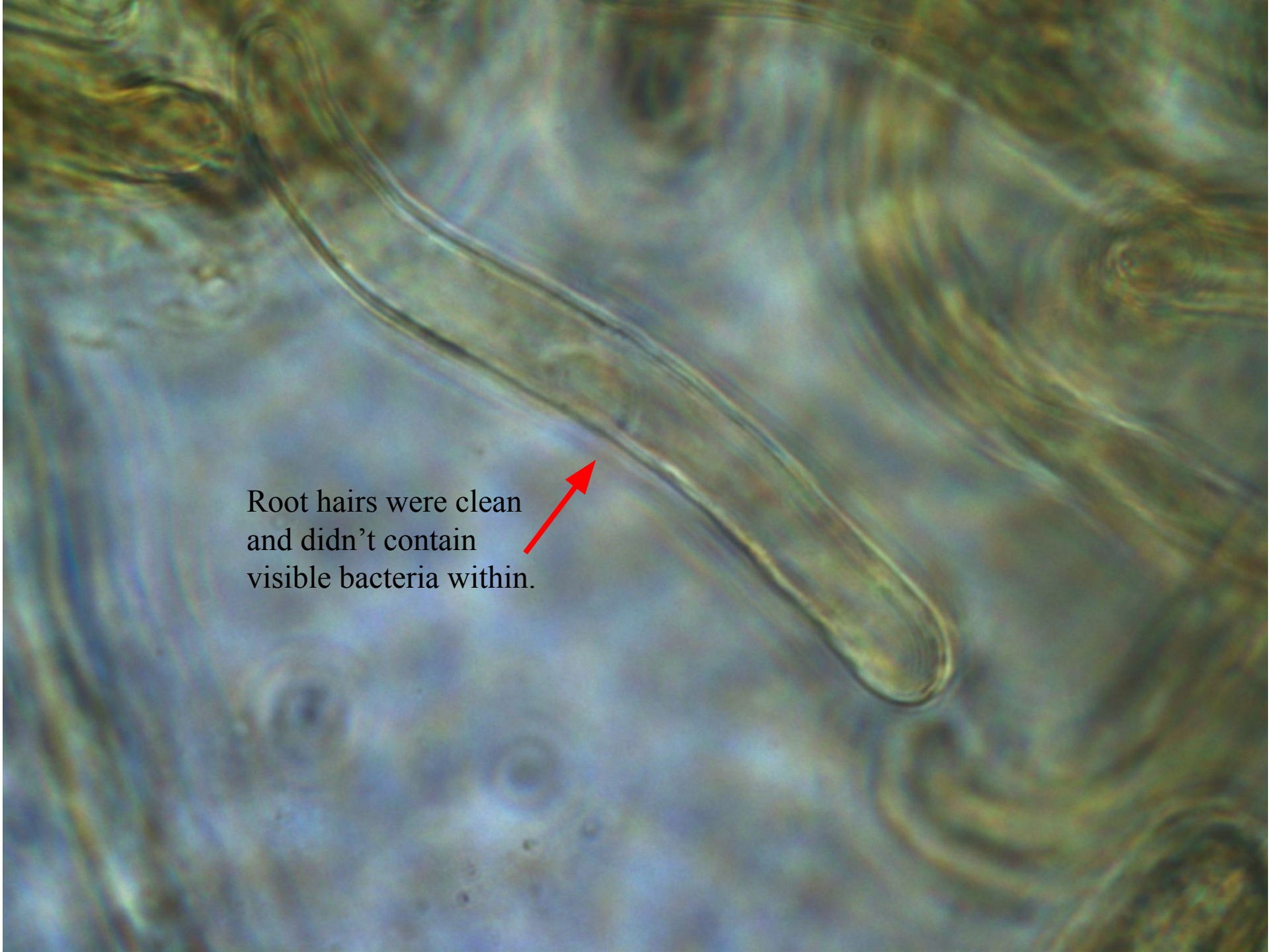
5%



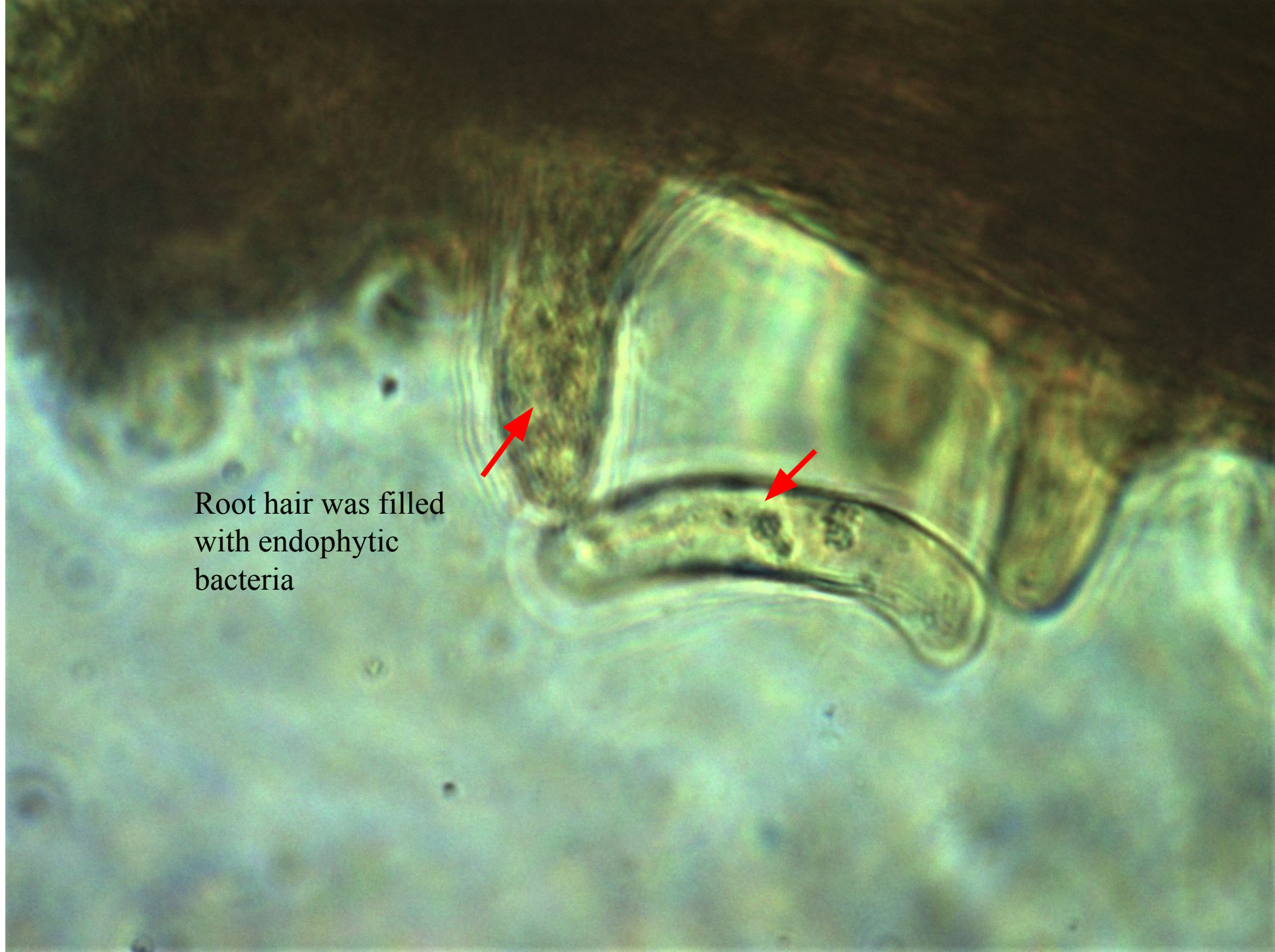


Control

Root hairs were clean
and didn't contain
visible bacteria within.



5 % Ensoil



Root hair was filled
with endophytic
bacteria

5 % Ensoil

Root hairs were filled
with endophytic
bacteria.

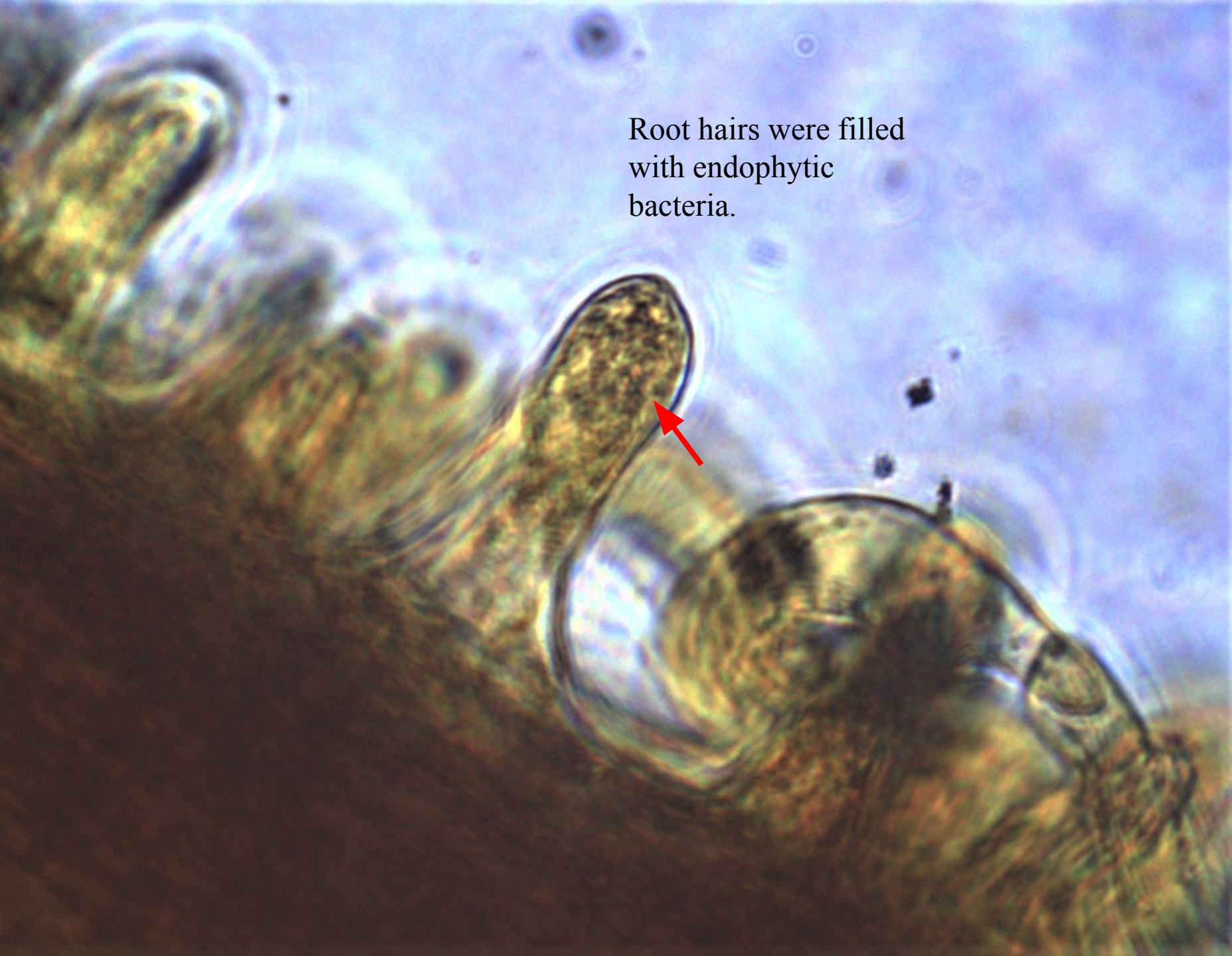


Table 3. Stimulation of Root and Root Hair Growth in *Soybean* Through Application of Ensoil Algal Product at Various Concentrations in agarose-based 8-day seedling assays

	Germination Rate	Gravitropic Response	Root Length	Root hairs
Control	83%	5	+	+
0.01%	94%	14	+	+
0.1%	83%	6	+	+
1%	83%	8	++	++
5%	83%	6	++	++

Results:

- Ensoil Algae Product application promoted seedling growth, exhibiting 4 positive growth promotional characters – germination, root length, root hair growth and Gravitropic Response.

Stimulation of Root and Root Hair Growth in Soyabean Seedlings Through Application of Ensoil Algal Product at Various Concentrations



Stimulation of Root and Root Hair Growth in Soybean Seedlings Through Application of 5% Ensoil Algae Product

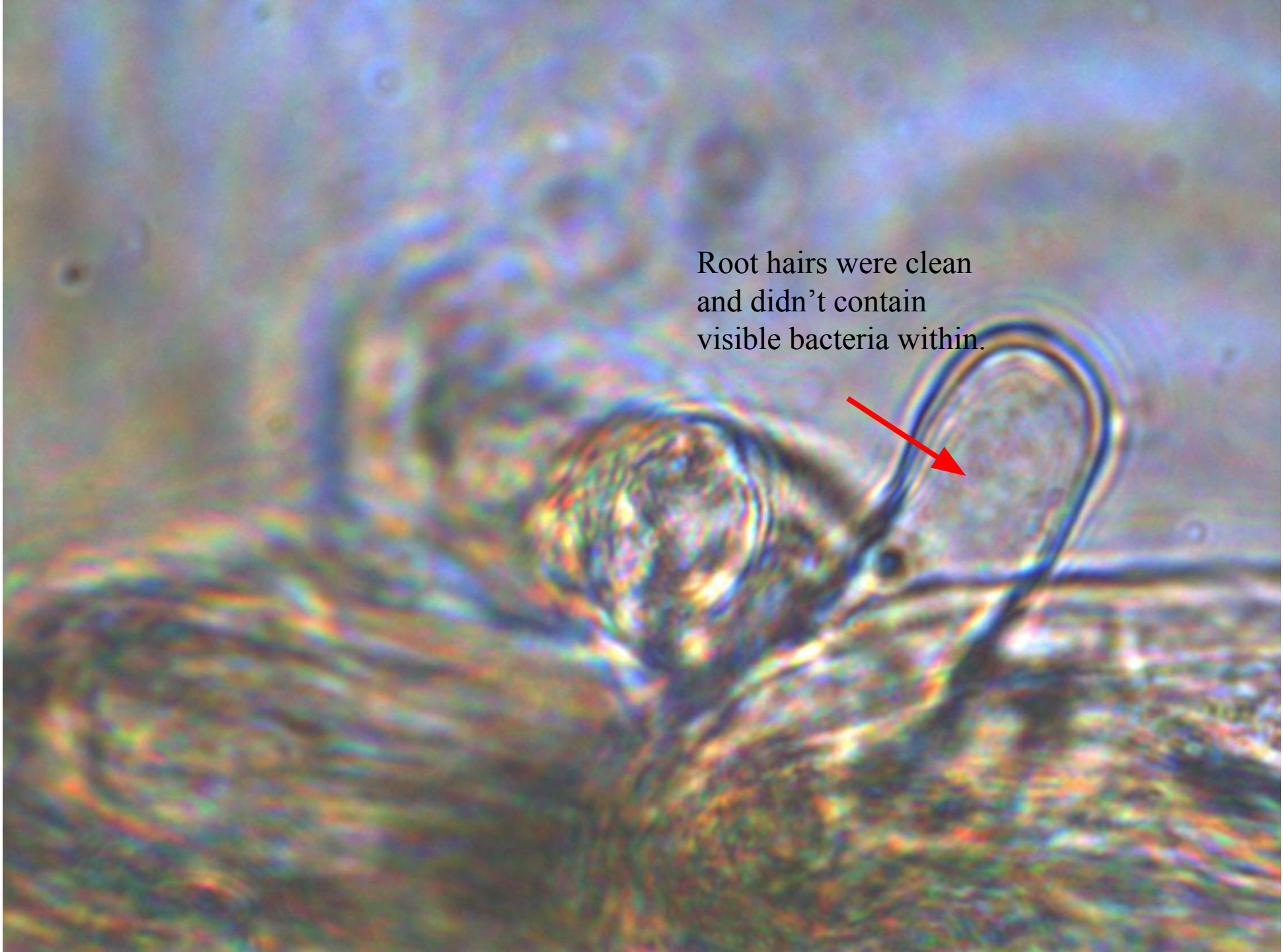
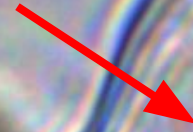


Control

5 %

Control

Root hairs were clean
and didn't contain
visible bacteria within.



5 % Ensoil

Root hairs were filled
with endophytic
bacteria



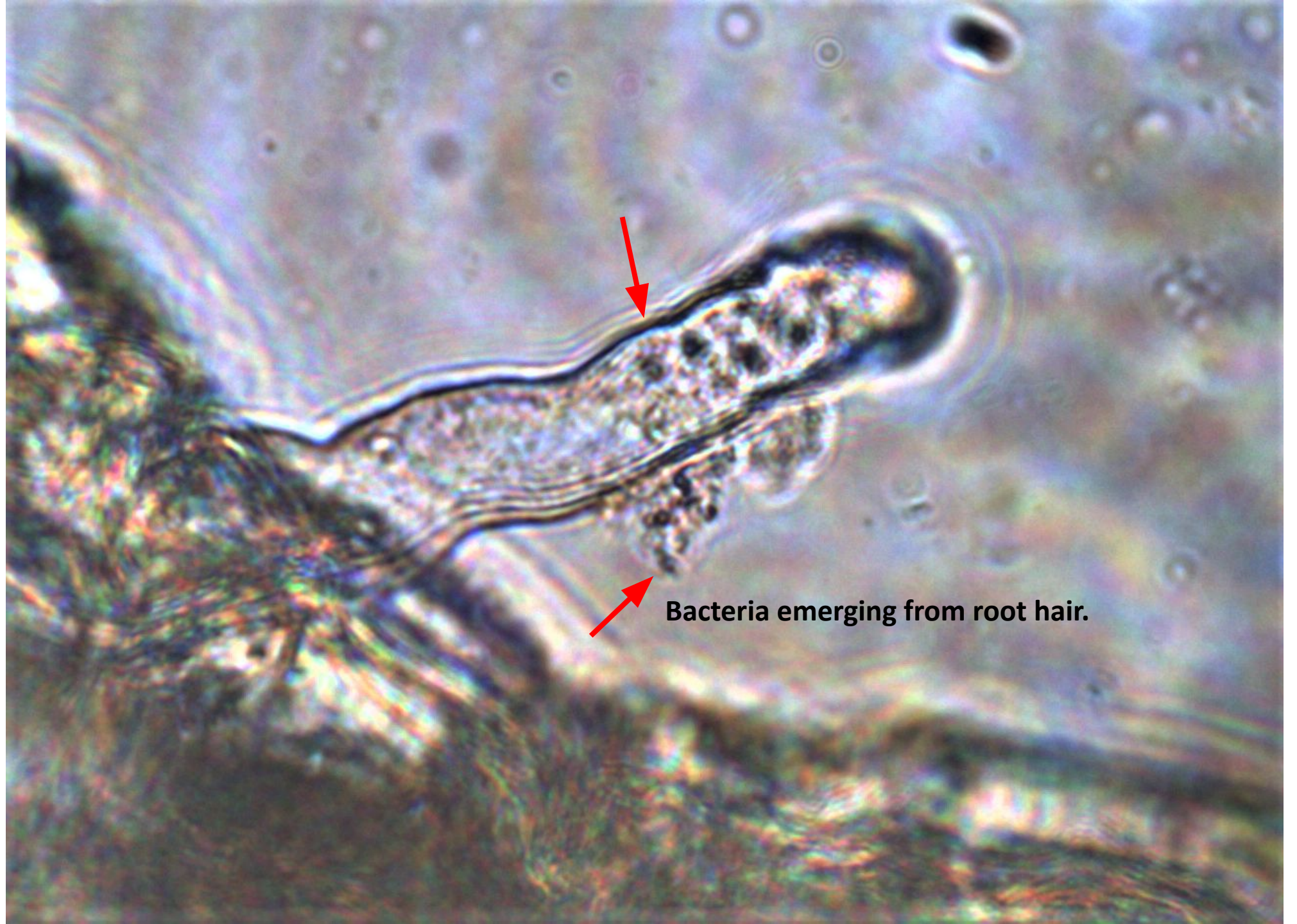
5 % Ensoil



5 % Ensoil



5 % Ensoil



Bacteria emerging from root hair.

Conclusion

Based on the experimental results, the application of algae improves the growth of creeping bluegrass, clover, and soybean. The mechanism behind this phenomenon is attributed to the symbiotic interaction between algae and endophytic bacteria and plants, whereby the algae eject or release bacteria and facilitate the colonization of the plant roots. This, in turn, promotes the growth of root hairs, ultimately leading to an overall enhancement of plant growth.