

Adventitious root formation in *Juglans nigra*: a time and place for everything[©]

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INTRODUCTION

Black walnut (BW; *Juglans nigra*) is an integral component of the Central and Eastern hardwood regions of the United States. Fine hardwood trees such as BW are used to manufacture high-end wood products such as veneer, cabinetry, gunstocks, and furniture, which are traded regionally and globally. Ecologically, BW serves an integral role as a riparian species, as well as providing food and shelter for wildlife. While BW continues to be cultivated commercially, significant effort and resources have been spent selecting for and breeding BW for improved timber characteristics.

As elite BW genotypes were developed it was quickly realized that clonal propagation was difficult. Traditional methods such as grafting requires a high level of skill, is time and labor intensive, has limited rates of success, and the resulting trees are not growing on their own roots, which can have an adverse effect on performance. Softwood cutting propagation is ideal for rapid multiplication of superior genotypes. However, the inability to predictably and reliably produce adventitious roots (AR) remains the greatest impediment to a routine BW propagation protocol. Recalcitrance to AR formation is common in most woody perennials such as BW. Adventitious roots formation is an extremely complex process controlled by many external and genetic stimuli; unfortunately, little is known about the underlying mechanisms controlling AR development.

Past attempts to improve adventitious rootability in BW have had limited success. Such gains were often difficult to reproduce, as rootability in BW is highly genotype specific, contributing to the inability to develop an optimized clonal propagation system.

RESEARCH OBJECTIVE

The objective of our research was to improve the frequency of AR formation in BW softwood cuttings, and investigate changes in anatomical patterning during root development. We tested multiple auxin types and concentrations, two environmental conditions during rooting, and two age classes of cuttings (easy-to-root half-sib seedling juvenile material vs. difficult-to-root mature grafted material).

CUTTING PROPAGATION

By using a fog system to maintain an elevated humidity, juvenile BW cuttings were successfully rooted as high as 72.2%. The use of high-density fog to root cuttings is commonplace in many horticulture systems, but has rarely been applied routinely to forest tree species. With this method, rooted cuttings were healthy and had well-developed root systems (Figure 1A, B). After transplanting to soil, cuttings continued to grow normally (Figure 1C). Cuttings rooted under intermittent mist however, often deteriorated as a result of superficial foliar salt accumulation and rooted less frequently than those in fog, independent of the auxin type used for root induction. Control cuttings without exogenous auxin application and cuttings taken from mature selections failed to root, regardless of rooting environment or auxin type and concentration.

After improving the rooting efficiency, we were then able to reliably study anatomical changes during AR formation in BW stems. By observing the timing and location of cellular changes during AR formation we hoped to better understand the underlying mechanisms

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regulating root formation. Stem tissue was collected on sequential days after root induction, and fixed in formaldehyde prior to paraffin embedding, serial sectioning, and staining. Histological analysis revealed that by Day 16, cell files were forming into what appeared to be root initials (Figure 2), and fully formed root primordia were evident by Day 18 (Figure 3). Adventitious roots were thought to originate from parenchyma cells located between gaps in phloem fibers. Location of root primordia formation and duration prior to emergence suggested an indirect pattern of root formation in BW cuttings. These preliminary findings are integral in the improvement of clonal propagation of elite genotypes, and will further allow for an elucidation of the molecular controls of AR formation in BW. Efficient and reliable propagation methods are also powerful tools for tree breeders and for conservation efforts.

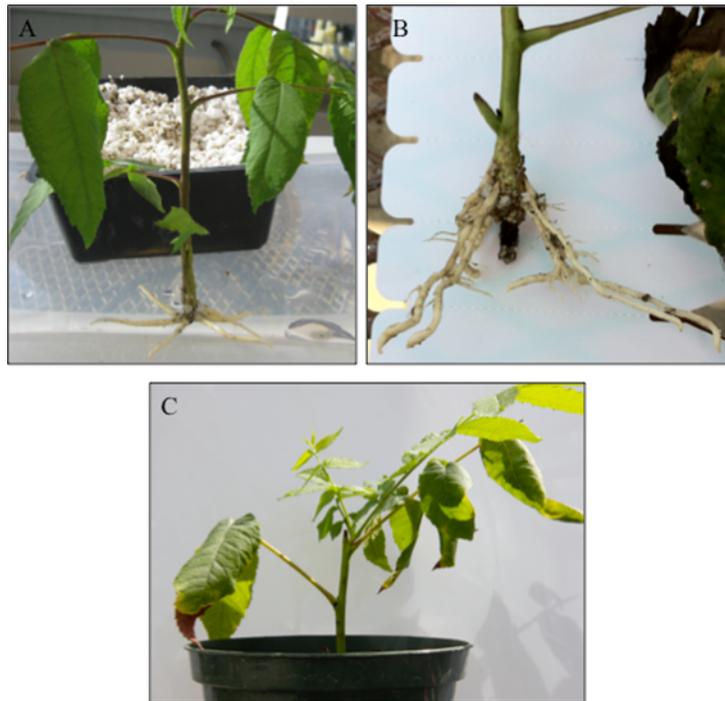


Figure 1. Successfully rooted black walnut (*Juglans nigra*) softwood cuttings. (A) Healthy rooted cutting 5 weeks after adventitious root initiation. (B) Close-up view of healthy root system of a rooted cutting. (C) Acclimatized rooting cutting transplanted in soil and continuing to grow normally.



Figure 2. Juvenile cutting transverse section 16 days after auxin application. Organized files of cells forming root initials (RI) first evident in gaps between phloem fibers (PF).

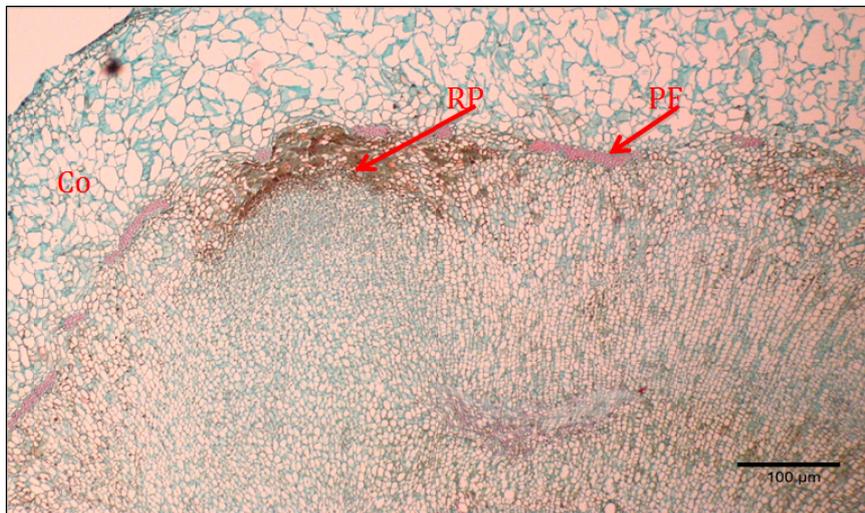


Figure 3. Juvenile cutting transverse section at Day 18. Root primordia (RP) is first visible with fully formed root cap. PF, phloem fibers; Co, cortex.

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