

# Long-term Freezer Storage of American Chestnut Pollen



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## Introduction

Effectively freezer storing limited & valuable transgenic pollen is necessary to perform as many cross-pollinations as possible. This study aimed to determine which storage container should be used to collect pollen (traditional vial or glass slides for easy pollen distribution), how long to desiccate the pollen, and the ideal freezer temperature.

## Methods

Pollen from 4 wild-type American chestnut trees was collected on 7 days during flowering. Vial collection: Catkins were stripped & pollen mixture was transferred into vial. Slide: Catkin was rubbed along a slide until grains were observed. Pollen was desiccated for 4, 24, or 48 hours and freezer stored at -80°C or -20°C according to treatment (Fig. 1). Pollen was frozen for 8 months.

Treatment	Container	Hours at 4°C	Desiccant Pellet?	Freezer Temp (°C)	Germination %
1	Vial	4	Yes	-20	34.88 ± 14.35
2	Slide	4	Yes	-20	5.50 ± 2.14
3	Vial	4	No	-20	39.75 ± 8.36
4	Slide	4	No	-20	2.10 ± 3.03
5	Vial	24	Yes	-20	44.61 ± 3.08
6	Slide	24	Yes	-20	5.96 ± 1.26
7	Vial	24	Yes	-80	46.43 ± 3.65
8	Slide	24	Yes	-80	12.65 ± 3.39
9	Vial	24	No	-20	35.50 ± 8.55
10	Slide	24	No	-20	5.87 ± 1.89
11	Vial	48	Yes	-20	27.00 ± 6.96
12	Slide	48	Yes	-20	5.79 ± 1.84
13	Vial	48	No	-20	31.50 ± 3.20
14	Slide	48	No	-20	3.79 ± 1.64

Fig 1. Pollen storage variables and corresponding percentage of germinated grains according to treatment.

## Pollen Analysis

- A grain that produces a pollen tube for gamete transfer is considered viable (Fig 2).
- 250 pollen grains were randomly per sample (Fig. 3).
- Germination: % = (number of pollen tubes germinated/total pollen grains)\*100



Fig 3. A typical view during pollen sample analysis. 40 +/- 10 pollen tubes are counted per field of view.

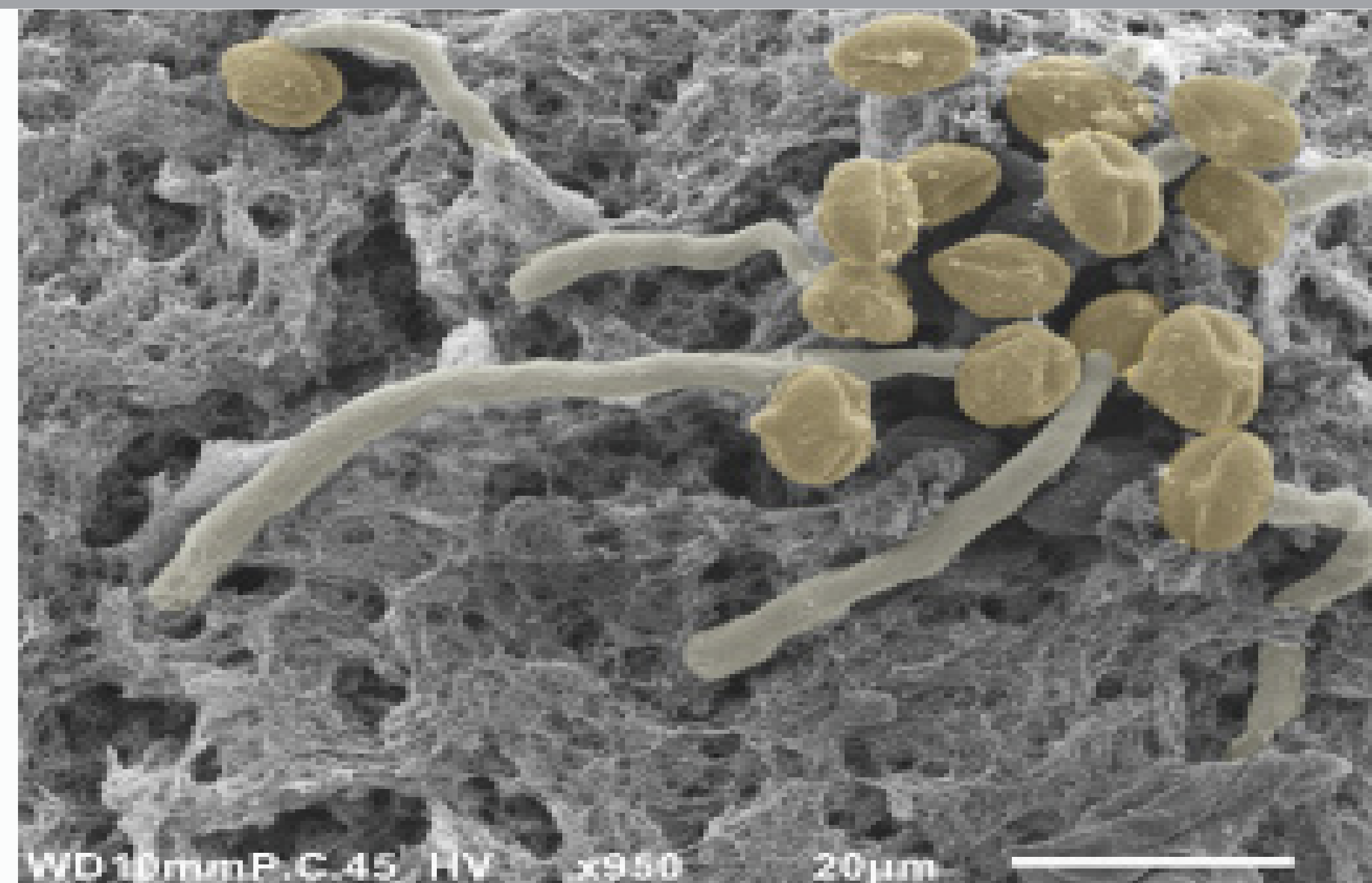
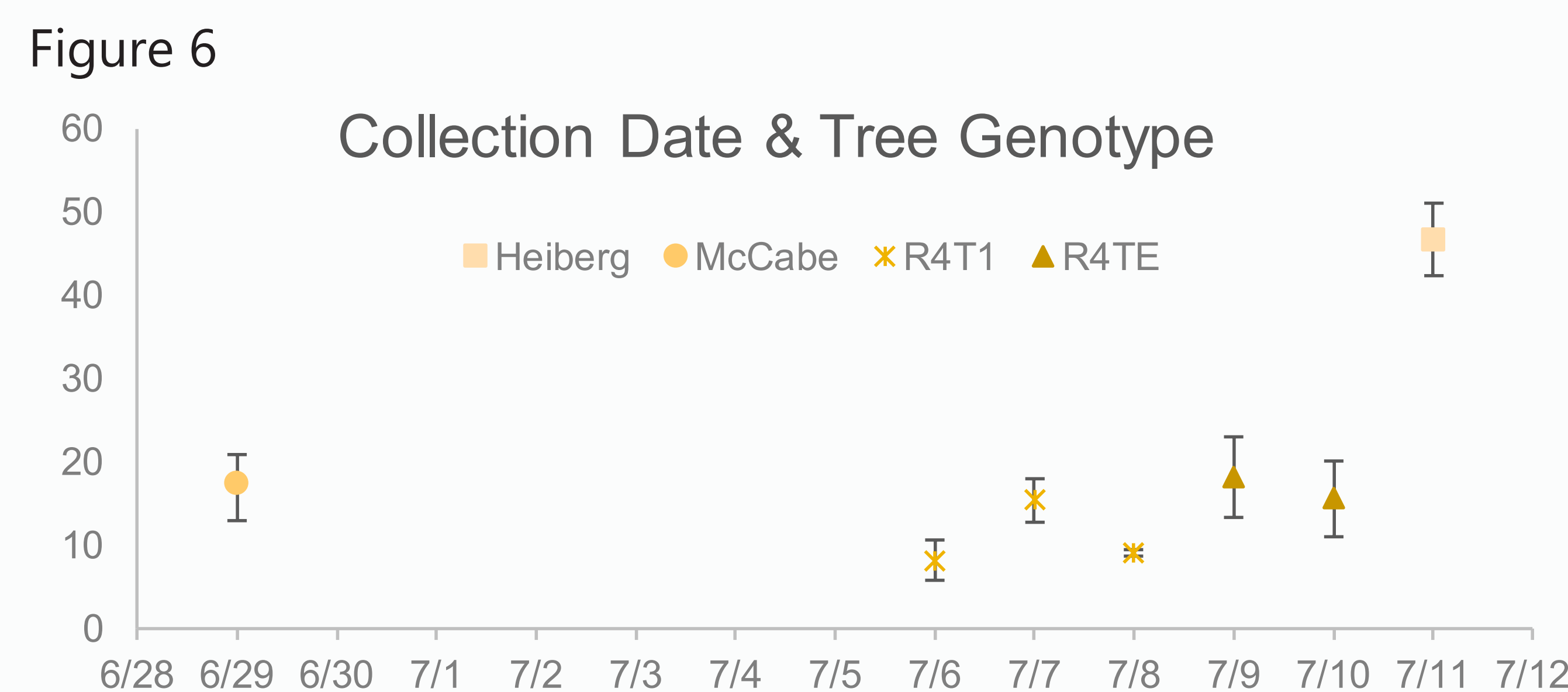
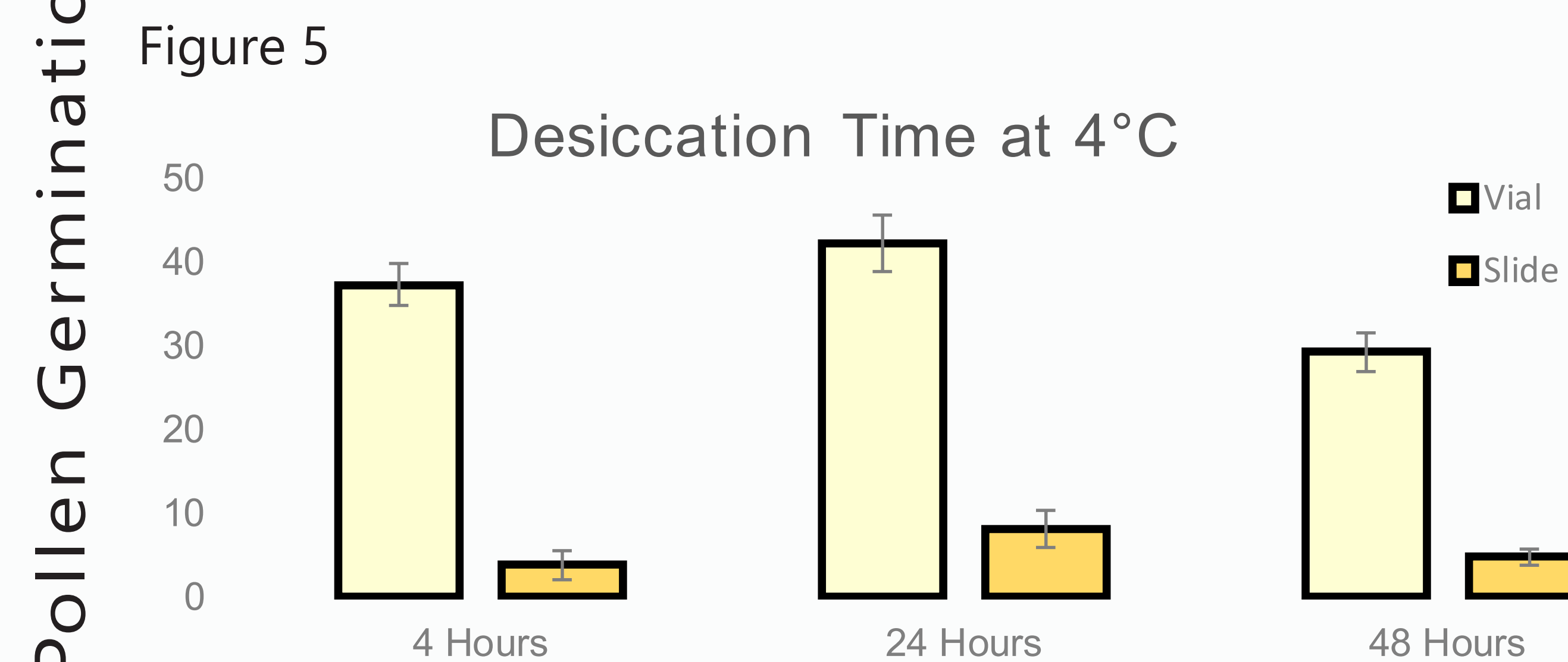
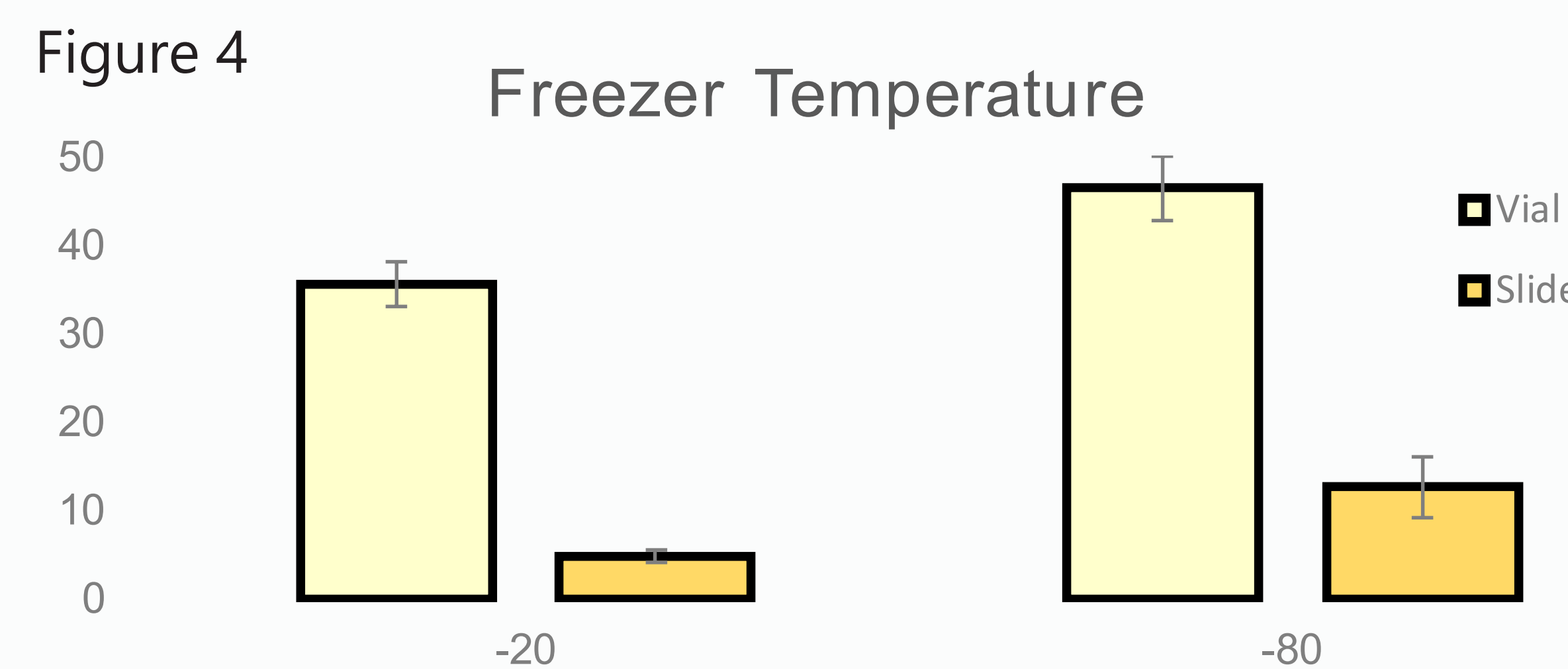


Fig 2. Colorized scanning electron micrograph of germinating American chestnut pollen grains.

## Which storage treatment maximizes pollen viability?



## How to store Pollen:

1. Strip catkin onto a glass plate.
2. Collect pollen mix in a vial with air-tight cap.
3. Place vial into container with desiccant rocks.
4. Dehydrate pollen for 24 hours at 4°C.
5. Store in -80°C freezer.

## Results

Storage container had the most significant effect on pollen viability. Storing pollen in vials with an air-tight lid will maintain greater pollen viability than slides (Fig. 4).

Pollen was more viable when stored at -80 °C than -20 °C (Fig. 4).

Pollen should be desiccated 4-24 hours before moving into the freezer. Desiccating for 48 hours resulted in less viable pollen (Fig. 5).

There was a significant effect of collection date and tree genotype on pollen viability (Fig. 6).

## Follow Up Study



Fig 7. A) Cordless Black & Decker vacuum. B) Modified nozzle to filter debris and trap pollen. C) Pollen grains collected on mesh in cluster.

- Study was repeated in 2018 with growth chamber pollen to minimize variability & optimize the protocol for the transgenic "Darling" genotype.

- A pollen vacuum was tested as a collection method for minimizing grain loss (Fig. 7).