The Effects of Silver Nanoparticles On Spore Germination, Gametophyte Size, Sexual Differentiation, and Sporophyte Root Growth in the Fern, *Ceratopteris richardii*

David Altom[1], Fayth Smith[2], Renee Lopez Swalls[2]

[1]SI Bridges to the Baccalaureate, [2]School of Biological Sciences, Southern Illinois University Carbondale.

ABSTRACT

Due to the expanding use of silver nanoparticles (AgNP) in manufacturing and agriculture, its release into the environment and toxic effects on plant growth are worrisome. Using the model fern, *Ceratopteris richardii*, we examined the effects of increasing concentrations of silver nanoparticles (0ppm, 20ppm, 40ppm, 60ppm, 80ppm, 100ppm) on spore germination, sexual differentiation, gametophyte size, and early sporophyte growth. Our results show that spore germination is significantly inhibited at 100 ppm AgNP. Regarding gender differentiation, generally the ratio of hermaphrodite to male gametophytes lessened. Hermaphrodite gametophytes in the treatments were smaller in size compared to the controls. AgNP severely damaged the sporophyte roots, and root growth was significantly shorter than the controls. Our findings are comparable to other studies that have observed similar effects of AgNPs on plant growth and development. The ecological consequences of having fewer hermaphroditic gametophytes would translate to fewer sporophytes, the diploid phase responsible for producing spores. Furthermore, exposure to silver nanoparticles severely damaged the roots, which would most likely negatively impact spore production.

INTRODUCTION

Silver nanoparticles are widely used in commercial products and agriculture due to their antimicrobial and antifungal properties[1]. However, the release of nanoparticles into the environment can pose a potential hazard to biological organisms[2]. Studies have shown that the effects of AgNPs on flowering plants are complex and can vary depending on factors such as nanoparticle concentration, plant species, exposure duration, and environmental conditions[3,4,5]. Few investigations have looked at the influence of AgNP on non-flowing plants such as bryophytes and pteridophytes[6].

Our study investigates the impact of (AgNP) on a model fern *Ceratopteris richardii,* a homosporous fern (produces one type of spore) that differentiates into distinct hermaphrodite or male gametophytes by hormonal sex determination[7].

MATERIALS AND METHODS

- Spores were sown on nutrient agar supplemented with AgNP (0ppm, 20ppm, 40ppm, 60ppm, 80ppm, 100ppm), with five replicates for each concentration.
- Cultures were maintained at 30 °C, 73% humidity, and under constant light.
- Plants were prepared and observed on Leica and Olympus microscopes and scanning electron microscopy (SEM) (Lopez and Renzaglia, 2008).
- Data were collected on 1) spore germination rates, 2) ratio of hermaphrodites to male gametophytes, 3) relative gametophyte size, and 4) roots (following fertilization).
- Spore germination rates were analyzed using T-test and One Way ANOVA in Excel (p-value 0.05).

RESULTS

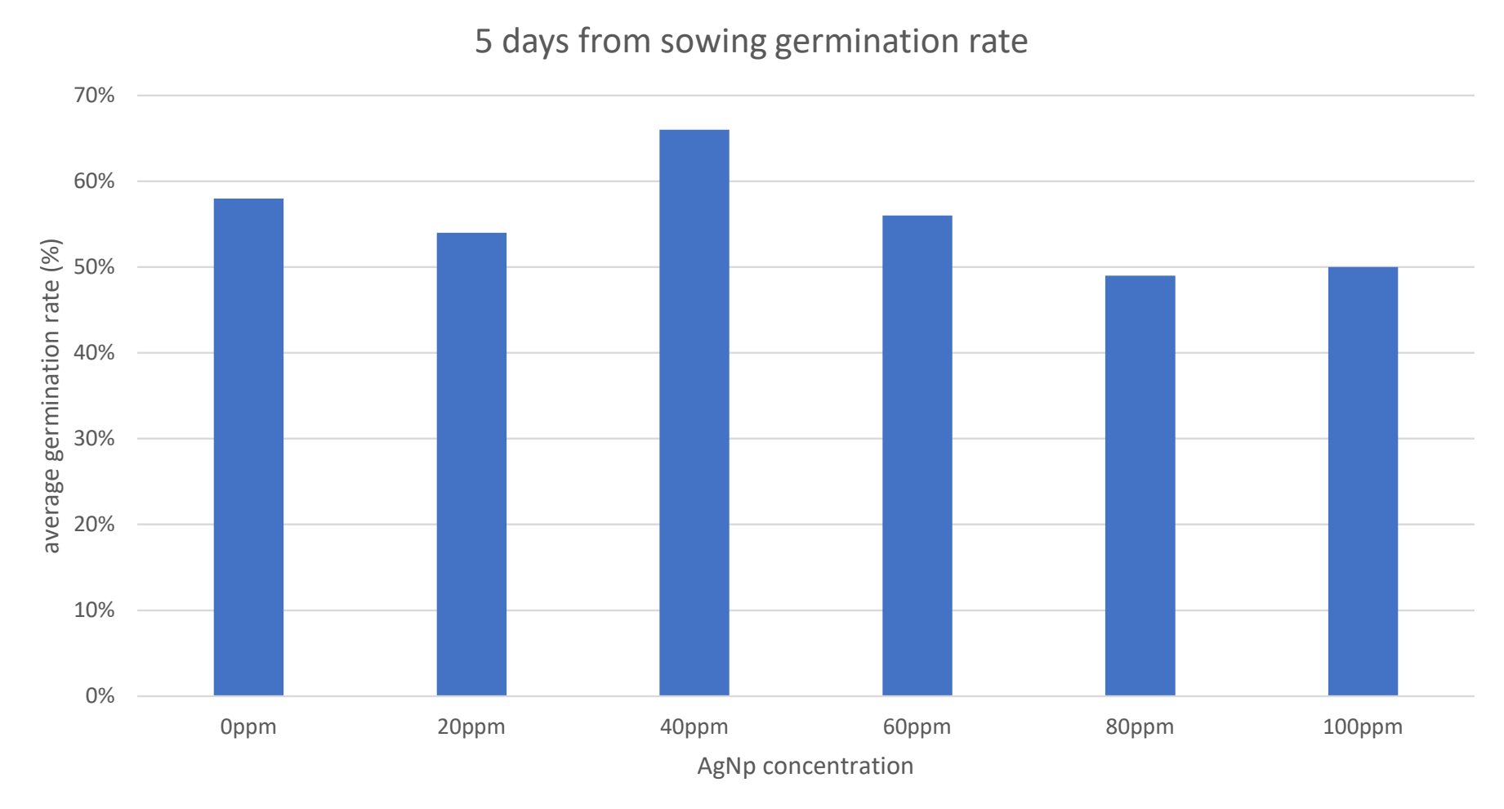

Figure 1: Spore germination at 5 days from sowing (dfs). No significant differences from the controls were observed (p=0.05).

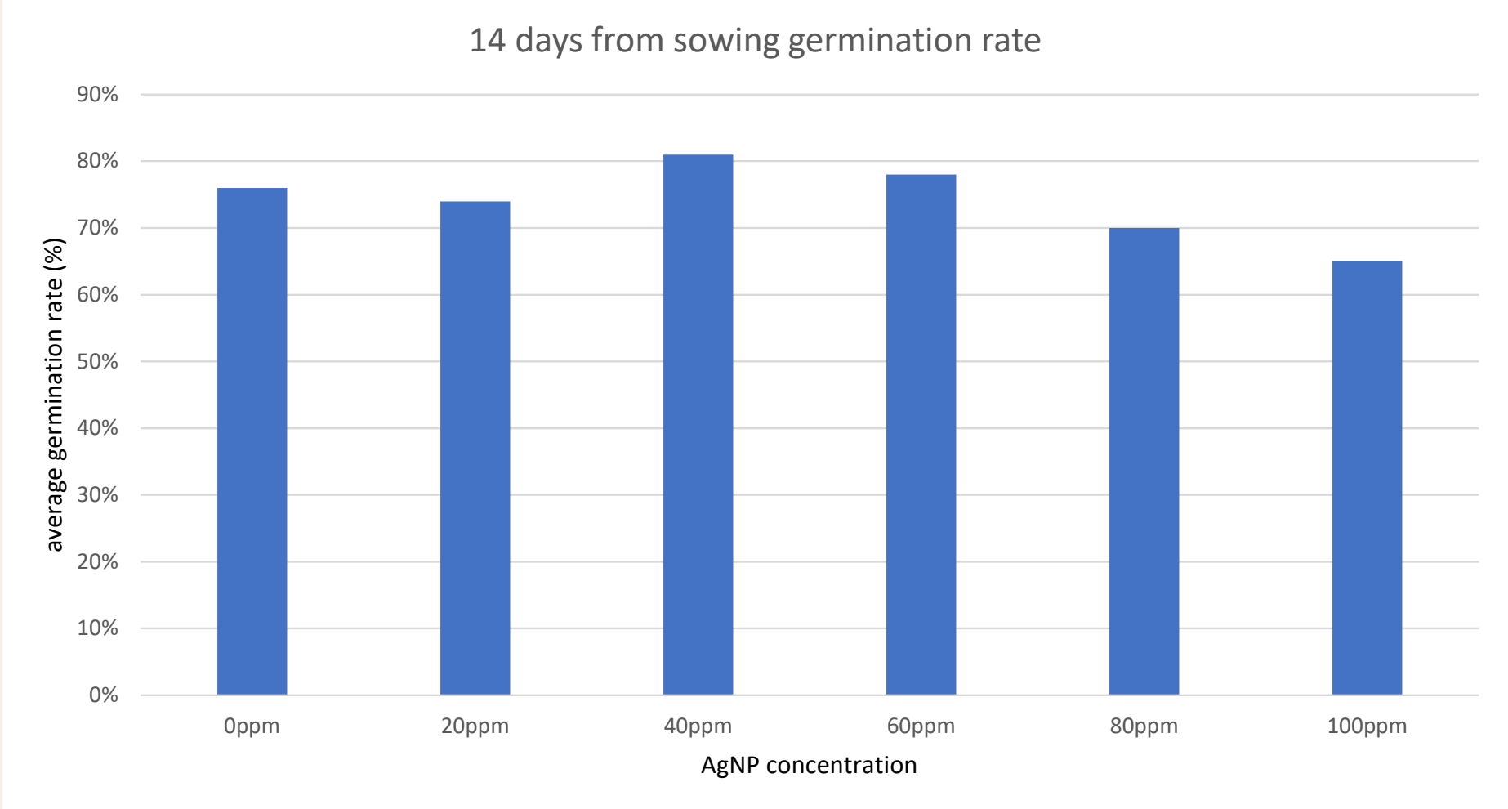

Figure 2: Spore germination at 14 days from sowing (dfs). All treatments except 100ppm were not significantly different from each other or the controls.

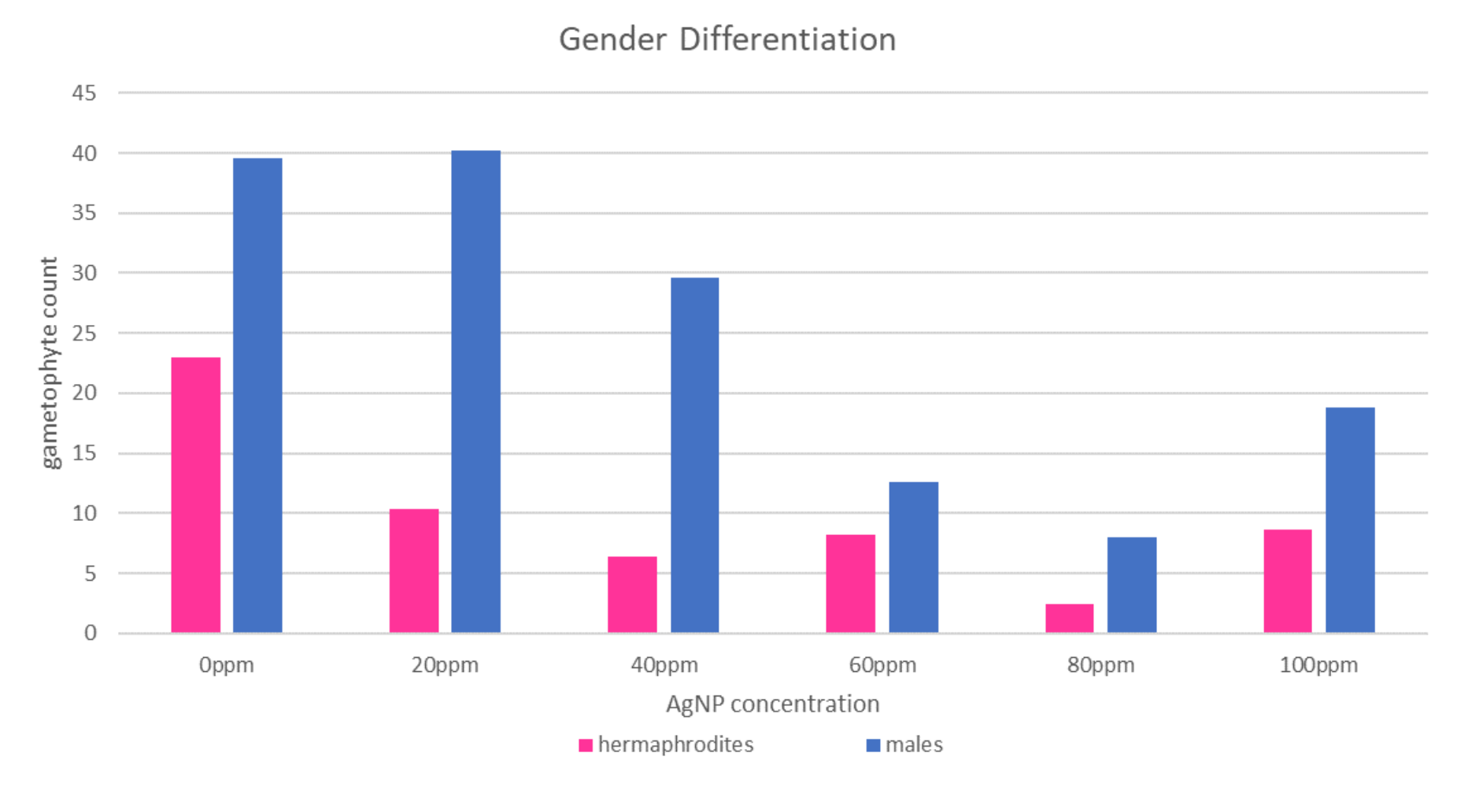

Figure 3: Gender ratios. Hermaphrodites compared with males are generally less across concentrations. 0ppm (22:40=55%), 20ppm (10:40 = 25%), 40ppm is (7:30 = 23%), 60 ppm is (8:12 = 67%), 80ppm is (2:7 = 29%), 100pp m is (8:18 = 44%).

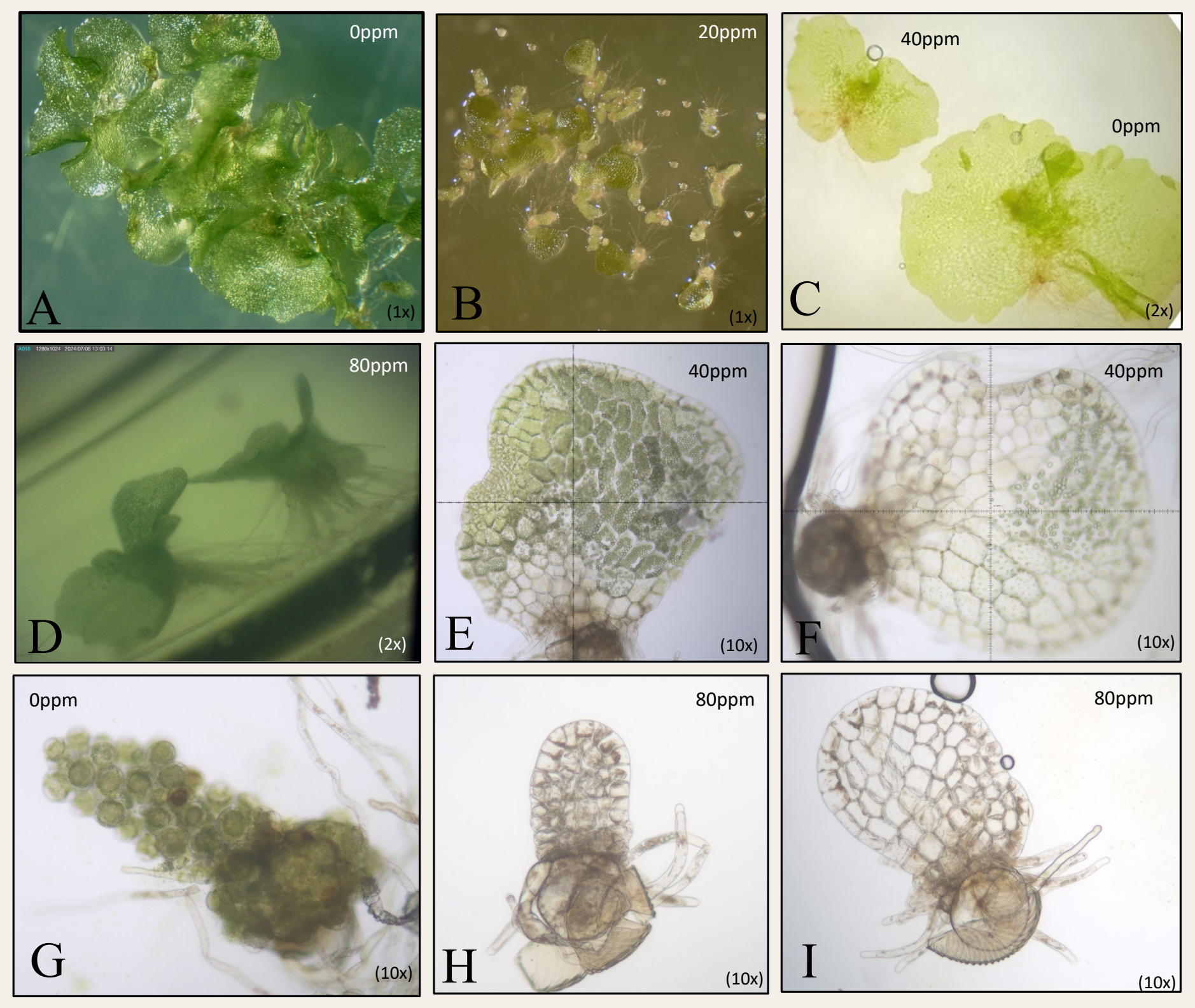

Figure 4: Gametophytes 26dfs. A. Mature hermaphrodites in 0ppm are green and large. B. Green, but not mature hermaphrodites in 20ppm are delayed compared to the control, which is true for all hermaphrodites grown under treatment conditions. C. Size comparison of hermaphrodites from 40ppm (left) and 0ppm (right). D. Mature green hermaphrodites observed in 80ppm were those growing on the edges of the petri dishes or growing upright away from the substrate (data not shown). E-F. Other gametophytes in direct contact with the substrate were delayed in development, and or chlorotic (lacked chlorophyll), and necrotic (dead). G. Mature green male gametophyte from 0ppm. H-I. Male gametophytes from 80ppm which are chlorotic and necrotic.

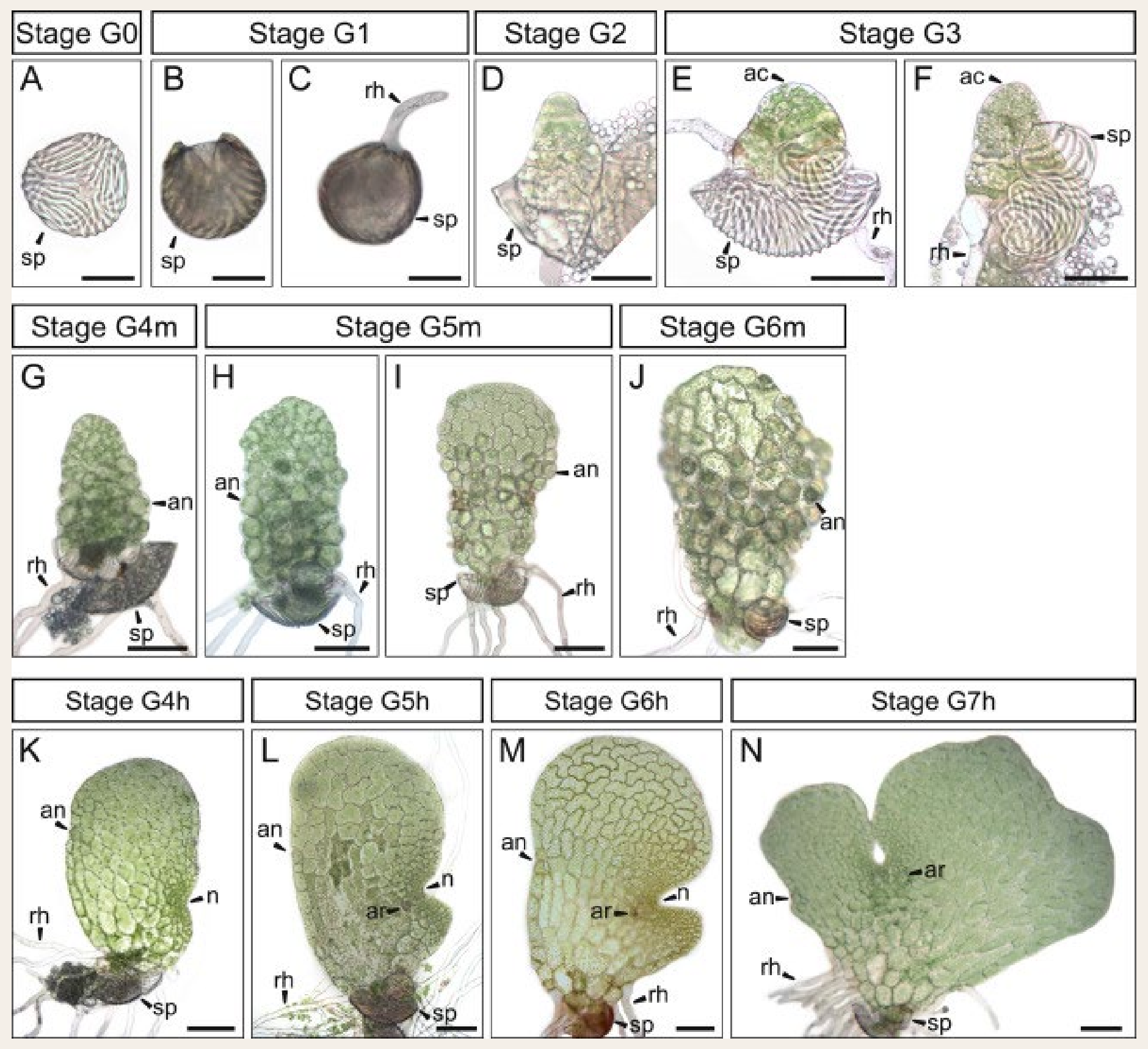

Figure 5: Normal gametophyte development in *C. richardii* (Conway and Di Stilio, 2020). A-F. Stages of spore germination. G-J. Developmental stages of the male gametophyte that will produce swimming sperm. K-N. Normal development of the hermaphrodite that produces the sessile eggs.

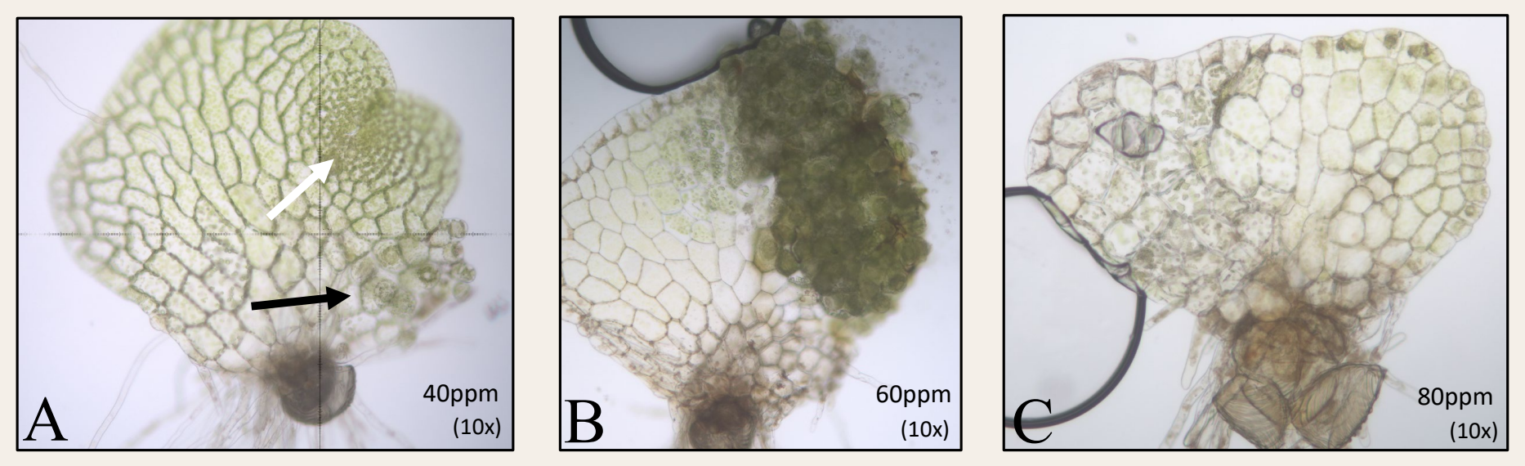

Figure 6: Abnormal gametophytes. A. Defined meristematic notch characteristic of hermaphrodites (white arrow) with abnormal cluster of antheridia (male sex organs) (black arrow). B. A cluster of green antheridia with chlorotic and necrotic tissue. C. Undifferentiated, chlorotic, and necrotic gametophyte.

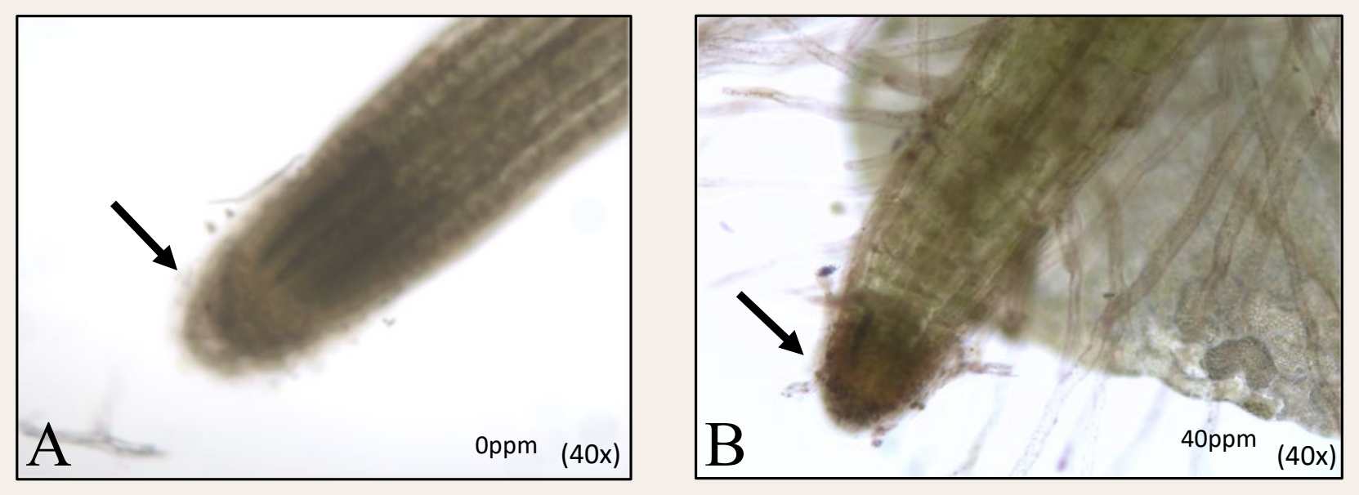

Figure 7: Sporophyte roots. A. Bright field micrograph (BFM) shows root tip (arrow) of sporophyte grown under control conditions (0ppm) B. Root tip (arrow) of sporophyte grown under treatment conditions (40 ppm) shows signs of necrosis.

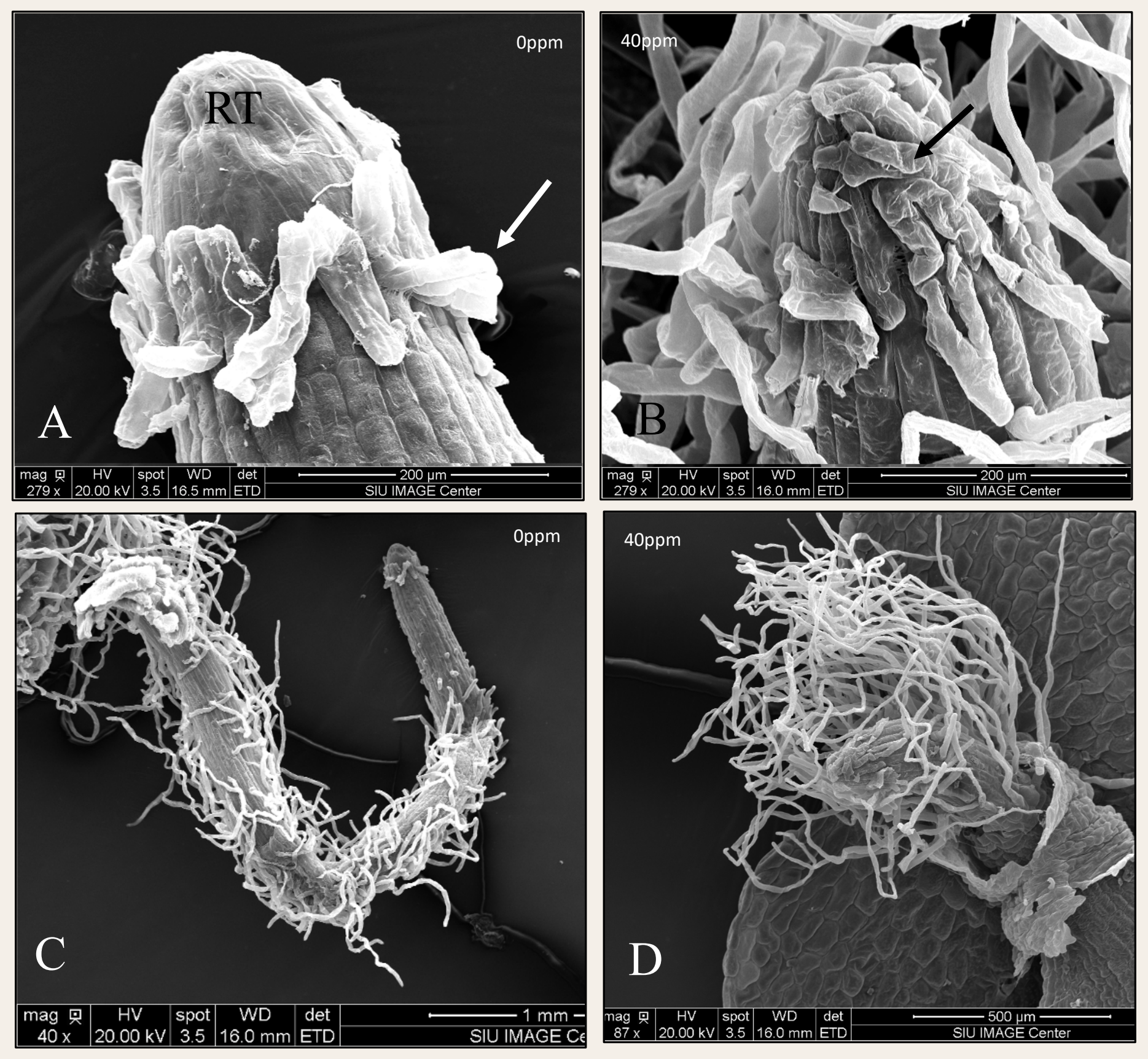

Figure 8. SEM of sporophyte roots. A. Root tip (RT) of control with outer cells sloughing off (arrow) shows normal growth behavior B. Sporophytes exposed to 40ppm (shown) – 100ppm AgNP have abnormal root tips that are not exposed, and cells are not sloughing off (arrow). C. At 31 days, control roots are elongated, and root hairs are present (arrow). D. Roots exposed to 40ppm (shown) – 100 ppm show signs of stunted growth and abnormal root tips (arrow).

DISCUSSION AND CONCLUSIONS

- We found that all concentrations of AgNPs (20-100ppm) had varying negative effects on growth and development of *C. richardii.* This study supports recent findings that AgNP released into the environment can potentially have negative effects on the ecosystem[1].
- Spore germination across treatments was on par with those of the control (Figs. 1 & 2). However, spore germination in *C. thalictroides* decreased as the concentration of AgNP increased [6]. However, other studies have shown that AgNP can have a beneficial effect on seed germination at low concentrations due to their antimicrobial and antifungal properties [2,5].
- AgNP significantly altered gender differentiation by reducing the number of hermaphrodite gametophytes across treatments (Fig. 3). Lu et al. (2022) obtained similar results in *C. thalictroides*. Reduced numbers of hermaphrodites within populations would reduce the number of offspring.
- Gametophytes were delayed in growth compared to the controls across all concentrations (20-100ppm) (Fig. 4).
- Hermaphrodite were smaller than those of the control (Fig. 4). A decrease in plant biomass is common under stressful, contaminated environments[1,2].
- By day 26, gametophytes found in the higher concentrations (60ppm-100ppm) suffered chlorosis and necrosis before reaching maturity (Figs. 4 & 5).
- Roots had damaged root tips (Fig. 7 A, B) and stunted in their growth (Fig. 8 D) compared to the control (Fig. 8 C). Soil pollutants like metal nanoparticles and microplastics inhibit root growth in a wide range of plants[2].
- While some studies show potential benefits of AgNPs at low concentrations[2-5] caution is warranted due to potential toxic effects at higher concentrations. Therefore, further research is needed to fully understand the mechanisms and long-term consequences of silver nanoparticle exposure on plant growth and ecosystems.

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