

Overall goal:

Overcome American chestnut's (AC) recalcitrance to adventitious root formation (AR).

Objectives:

- Develop rooting techniques.
- Reveal phytohormone levels and metabolites profile.

Main preliminary findings:

- Spring shoots broke buds but did not root (Fig. 1 A&B).

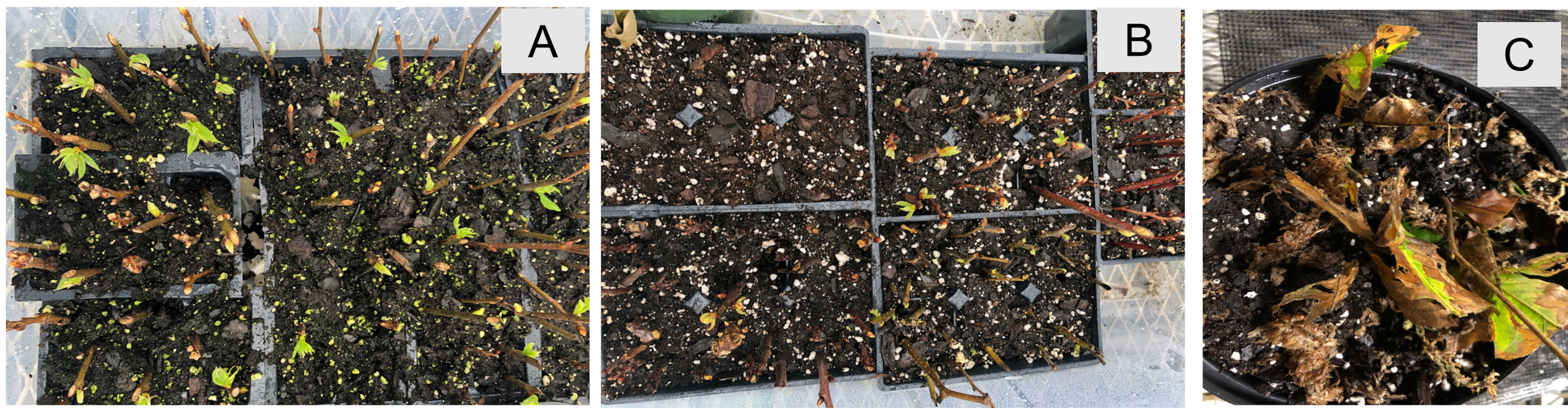


Figure 1A&B. Spring shoots: young leaves emerged then wilted. No roots were formed. 1C. Dead leaves.

- Leaves tend to become brown and dropped (Fig. 1C).
- Sphagnum moss and perlite are suitable (Fig. 2).



Figure 2.
Surviving cuttings

- Two kinds of adventitious roots can be induced with 1-Naphthaleneacetic acid NAA (Fig. 3).



Figure 3A. Adventitious rooting without callus formation.

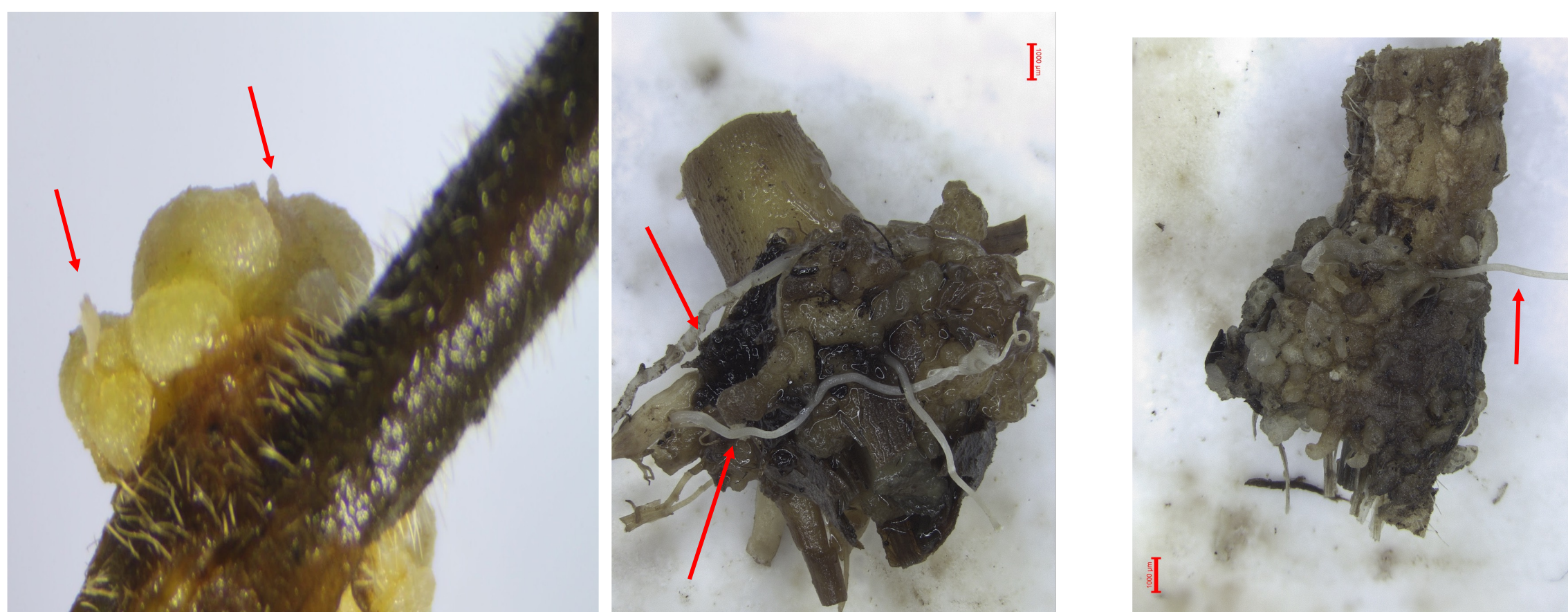


Figure 3B.
Adventitious rooting
without callus
formation.

Main preliminary findings (Continued):

- Compared to easy-to-root poplar, levels of known AR-inhibiting hormones, cytokinin (CK), inactive form of indole-3-acetic acid-aspartate (IAA-ASP), jasmonic acid (JA), abscisic acid (ABA), JA-Isoleucine (JA-ILE), salicylic acid (SA), and oxylipin 12-oxo-phytodienoic acid (OPDA), are significantly higher in AC cuttings (Fig. 4). For IAA, conflicting results were obtained between the two facilities that performed the analyses.

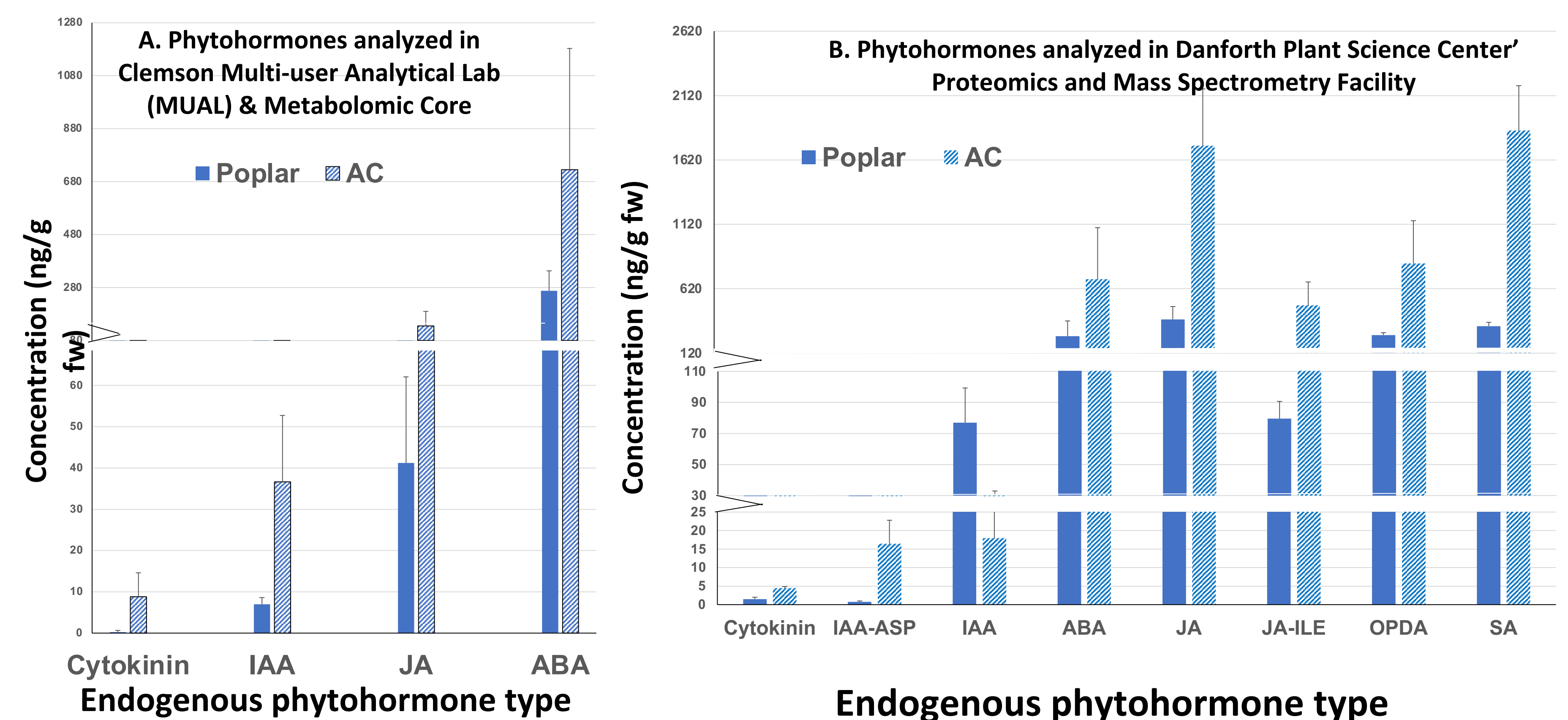


Figure 4. Comparison of endogenous hormone levels in AC and poplar stems.

- AC cuttings seem to have a different metabolite profile from poplar, since their secretion in ethanol and water led to different coloration (Fig. 5).

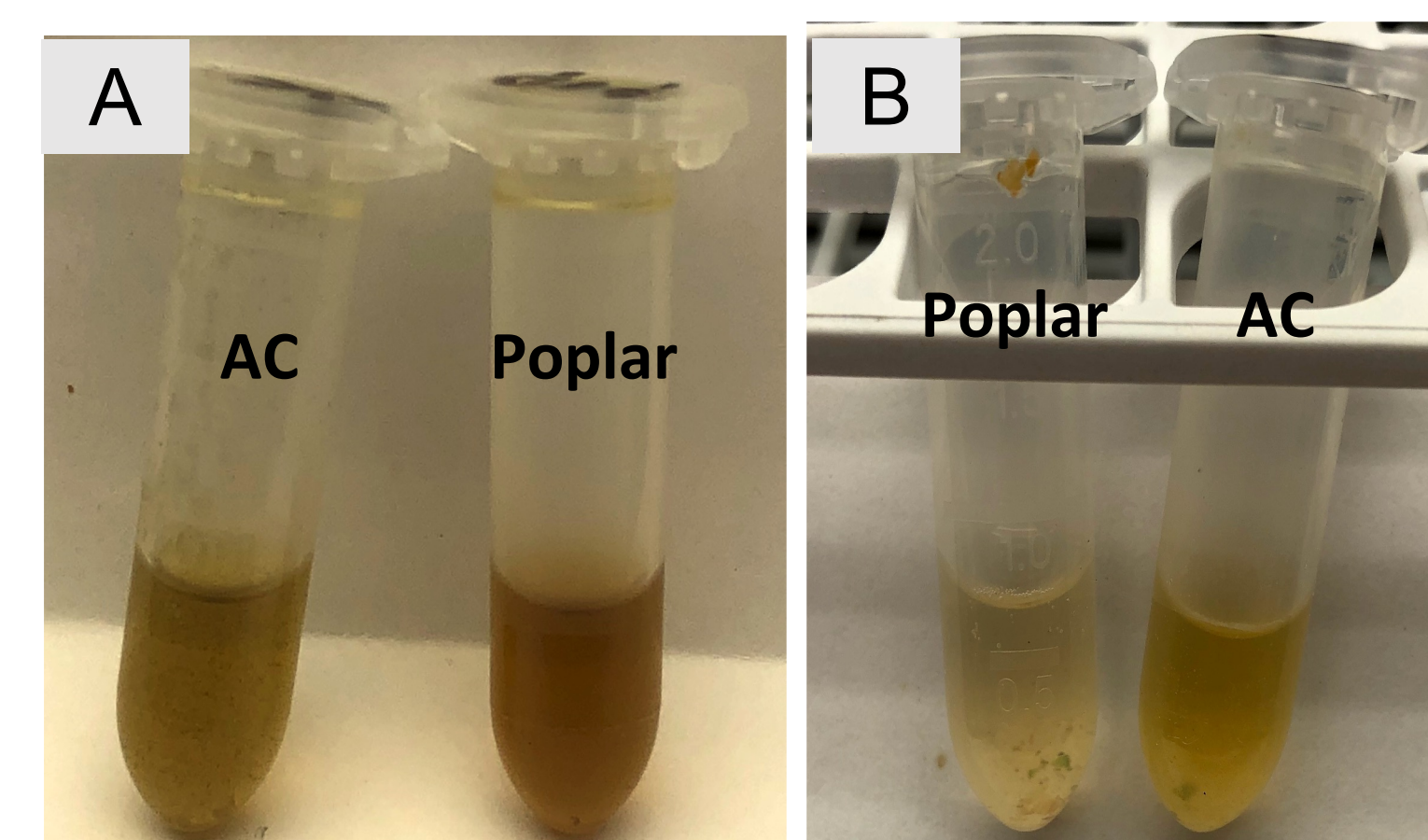


Figure 5. Color change in ethanol (A) and water (B) solutions after soaking with American chestnut and poplar stems (2 mm in length, 0.6g, submerged in 1 ml water or 75% ethanol at room temperature overnight).

Conclusions:

- AC cuttings take 1.5 to 2 months to root. AR system without callus formation has more and longer roots.
- Induced roots obtained so far were from juvenile AC plants. Rooting experiments mature tree donors are ongoing.
- Our data suggest high levels of unfavorable endogenous phytohormones and some metabolites in AC shoot cuttings may contribute to AC's recalcitrance to AR induction.

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