

Abundant, Geographically-Diverse Darling58 Pollen in Preparation for Deregulation

Virginia Grace May & Thomas Klak (tklak@une.edu)

School of Marine and Environmental Programs University of New England (UNE), Biddeford, Maine 04005

SUMMARY

- After years of trial and error, Team UNE has recently been able to **produce an unprecedented quantity and diversity of T3 & T4 Darling58 (D58) pollen**.
- Since July 2022, we have collected T3 & T4 D58 pollen from **at least 20 seedlings, from crosses that originated in ME, NY and VA**.
- We have **reduced the time** a seedling requires to reach pollen maturity, so that some D58 pollen is now available **in the same chestnut year**.
- We are preparing to ship out around **1,000 vials of D58 pollen** on dry ice upon deregulation to TACF members; this is conservatively enough pollen to fertilize 40,000 female flowers in the field.



Fig.1: A UNE greenhouse seedling yielding T4 D58 pollen in July 2022, and used that month to pollinate trees in Maine & at ESF.



Figs.2 & 3: D58 pollen collected on microscope slides in dense quantity, and stored in vials at -80C. Each slide holds many 1000s of D58 pollen grains, suggesting the value of increased efficiency of D58 pollen deployment in the field.

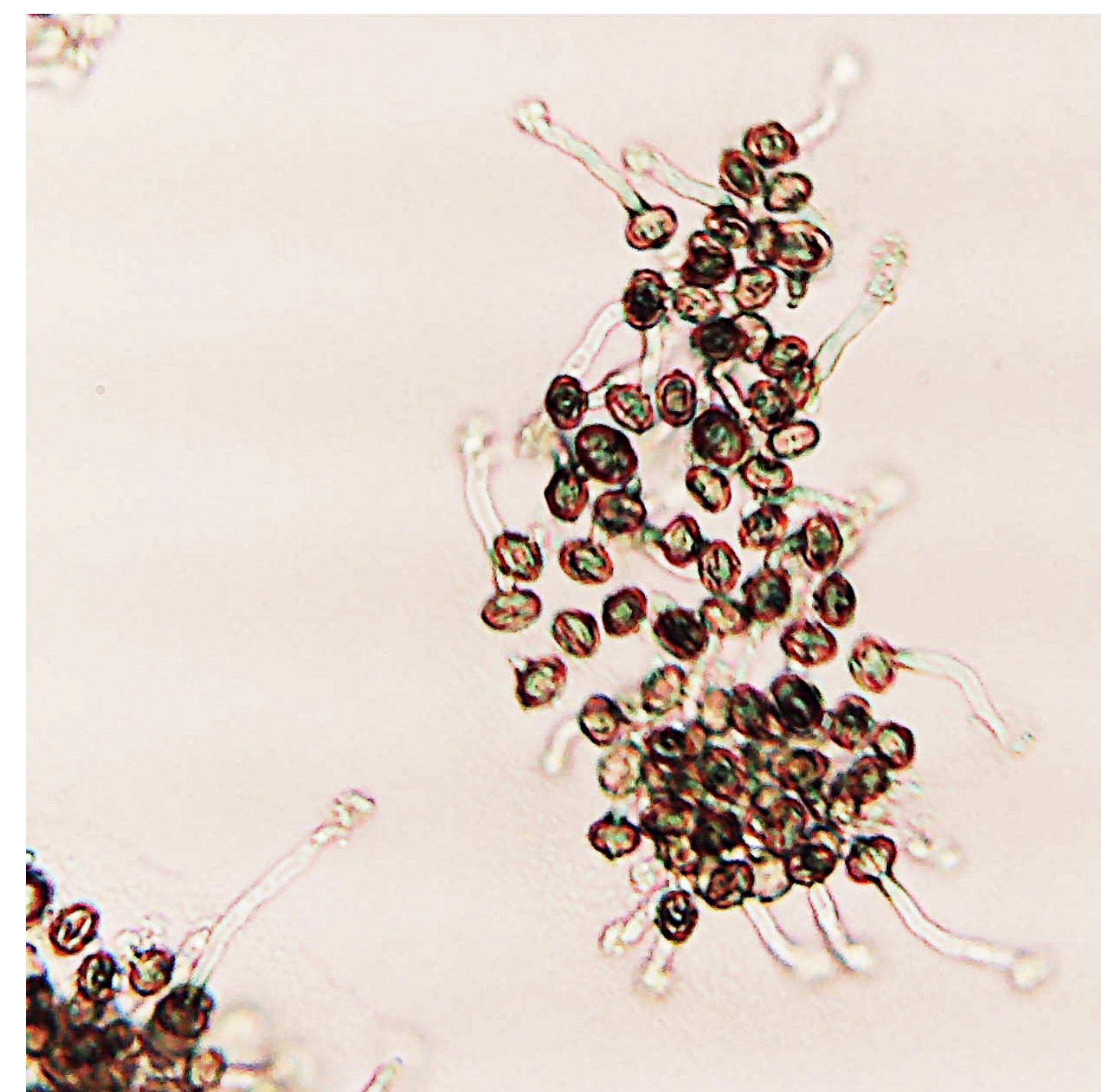


Fig.4: D58 pollen recently tested at UNE for viability (note tubes) through an assay deploying an Agar Sucrose medium.

UNE's GREENHOUSE POLLEN PRODUCTION METHODS

Speed Breeding Components:

- High Intensity **LED Lighting**:~16 hrs/day
- Temperature & Humidity Control
- Flush out accumulated salts from soils** every other day
- Rotation of **6 or more fertilizers** & adjusting their pH to **5.5**
- Experimenting with 1 vs. 2-gallon pots

Pests & Pathogens and their Treatment:

- Fungal gnats, spider mites, powdery mildew fungus, & others wreak havoc
- Treated with soil application of Gnatrol (Bt bacteria) & with ~70% Isopropyl alcohol wipes and folic spray (209, Clean Leaf, Neem oil)
- Close **monitoring to address pest outbreaks**

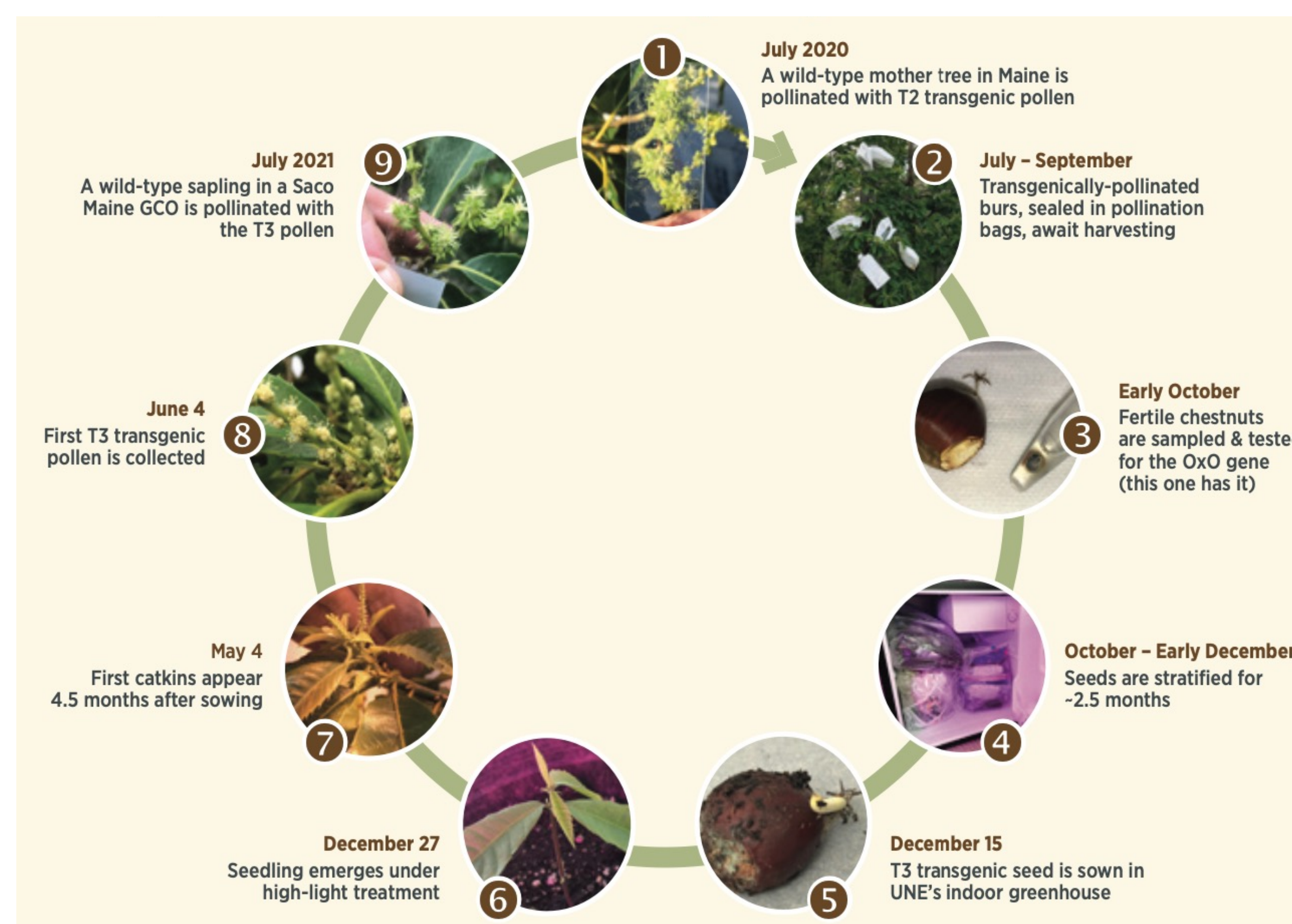


Fig.5: "The Chestnut Year" depicts how UNE was able to produce T3 & T4 D58 pollen in 2021 & 2022 in time for July field pollination (Klak, Spiers & Powell 2021).

ME	240
Cape Elizabeth	115
Georgetown GCO	102
Saco GCO	23
NY	170
ESF	170
VA	129
Lesesne St Forest	20
Meadowview	109
T2 Pollen	183
Total Darling58 Vials Frozen @ 9/17/22	722

Fig.6: UNE Darling58 Vials -80C Frozen, by State & Mother Tree Location, @ 9/17/22

T4	129
T3	410
T2	183
Total	722

Fig.7: UNE Darling58 Pollen Vials in Frozen Storage, by Generation, @ 9/17/22

FUTURE DIRECTIONS

American Chestnut Suitable Habitat, 2050, Low & High Greenhouse Emissions Projections

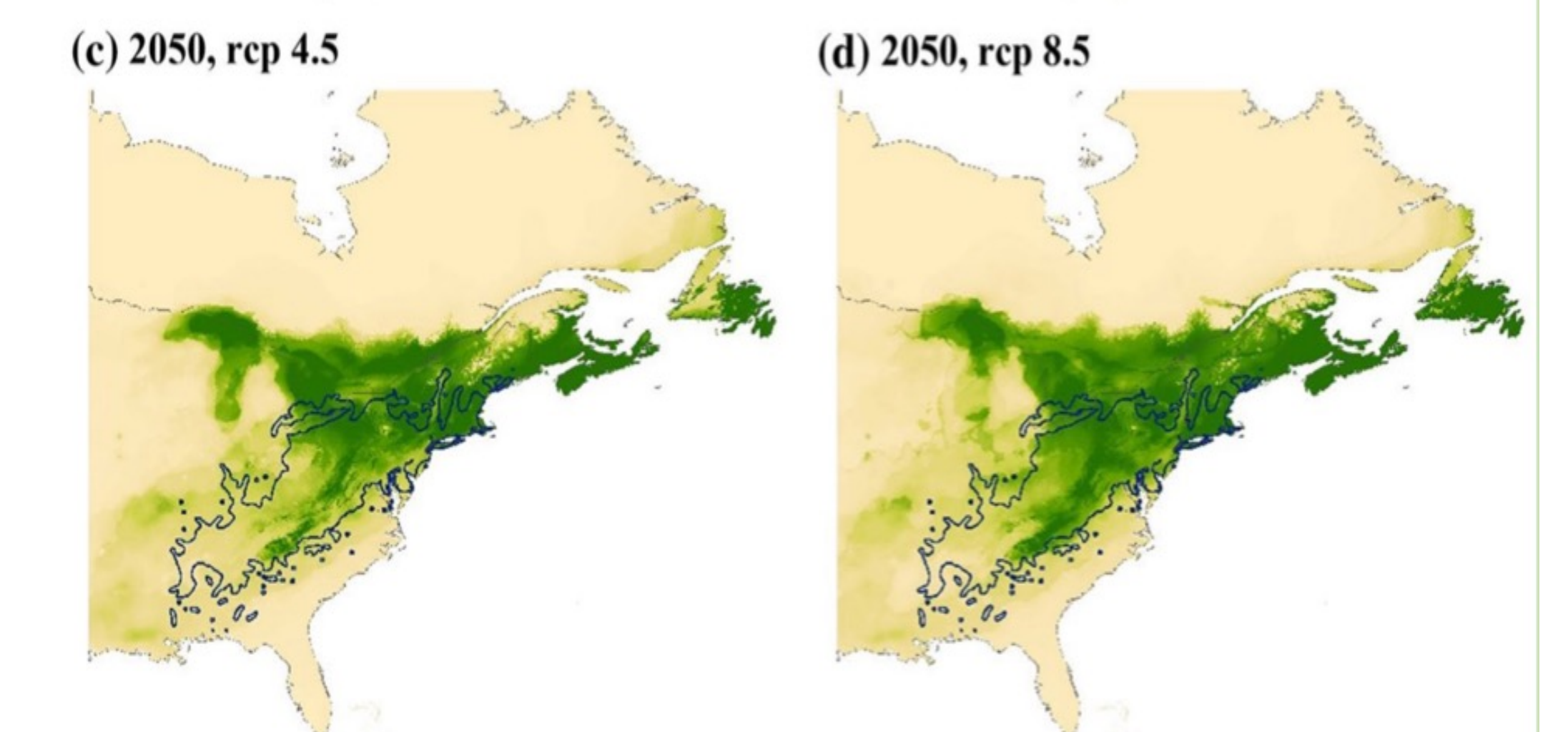


Fig.8: Two maps projecting suitable habitat for the American chestnut in 2050 show the emerging centrality of New England for chestnut outplanting (Barnes & Delbourne 2019).



Fig.9: Burs from a TGxTG cross in UNE's greenhouse. Fig.10: Live embryos from UNE's TG crosses, now in tissue culture in Hannah Pilkey's ESF lab. If she can advance them to seedling stage, some could be among the first homozygous D58s.

SOURCES

- Barnes, JC & Delborne, JA. 2019. "Rethinking restoration targets for American chestnut using species distribution modeling" *Biodiversity and Conservation* 28:3199-3220.
- Johnson J. 2020. "The American Chestnut" Half Earth Project; [accessed April 24, 2022]. <https://www.half-earthproject.org/the-american-chestnut/>
- Klak T, Spiers E, and Powell W. 2021. "Breakthrough: Transgenic Pollen in Less Than a Year" *Chestnut: The Journal of The American Chestnut Foundation* 35(3 Fall):27-29.
- Westbrook JW, Holliday JA, Newhouse AE, Powell WA. 2020. A plan to diversify a transgenic blight-tolerant American chestnut population using citizen science. *Plants, People, Planet* 2:84-95. <https://nph.onlinelibrary.wiley.com/doi/pdfdirect/10.1002/ppp3.10061>

ACKNOWLEDGEMENTS

We thank the students in "Genetic Engineering for Ecological Restoration" for their contributions to this project, & UNE's Schools of Marine & Environmental Programs and Biological Sciences for supporting our American Chestnut Research restoration work. We also thank the UNE Facilities who have been very helpful through their maintenance work on the greenhouse. Funding support from The Cricket Foundation and the American Chestnut Foundation is gratefully acknowledged.