

Darling 58 Transgenic American Chestnut

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Controlled inoculations demonstrate blight tolerance



Inoculation Results (3 mo.)



At least half girdled



Girdled, dead above inoculation site





- 23 full-sibling pairs of T2 trees were inoculated with *Cryphonectria parasitica* strain EP155 in June 2022
- Darling 58 stems had consistently smaller cankers than non-transgenic controls
- All non-transgenic controls were at least half girdled after 3 months, 10 were dead
- Most Darling 58 stems remained healthy, one was girdled, none were dead









Federal regulatory decisions are expected soon

USDA Submitted 1/2020, decision expected by 8/2023

(APHIS Petition for Nonregulated Status: general human and environmental safety)



EPA Submitted 9/2021, expected 12/2022

(FIFRA Registration and tolerance exemption: environmental safety of "pesticides")



FDA Submitted 10/2021, expected ~4/2023

(Biotechnology Consultation: food and nutritional safety)

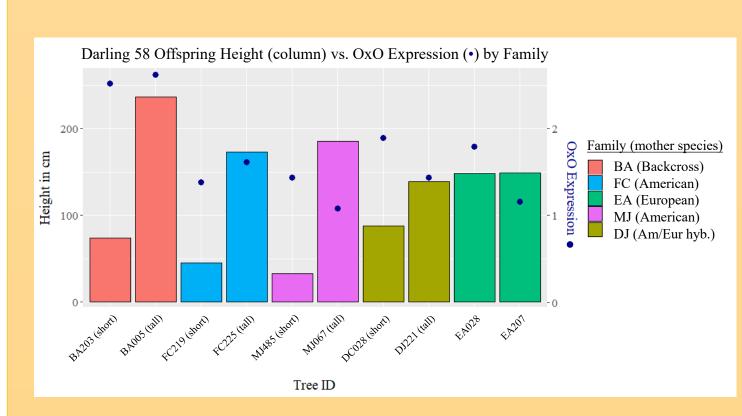
- 6,453 total pages submitted to 3 agencies (so far)
- Additional exemptions likely needed for EPA and FDA
- Discussions with Canadian regulators are underway





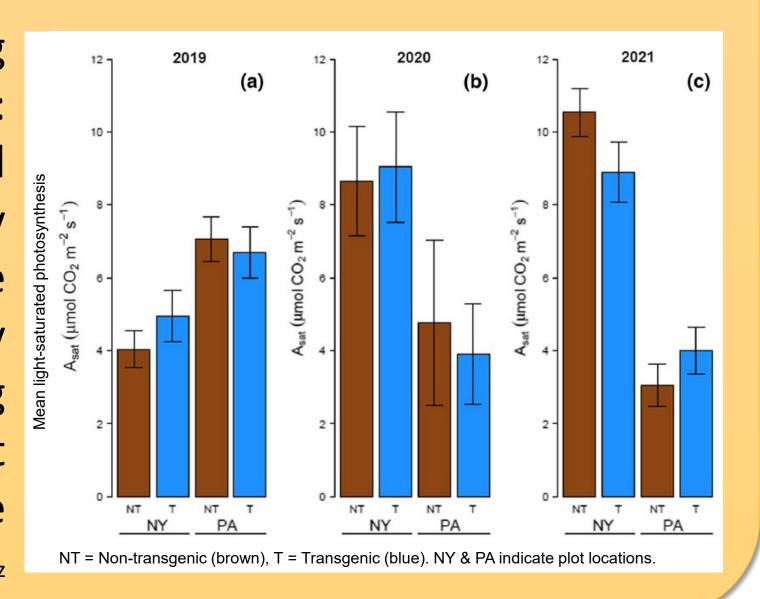


OxO transgene expression levels do not significantly affect tree performance

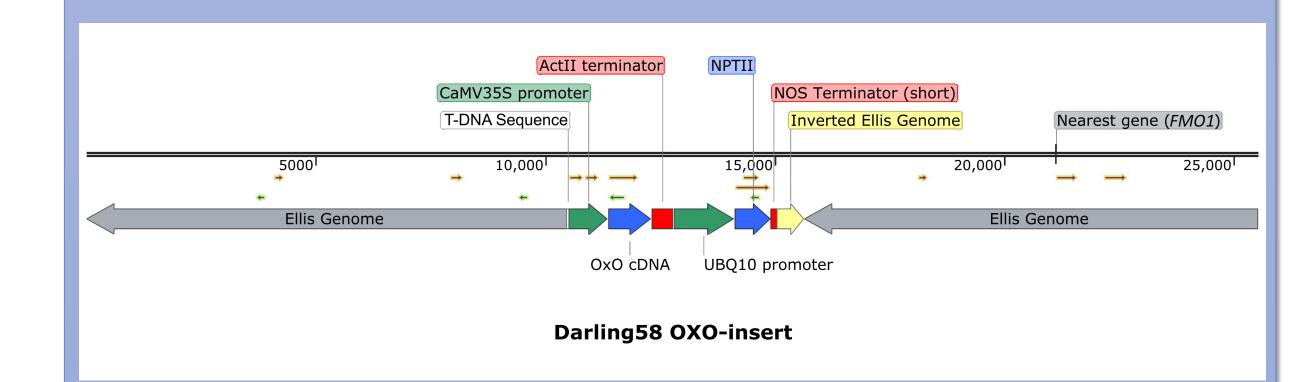


OxO isn't stunting growth: transgene expression is not strongly correlated to species background or tree height, but varies by individual family

OxO isn't changing tree physiology: Light-saturated photosynthesis (a key measure of tree physiology) varies by year and planting location, but not transgene presence Onwumelu et al. 2022 bit.ly/3xLM8jz



Modern techniques confirm initial DNA tests



- Long-read genome sequencing (PacBio) performed on Darling 58
- Single OxO insert on Chromosome 7, ~6,000 bp away from nearest gene
- Minimal rearrangement to nearby DNA (though natural genome rearrangements are ubiquitous)
- No extraneous Agrobacterium inserts (though bacterial genes are naturally found in plants)