Morpho-physiological responses of butternut (*Juglans cinerea* L.) and naturally-occurring hybrids to drought and flood

Philip A. Crystal[1] and Douglass F. Jacobs[1]

[1]Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana, 47907

Abstract

Rates of natural butternut (*Juglans cinerea* L.) regeneration cannot maintain current populations and lack of information regarding species' site preference hampers artificial regeneration success. Hybrids with Japanese walnut (*J. ailantifolia* Carr.) have been proposed as a restoration method, but it is unclear if these have similar site preference. To simultaneously evaluate both taxa, we exposed black walnut (*J. nigra* L.), Japanese walnut, butternut, and hybrid butternut seedlings to control, drought, and flood conditions in a controlled environment. Flood assigned butternut had reduced photosynthetic assimilation (*A*), *Fv/Fm*, and total leaf area (LA) compared to controls. Japanese walnut *A* and LA were reduced under drought conditions, while hybrid *A* and LA reductions occurred under both stress conditions. Results indicate butternut is intolerant of flooded conditions, implying for successful establishment, avoidance of poorly-drained areas is critical. The hybrid response indicates a shift in moisture tolerance from genetic contributions of extremely dissimilar parental taxa.

Figure 1. (a) Agarose gel of seedling chloroplast RAPD genotype. Double bands indicate Japanese walnut maternity, single bands, butternut maternity. (b) Seedlings growing in Bay 26, Horticulture and Landscape Architecture's Plant Growth Facility, Purdue University. (c) Assessing chlorophyll fluorescence with a LI-6400.

Introduction

- Butternut has been virtually extirpated by butternut canker disease (*Ophiognomonia clavigignenti-juglandacearum*) (Woeste and Pijut, 2009).
- Natural regeneration is not sufficient to maintain the current population and will continue to decrease (Woeste *et al.*, 2009).
- Minimal knowledge of species' site preference, notably conflicting reports of moisture tolerance, impedes outplanting success (Rink, 1990; Ostry *et al.*, 2003).
- Black walnut, a well-studied congener, may share silvical and ecological requirements, allowing research to progress rapidly (Ostry *et al.*, 2003).
- Naturally-occurring, potentially more disease resistant, hybrids with Japanese walnut have been suggested as a potential species restoration method (Michler *et al.*, 2006). While morphologically similar, it is unclear if these individuals have similar site preference or can fulfill the ecological niche occupied by butternut
- **Is butternut tolerant of both drought and flood conditions?**
- **Do hybrids have similar moisture tolerances?**

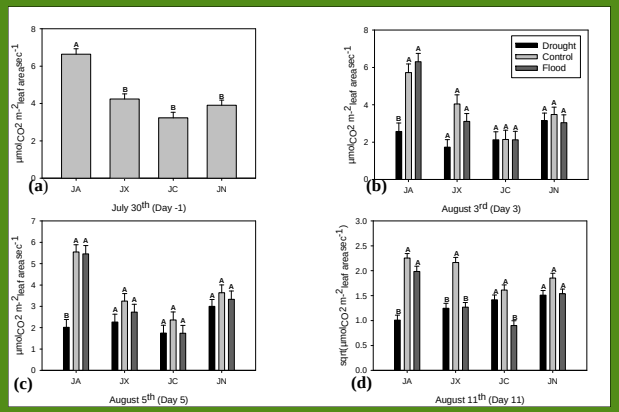

Figure 2. Photosynthetic rate variations between species and treatments. No significant treatment × species interaction was detected on Day -1 (a), but were detected on Day 3 (b), 5 (c), and 11 (d). Letters denote significant differences between treatments and controls within species at the α = 0.05 level. Bars represent standard error.

Materials and Methods

- Plant material obtained from two locations
  - IDNR Vallonia Nursery, Bloomington, Indiana: 1+0 black walnut (JN), butternut (JC), and hybrid seedlings (JX)
  - Grimo Nut Nursery, Ontario, Canada: 2+0 Japanese walnut (JA)
- Identity of morphologically similar JA, JC, and JX, confirmed with 1 chloroplast and 5 nuclear RAPD markers using root DNA (Figure 1a)
- From April 30th to May 5th, 2011 seedlings root-pruned and potted in 12.04L pots containing a 2:1:1 (v:v:v) mixture of sand, peat, and silt-loam soil and fertilizer.
- Plants were grown under controlled conditions (one seedling / 0.093m^2) in a completely randomized design across two adjacent benches (Figure 1b).
- Moisture stress treatments applied August 1st to August 17th, 2011.
- Photosynthetic assimilation (*A*) and chlorophyll fluorescence (*Fv/Fm*) assessed during the treatment period (Figure 1c).
- Stress morphology and leaf area (LA), assessed during destructive harvest
- Statistical analysis analyses were performed in Statistica version 10

Results

- Taxon response to moisture stress extremely variable
- Butternut *A* , *Fv/Fm*, and LA reduced in response to flood stress compared to control conditions (Figures 2d, 3, and 4b)
- Japanese walnut *A* and LA reduced in response to drought stress compared to control conditions (Figures 2 and 3b)
- Hybrid *A* and LA reduced under both stress conditions (Figures 2 and 3)
- No significant differences detected between stressed and control black walnut seedlings (Figures 2, 3, and 4)

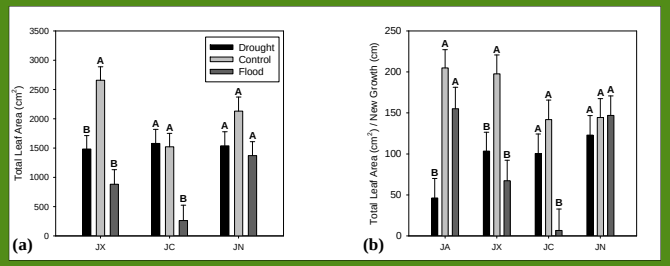

Figure 3. Leaf area variances between treatments within species. Total leaf area. Significantly taller Japanese walnut was excluded (a). Leaf area correlated with seedling new growth (b). Letters denote significant differences between treatments and controls within species at the α = 0.05 level. Bars represent standard error.

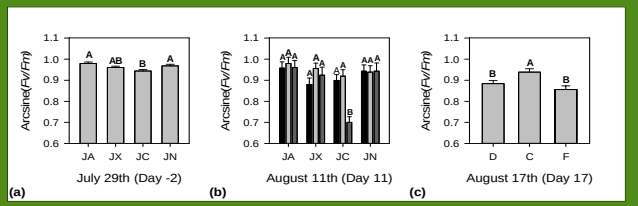

Figure 4. Significant F_v/F_m interactions. Differences between taxa detected prior to stress initiation (a). Flood assigned butternut reduced on Day 11 (b). Treatment effects detected on Day 17 (c). The species effect was the same as (a) on this date. D, C, and F represent drought, control, and flood treatments, respectively. Letters denote significant differences between treatments and controls within species at the α = 0.05 level. Bars represent standard error.

Conclusions

- Butternut families tested are extremely intolerant of water-logged conditions, implying for successful establishment avoidance of poorly-drained conditions is critical.
- Careful selection for butternut character in hybrids is essential to avoid a shift in moisture requirements from genetic contributions of flood tolerant Japanese walnut.
- Reduced hybrid *A* and LA under both stress conditions was likely due to pooling half-sib families introgressed towards species extremely dissimilar in moisture tolerance.
- Research is currently underway to assess root to shoot ratios and water use efficiency of all taxa grown from seed, which will preserve root morphology.

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For additional information contact: **pcrystal@purdue.edu**