Leucaena Hybrids & Sterility: the University of Hawaii Collection

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Outline

• Interspecific crosses: natural & experimental crosses & compatibility
• Variable sterility of crosses
• Sterile hybrids of interest
• Vegetative propagation
• Future needs & directions
Interspecific Crosses: Natural Hybrids

- Geographic isolation has resulted in few natural hybrids
- *L. esculenta* (2n = 52) x *leucocephala* (2n = 104), completely sterile
  - Recreated at UH as variety 'KX4'
- *L. diversifolia* (2n = 104) x *L. leucocephala*, highly fertile
  - Recreated at UH as variety 'KX3'
Interspecific Crosses: Experimental Hybrids

- Sorrensson crossed 17 species in 232 of 240 possible combinations
- 141 produced viable seeds
- All tetraploid crosses fertile
- $3n$ hybrids often had aborted seeds
- 1-way incompatibility in 1/3 of crosses

Fig. 2.3. Sorrensson’s hybridization chart summarizing his experience with 15 species of *Leucaena*, connecting bars indicating whether crosses were fertile both ways, one-way only, or with only abortive or no seeds. Abbreviations include TRI (*L. trichodes*) and TRIC (*L. trichandra*). Adapted from Sorrensson 1993.
Variable Sterility of Crosses

• Seed production from crosses ranges from fully fertile to fully sterile
• Many $3n$ crosses have proportion of aborted seeds or truly sterility, e.g. 'KX4'
• Others are fully fertile, e.g. 'KX3'
• Some tetraploid crosses are self-sterile, e.g. $L. pallida$ ($2n = 104$) $\times L. leucocephala$, 'KX2'
• Fertile hybrids may be good choices for future breeding
Sterile Hybrids of Interest

**KX2** *L. pallida (2n = 104) x L. leucocephala, 'K376' x 'K8'*
Sterile Hybrids of Interest

**KX2**  *L. pallida* (2n = 104) x *L. leucocephala*, 'K376' x 'K8' (Brewbaker 2008)

- Has been recreated with different varieties, e.g. cv. K636 as *L. leucocephala* parent
- *L. pallida* confers cool tolerance
- Self-incompatible (S-allele type), usu. low seed yield in seed orchards
- Good psyllid resistance & forage quality
- Forage yield at 900 m elev. in Hawaii: 2-3 tons ha\(^{-1}\) y\(^{-1}\)
Sterile Hybrids of Interest

**KX2** *L. pallida (2n = 104) x L. leucocephala*, self-sterile

<table>
<thead>
<tr>
<th>Trait</th>
<th><em>L. pallida</em></th>
<th><em>L. leucocephala</em></th>
<th>KX2-Hawaii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree height (m)</td>
<td>7</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Leaf length (cm)</td>
<td>26</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Pairs of pinnae per leaf</td>
<td>16</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Pairs of leaflets per pinna</td>
<td>45</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Pod length (cm)</td>
<td>20</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>1000 seed wt. (g)</td>
<td>30</td>
<td>63</td>
<td>42</td>
</tr>
<tr>
<td>Head diam. (mm)</td>
<td>20</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Anther color</td>
<td>Pink</td>
<td>White</td>
<td>Pale pink</td>
</tr>
</tbody>
</table>

(Brewbaker, 2008)
Sterile Hybrids of Interest

**KX2** *L. pallida* (2n = 104) x *L. leucocephala*, 'K376' x 'K8'

- Vigorous coppicing: 10 tons ha\(^{-1}\) y\(^{-1}\) wood yield (Youkhana and Idol, 2011a)
- Excellent source of high-quality mulch in agroforestry systems (Youkhana and Idol, 2009)
- Improves coffee growth and soil C and N as compared to inorganic fertilization (Youkhana and Idol, 2011b, 2016)
Sterile Hybrids of Interest

**KX4** *L. leucocephala, 'K636' x L. esculenta (2n = 52), 'K838' (Brewbaker 2013)

Fig. 1. Trees of ‘KX4-Hawaii’ at Waimanalo, HI; (A) mature 10-year-old tree; (B) coppice regrowth at age 2.5 years; (C) narrowly spaced shade planting around Quonset hut at age 2.5 years.
Sterile Hybrids of Interest

**KX4** *L. leucocephala, 'K636' x L. esculenta (2n = 52), 'K838'
(Brewbaker 2013)
**Sterile Hybrids of Interest**

**KX4** *L. leucocephala*, 'K636' x *L. esculenta* (2n = 52), 'K838' (Brewbaker 2013)

- Fully sterile 3n hybrid; flowers year-round
- Generally outperforms best *L. leucocephala* cultivars; mature height = 15 m in 4-5 yr
- Psyllid-resistant
- Vigorous coppicing; wide crown spacing
- Lower forage quality than KX2 or K636
Sterile Hybrids of Interest

KX5  *L. diversifolia* (2n = 104), x *L. pulverulenta* or *trichandra* (2n = 52)

- Fully sterile 3n hybrid
- Cool-tolerant & psyllid-resistant
- Vigorous coppicing
- Poor rooting ability of cuttings
Sterile Hybrids of Interest

**KX5**  *L. diversifolia* (2n = 104), x *L. pulverulenta* or *trichandra* (2n = 52)
## Sterile Hybrids of Interest

### Other Hybrids of Interest

<table>
<thead>
<tr>
<th>Female Par</th>
<th>Male Par</th>
<th>2n</th>
<th>Fertility?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Div4</td>
<td>Leuc K500</td>
<td>104</td>
<td>High</td>
</tr>
<tr>
<td>Leuc K8</td>
<td>Trich K738</td>
<td>78</td>
<td>Low</td>
</tr>
<tr>
<td>Macr K158</td>
<td>LancS K393</td>
<td>52</td>
<td>??</td>
</tr>
<tr>
<td>Div4 K156</td>
<td>Pall K376</td>
<td>104</td>
<td>High</td>
</tr>
<tr>
<td>Div2 K11</td>
<td>Leuc K8</td>
<td>78</td>
<td>v. low</td>
</tr>
<tr>
<td>Pulv K19</td>
<td>Leuc K8</td>
<td>78</td>
<td>??</td>
</tr>
<tr>
<td>Coll K185</td>
<td>Lanc K264</td>
<td>54</td>
<td>High?</td>
</tr>
<tr>
<td>Lanc K10</td>
<td>LancS K393</td>
<td>52</td>
<td>??</td>
</tr>
<tr>
<td>Div4 K156</td>
<td>LancS K393</td>
<td>78</td>
<td>??</td>
</tr>
</tbody>
</table>

“Div2” = Trichandra
Expected high sterility for n = 78

- All trees in a single orchard at UH Waimanalo station
- Recently coppiced to produce new shoots for veg prop
- Cool winter weather resulted in psyllid outbreak
- All hybrids of interest affected (incl. KX2 and KX4)
- Seasonal only; trees recover
Vegetative Propagation
Vegetative Propagation

**Cut Stakes**
Vegetative Propagation

**Cut Stakes**

- Low-tech, in-field or nursery, low success (~20% for KX4)
- 3-6 mo coppice regrowth
- 10-20 usable stems per stump max
- Shoots resprout in 7-10 days
- Root formation, if at all, in 4-6 weeks
- No effect of girdling and rooting hormone application as stimulating treatments
Vegetative Propagation

Air Layering
Vegetative Propagation

**Air Layering**
- Low-tech, in-field, high success
- 3-6 mo coppice regrowth
- 10-20 usable stems per stump max
- 4-6 weeks to generate roots
- Rapid growth after outplanting
- Do not perform well for long as nursery stock plants
Vegetative Propagation

Rooted Cuttings
Vegetative Propagation

Rooted Cuttings

- Rapid production, med-tech, controlled nursery environment, variable success
- 3-6 week coppice regrowth
- 30-80 usable shoots per stump
- 4-6 weeks to generate roots
- 6-8 week nursery phase after transplant (highly variable, based on root generation)
- Good stock plants for nursery production
Vegetative Propagation

Rooted Cuttings

<table>
<thead>
<tr>
<th>Variety</th>
<th>Rooting % Summer</th>
<th>Rooting% Winter</th>
<th># Roots Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>KX4</td>
<td>84</td>
<td>04</td>
<td>09</td>
</tr>
<tr>
<td>KX2</td>
<td>25</td>
<td>00</td>
<td>04</td>
</tr>
<tr>
<td>KX3</td>
<td>56</td>
<td>09</td>
<td>08</td>
</tr>
<tr>
<td>KX5 c1</td>
<td>72</td>
<td>07</td>
<td>09</td>
</tr>
<tr>
<td>KX5 c2</td>
<td>93</td>
<td>38</td>
<td>15</td>
</tr>
<tr>
<td>KX5 c3</td>
<td>34</td>
<td>00</td>
<td>07</td>
</tr>
</tbody>
</table>

Shi and Brewbaker 2006
Vegetative Propagation

**Grafted Cuttings** (top-wedge or saddle graft, 5-mm dia)

KX4 scion

K636 rootstock

10 days covered
Vegetative Propagation

**Grafted Cuttings** (top-wedge or saddle graft, 5-mm dia)

- Medium production, medium-tech, nursery environment, good success with KX4
- 4-8 week coppice regrowth (scion)
- 20-40 usable shoots per stump
- 8-10 week seedling growth (root stock)
- 7-10 days for new shoot generation
- 6-8 week nursery phase after transplant (more uniform growth than cuttings)
Vegetative Propagation

**Micropropagation** (tissue culture)

- Demonstrated for immature zygotic embryos (Pal et al. 2012) and seedling cotyledons (Saafi and Borthakur 2002) of *L. leucocephala*
- High-tech, sterile lab environment, controlled nursery environment, high success
- Hundreds of seeds/tree; seasonalality?
- 6-8 weeks from explant to transplant stage; 5-8 shoots/explant (zygotic embryos)
Vegetative Propagation

Micropropagation (tissue culture)

- Explants (zygotic embryos)
- Shoot initiation (2-3 d)
- Multiple-shoot initiation (3 wk)
- Shoot elongation after 6 wk
- Hardening and acclimatization: 2 more wks

(Pal et al. 2012)
Vegetative Propagation

**Micropropagation** (tissue culture)

Hardening and acclimatization: 2 wk

(Pal et al. 2012)
Future Needs & Directions

1. Standard evaluation of interesting hybrids for utilization and vegetative propagation
2. Refinement of vegetative propagation methods, especially rooted cuttings
3. Micropropagation protocols for vegetative explants of sterile hybrids
4. Optimal nursery conditions for generating vigorous rooted plants and stock plants
Acknowledgments

- Dr. Adel Youkhana, Paul Santiago, Erin Hickey, Kalani Matsumura, and other students researchers for working with me on leucaena the past 10 yrs
- Roger Corrales and the Waimanalo station crew for field support
- 50+ of students and collaborators working with Dr. Brewbaker on leucaena
- Dr. Dulal Borthakur and his team for their work on micropropagation
Bibliography