Oxalic acid leaf disk assays may be another method of screening phenotypic variability in "American" looking Castanea hybrid trees

Harden, K.E. and Craddock, J.H.
The University of Tennessee at Chattanooga, 615 McCallie Ave, Chattanooga, TN

Background

Cryphonectria parasitica effectively uses oxalic acid to kill *Castanea* species (Zhang, et al. 2013). By studying oxalic acid's effects on several linages, we may be able to characterize trees with relative tolerance to oxalic acid. The acid causes browning of leaf tissue. The newest way to fight blight is with the oxolate oxidase gene from wheat (Carlson et al. 2021). The role of oxalic acid in this patho-system led to the first attempt to use OxO in genetically modified chestnut trees. As we study the transformed *Castanea* with OxO, we may be able to characterize trees using relative tolerance. We can use this knowledge to characterize our hybrid germplasm in seeding population that we expect to vary.

Objectives

I hypothesize that there will be measurable differences in area of leaf disk necrosis following an oxalic acid soak of leaves of C. alabamensis, C. dentata, C. mollissima, C. ozarkensis, C. henryi, C. alnifolia, C. pumila, and open-pollinated seedlings of selected backcross hybrids. The resistance of C. pumila species to C. parasitica are understudied, and I think their endogenous tolerance will be somewhere between C. dentata and C. molissimia.

Table 1: 2022 SSA backcross hybrid families and selected *Castanea* species

Family	Seedling Type	Quantity
C. dentata (CT-EL007)	American	4
C. mollissima (AU-1-26)	Chinese	4
C. pumila	Chinquapin	1
C. pumila var. albamensis	Alabama Chinquapin	2
C. pumila var. ozarkensis	Ozark Chinquapin	1
C. henryi	Henryi	2
C. pumila var. alnifolia	Trailing Chinquapin	2
TN-DC12-2-8	B4	4
TN-DC12-4-6	B4	4
TN RC09-2-22	B4	4
TN-RC09-2-35	B4	4
TN-RC09-3-62	B4	4
TN-RC09-3-9	B4	4
TN-RC09-5-15	B3	4
TN-RC09-5-30	B1	4
TN-RC09-6-46	B3	4
TN-RC09-7-33	F1 (*dentata x crenata*)	4
TN-TTU-A34	F1 (*dentata x mollissima*)	4

Methods and Materials

- Table one includes the selected trees, each individual species available, and the 13 families in the Fortwood Greenhouse at UTC, other specimens were collected at TN-RC09. I used one leaf to create 10 disks, and the quantities of individual plants vary between families. However, the controls and hybridized species all have 4 plants per family. Fresh leaves were collected from non-adjacent blocks.
- I used a steel hole punch to create 1.5 cm disks that were then placed in 50 mM oxalic acid solution. The disks were all placed in closed tubes and then taped horizontally to an orbital shaker to prevent the disks from clumping.
- Time trials (anywhere from 2-24 hours) were performed in order to accurately predict how long the leaves could soak in my solution before turning completely brown.
- I also completed a trial to see if different inversions of the acid solution tubes on the orbital shaker affected the areas. I concluded that horizontal placement of the tubes on the orbital shaker was most effective.
- Each disk was rinsed with distilled water and then dried for photographs to calculate areas. The areas (mm^2) of brown to green (healthy) of the disks were computed with ImageJ. The average percentages were then calculated for each family.

Results

- Preliminary results show that the area of browning differs by species and varies greatly between backcrossed species.
- Unexpectedly, C. pumila had the lowest average area of browning on the disks. Since C. mollissima is inherently resistant to blight, it was expected to have the lowest percentage, or highest tolerance to the oxalic acid solution. Somehow, the chinquapin has a higher tolerance than the Chinese chestnut leaves. The hybrid species are highly varied and are more like the American's tolerance to oxalic acid. The least tolerant species was C. dentata.

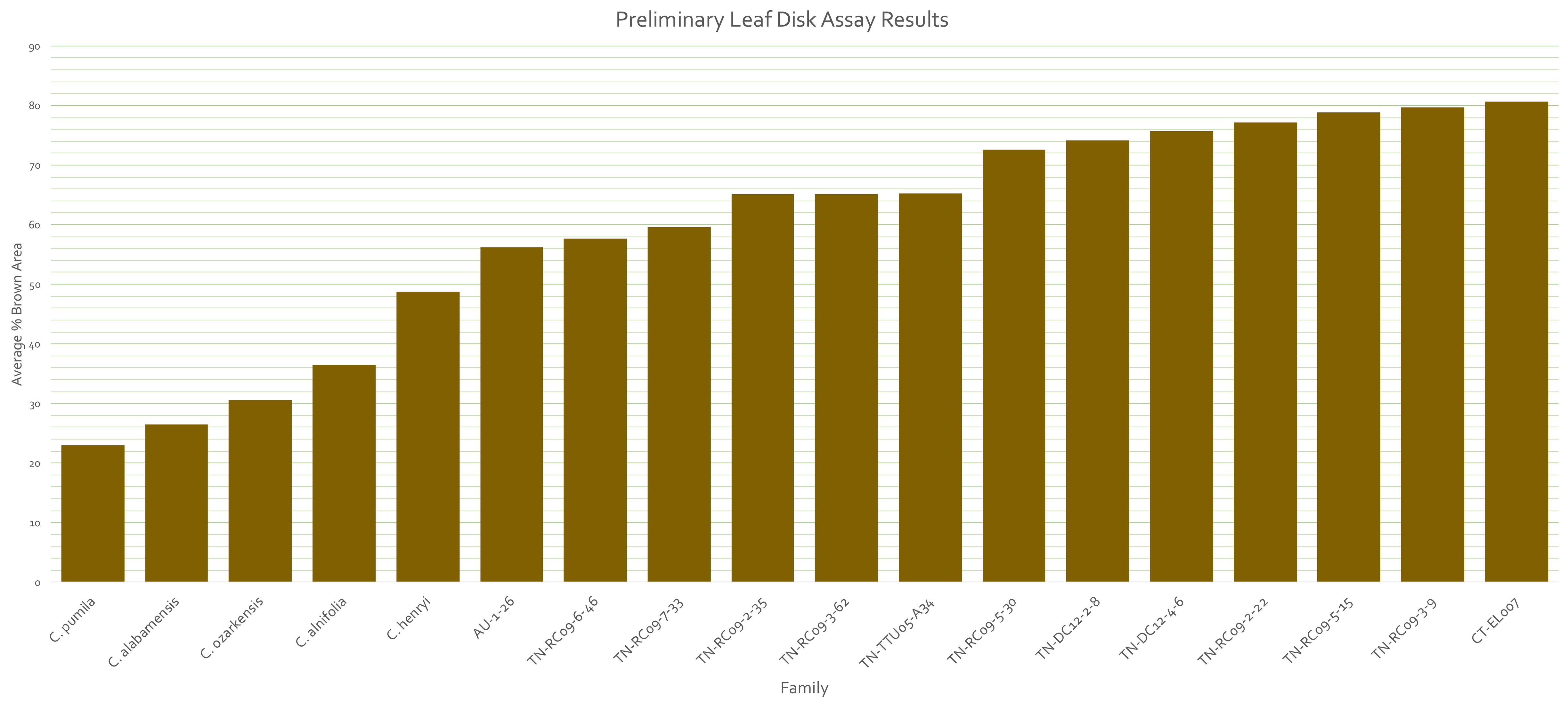

Figure 1: Histogram of average % of browning on leaf disks

Discussion

The B3 and B4's of the hybrid species appear to have similar tolerances to the American chestnut. However, the F1 is still more similar in tolerance to the Chinese. It appears that these B3 and B4 trees might look more like C. dentata, but their tolerance to blight is lessened because of it. If they cannot tolerate the oxalic solution, it is possible that they may suffer in blight resistance terms. I am surprised about the chinquapin results, and will continue to see if C. pumila still has higher oxalic acid tolerance than Chinese.

Future Directions

- Assuming there is a correlation between OA tolerance and SSA, the OA leaf soak might be a fantastic and short-term indicator for relative blight tolerance for future growing seasons. This could be replicated in various TACF chapters, allowing for ample data to go with SSA's.
- As I continue looking at relative OA tolerance, I want to look at as many trees as possible within the SSA.
- When my fellow classmate finishes her statistical analysis of the SSA, we will compare data and see if there will be a correlation.
- Final results will be published by May 2023.

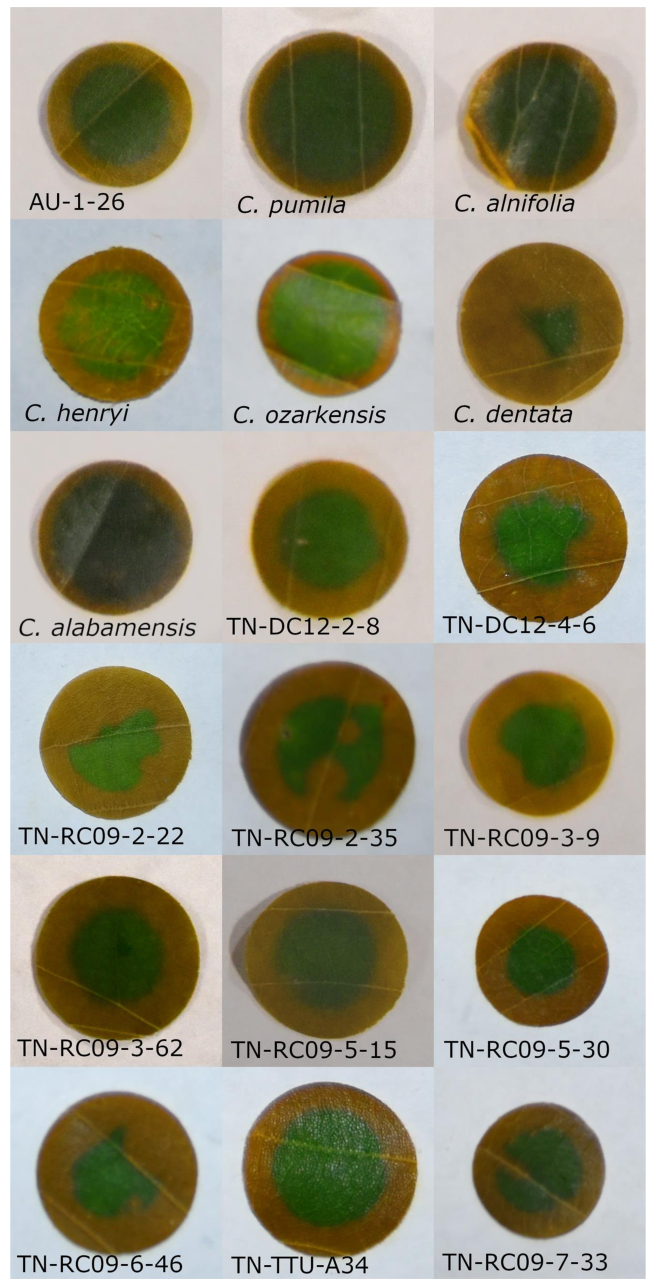

Figure 3: Photographs of individual 1.5 cm diameter disks after oxalic acid solution soak.

Contact Information

Kaitlyn Harden
The University of Tennessee at Chattanooga
Email: cqc815@mocs.utc.edu
Phone: +1(423) 618-4154

References

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