

Screening full-sib and half-sib families of chestnut seedlings for resistance to *Cryphonectria parasitica* using a small stem assay and to oxalic acid using a leaf-disc assay

Uma Plambeck and J. Hill Craddock

Department of Biology, Geology, and Environmental Science

Research Topic

We expect to find significant differences between the resistance of *C. dentata*, *C. dentata* hybrids, and other resistant *Castanea* species using the small stem assay and leaf disk assay.

Introduction

Cryphonectria parasitica is the ascomycete fungus species that causes chestnut blight disease. Symptoms of chestnut blight include bark cankers and eventually the death of American chestnut, *Castanea dentata*. But not all *Castanea* species are equally affected by the disease, and some Asian species appear highly resistant. We investigated the effect of *C. parasitica*, on three North American species, one European species, four east Asian species of *Castanea*, and nine full-sib families of TACF backcross hybrids (Table 1). Our study used both a small stem assay to measure canker lengths and an excised leaf disk assay to measure variation in tolerance to oxalic acid between the different *Castanea* species and hybrids.



Figure 1. Chestnut blight canker on cut stem

Family

C. alabamensis
C. crenata
C. dentata
C. henryi
C. henryi DC-13-3-13
C. mollissima AU
C. mollissima GAFL1
C. ozarkensis
C. sativa
C. seguinii
CCCG-61 x GABE001-165
CCCG-61 x GABE001-297
CCCG-78 x GABE001-165
CCSP-1-79 x CCSP-3-50
CCSP-1-79 x CCSP-B2-3-7
CCSP-13-140 x CCSP-7-134
CCSP-13-140 x TN-RC09-3-9
TN-RC09-2-22 x GAHR001-D39
TN-RC09-6-46 x GABE001-297

Provenance

Alabama
Japan
North America
China
China
China
China
Missouri
Europe
China
TACF
TACF
TACF
TACF
TACF
TACF
TACF
TACF
TACF

Table 1. List of species, provenance, and TACF Backcross hybrid families.

Methods

The experiment was conducted throughout the summer and early fall at the Fortwood Street Greenhouse and Nursery at the University of Tennessee of Chattanooga. We inoculated 967 container-grown seedlings with *C. parasitica* strain EP155 and allowed cankers to develop for 12 weeks (Figure 1). Two measurements were recorded for each canker: orange zone, and full length of necrosis, following the method of Cipollini et al. 2021 (Figure 2). The leaf disk assay utilized only two hundred of the trees in the nursery. Ten fifteen mm disks were cut from leaves. Excised leaf discs were soaked in a 50 mM solution of OA for 8 hours and then digitally imaged for measurement of browning with Image J, following the method of Hardin 2023 (Figure 3).



Figure 2. A digital caliper was used to measure canker lengths in millimeters.



Figure 3. OA causes browning of the leaf tissues from the cut margin of the disk.

Results

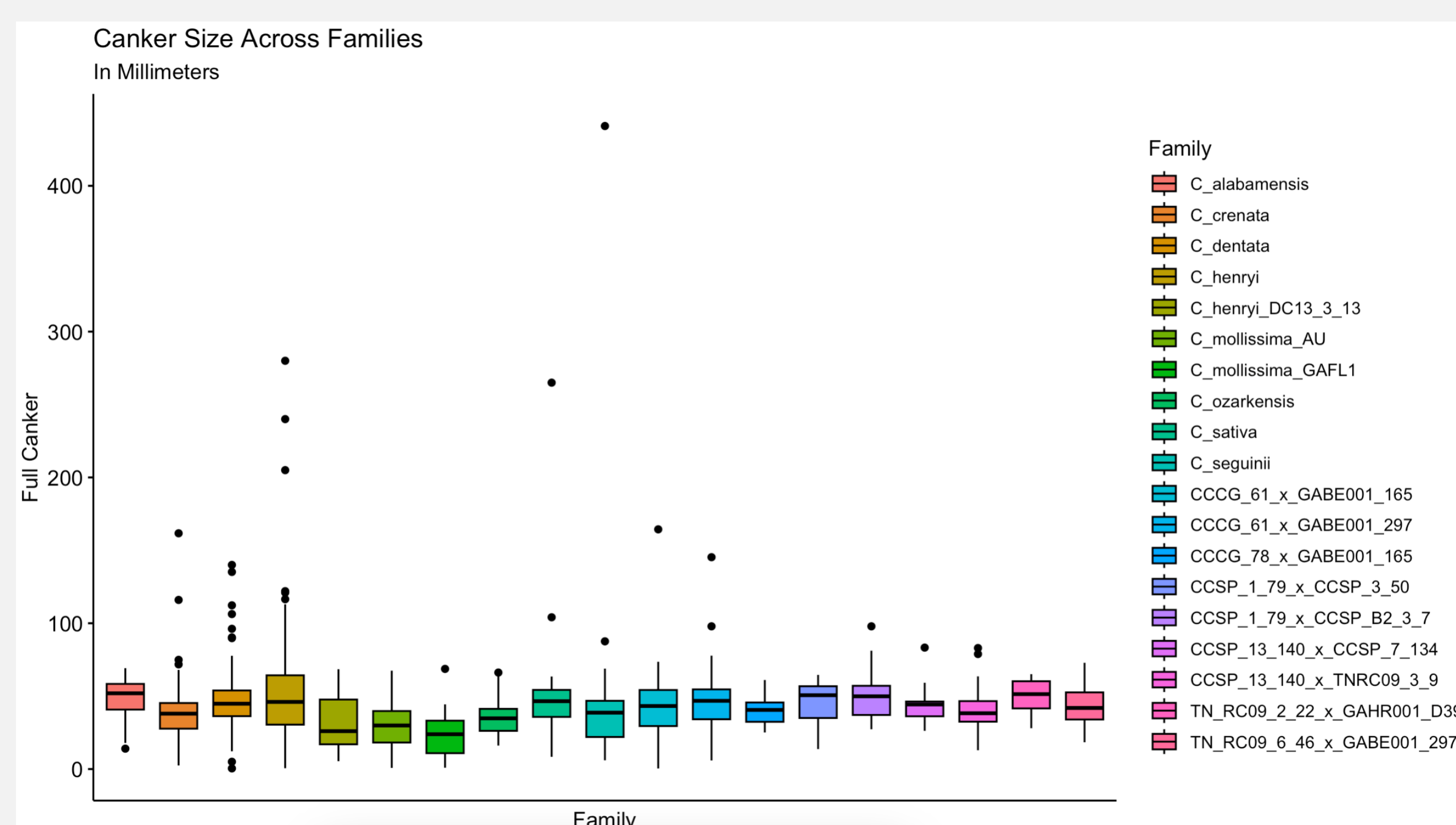


Figure 4. Variation by family in resistance to *C. parasitica*, as measured by canker lengths in the Small Stem Assay

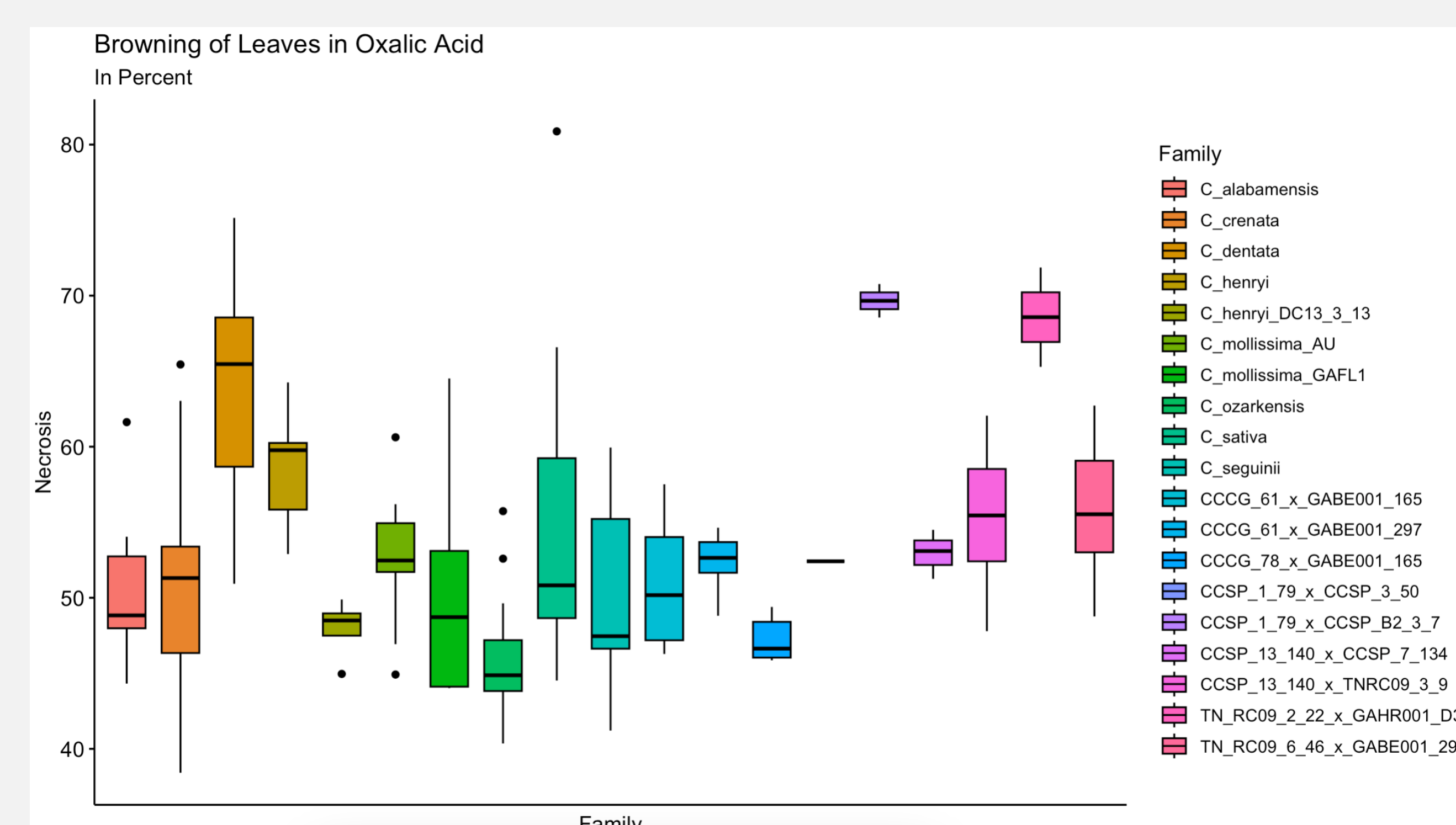


Figure 5. Variation by family in resistance to oxalic acid as measured by browning in excised leaf disks

Preliminary results show differences that vary by *Castanea* species in resistance to *C. parasitica*, as measured by canker lengths in the SSA (Figure 4), and differences to OA degradation (browning) as measured by the leaf disk assay (Figure 5). The TACF hybrids appear to be intermediate in their responses to *C. parasitica* and OA. (Figures 4 and 5). The data will be analyzed statistically using the program R.

Further Investigation

- Test the variation in OA response and *C. parasitica* response for statistical significance
- Run correlation between OA resistance and *C. parasitica* resistance
- Plant TACF hybrids in experimental orchards for long term studies
- Plant the *Castanea* species in demonstration plots throughout the eastern United States



References

- Cipollini, Moss, J. P., Walker, W., Bailey, N., Foster, C., Reece, H., & Jennings, C. (2021). Evaluation of an Alternative Small Stem Assay for Blight Resistance in American, Chinese, and Hybrid Chestnuts (*Castanea* spp.). *Plant Disease*, 105(3), 576–584. <https://doi.org/10.1094/PDIS-06-20-1272-RE>
- Harden, Kaitlyn, "Oxalic acid leaf disk soak assay is a new possibility in screening for blight resistance in *Castanea* species" (2023). *Honors Theses*. <https://scholar.utc.edu/honors-theses/406>

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