EMERGENCY CHESTNUT C-SECTIONS

Using immature embryo rescue of American chestnut (*Castanea dentata*) to conserve germplasm and accelerate breeding

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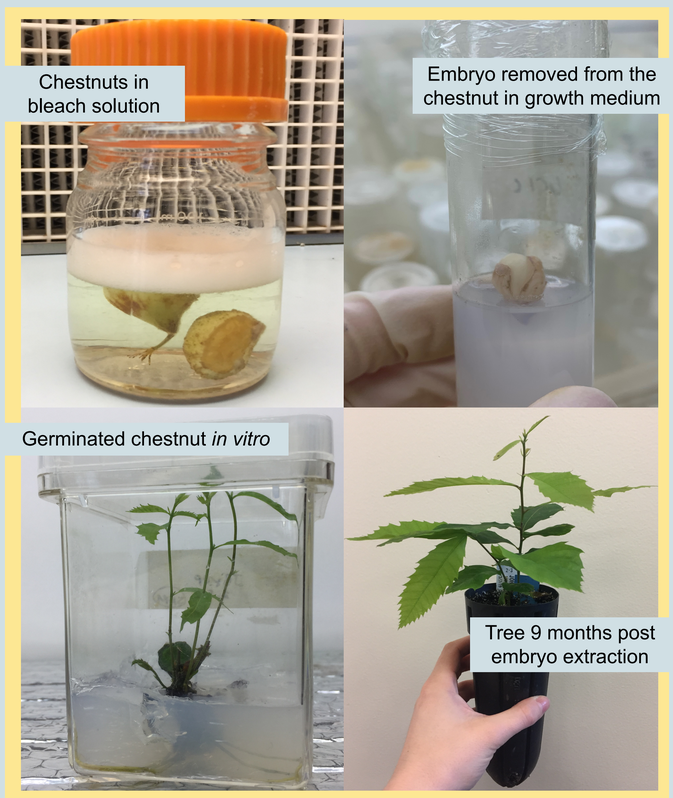
INTRODUCTION

The production of American chestnut seeds are crucial for conserving this imperiled species. Following fertilization, chestnuts take approximately 12-14 weeks to mature until harvest [1]. In that time, developing zygotic embryos can killed by chestnut blight, storms, insects, frost, and other stressors. Given the importance of chestnut progeny, an emergency protocol was developed to remove and rescue the at-risk immature embryos from the seed. Germinating seeds *in vitro* allows them to be clonally propagated and grown into whole plants. **The purpose of this study was to determine the earliest and most optimum time immature embryos can be excised from the seed and germinated *in vitro.***

METHODS

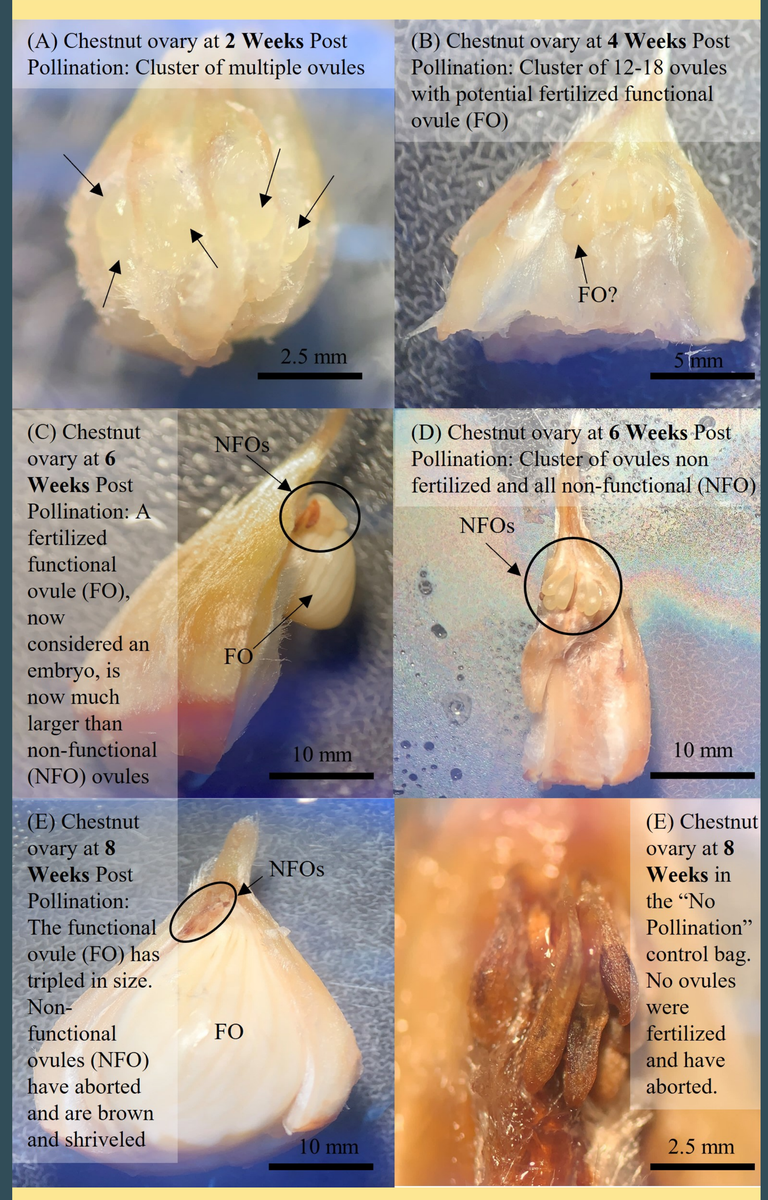
Multiple clusters of pistillate flowers were covered with bags to prevent open pollination. Once receptive, all bagged flowers were hand pollinated. At **2-weeks, 4-weeks, 6-weeks, and 8-weeks post pollination, developing burs were removed from the trees.** At each timepoint, seeds were removed from the burs and sterilized in a bleach solution. Seeds were carefully sliced open and all healthy ovules were excised. Embryos were grown in a semi-solid agar growth medium to stimulate the proliferation of the shoot apical meristem.



Germination rate and time elapsed until germination were recorded for each embryo for each extraction time. Once an embryo germinated, shoots were clonally propagated. Shoots were *ex vitro* rooted before potting in soil [2]. Dry biomass of trees from each timepoint were taken at the end of the study.

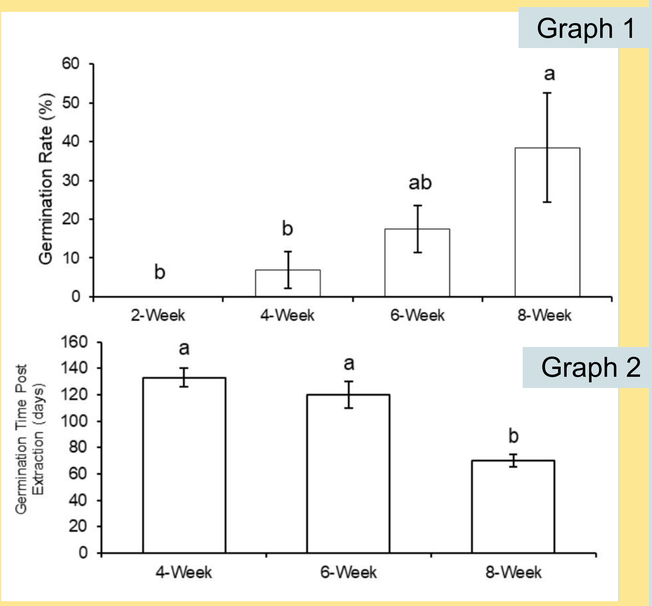
RESULTS

The differentiation of ovules into zygotic embryos was drastic in the eight weeks post pollination (shown below).



Embryos extracted at 8-weeks had the highest germination rate (38.46% ± 14.04%). The earliest *in vitro* germination was observed at 4 weeks (6.90% ± 4.79%) (Graph 1). Embryos extracted at 8-weeks germinated significantly faster (Graph 2). Biomass measurements of trees were not significantly different by extraction time (p= 0.261). **Embryos were planted in the field in less than a year following extractions.**

Graph 1



Graph 2

DISCUSSION

The earliest time the immature chestnut can be removed from the developing seed is at 4 weeks post pollination. However, germination rate is low and it is difficult to distinguish fertilized, functional ovules from non-functional ovules. To prevent initiation of non-functional ovules, an enlarged embryo sac is a good indication the ovule will not abort [3]. Therefore, **the most optimum time to remove the embryo is at 8 weeks when the functional ovule and embryo sac is obvious. Germination is also quicker and more successful at 8 weeks.** Overall, embryo cultures from 4, 6, and 8-week extraction time all grew vigorously, survived into whole plants, and did not differ in biomass. As the remaining American chestnut germplasm diminishes, this protocol may be used to protect pollinations and preserve genotypes through micropropagation.

EMBRYO RESCUE FOR CHESTNUT BREEDING

Embryo rescue can be used intentionally to initiate offspring from tree genotypes of interest. Embryos are selected, initiated *in vitro*, and multiplied. **Winter dormancy of the chestnut is bypassed which accelerates production of the trees.** Whole plants are put into high light growth chambers to stimulate pollen production [4]. Embryo rescue has been useful in the ongoing breeding effort of ESF's transgenic, blight-tolerant, Darling 58 chestnut.

This technique is utilized to rescue and successfully germinate embryos produced indoors. Juvenile trees sometimes produce female flowers while growing indoors or under high light treatments but they typically do not have enough energy to produce mature nuts. Embryo rescue allows for germination of the immature embryos which would otherwise not survive.

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