POLLINATING AND EMASCULATING TECHNIQUES FOR LEUCAENA SPECIES

Theses of Pan (1985) and Sorensson (1987) described emasculation and pollination methods that have been refined in the past five years (over 30,000 pollinations). This paper describes these procedures and emphasizes appropriate changes for species other than *Leucaena leucocephala*.

Self-fertile Species and Hybrids Needing Emasculation: There are two recognized self-fertile species, *L. leucocephala* (4x = 104) and *L. diversifolia* (4x = 104). These species, and their self-fertile hybrid KX3, are trees we commonly emasculate for use as females in crosses. The tentative species "*L. cuspidata*" (K745, probable 4x = 112) was recently proven to be self-fertile in Hawaii (Sorensson, unpublished) and one tree of *L. retusa* K280 (2x = 56) was also found to be weakly self-fertile (Sorensson, 1987). All other species were self-sterile.

In addition to emasculating self-fertile trees, we may perform late morning emasculations on inflorescences of other species whose styles do not extend past the anther halo, which interferes with pollination. The species whose stylar length is comparable to anther filament length are *L. collinsii* and *L. lanceolata*, and some *L. shannonii*. Styles of some *L. lanceolata* continue lengthening after the morning of anthesis, so late pollinations are easier to make as long as the pollen is still usable.

Emasculating/Pollinating Equipment (carry these in waist aprons).

1. Hand-held stapler. We use Ace Clipper model 702 (W. Germany).
2. A fingernail clipper which has a nail file with a triangular, rather than a curved, tip.
3. A squirt bottle containing 75% ethanol.
4. Small heavy-duty scissors.
5. Small (8 x 11cm) ziploc-style polyethylene bags.
6. Wax-paper pollination bags: We use 5 x 16cm corn shoot bags (No. 217 Lawson, 480 Central, Northfield, Ill.) that are cut with pinking shears to 5 x 12cm for most species (5 x 15cm for species like *L. greggii* with long peduncles). We also cut 3.5cm vertically into the bag with pinking shears, starting halfway between the edges of the unopened bag.
7. A miner's head lamp.
8. Sharp-pointed dissecting tweezers. We use No.4 BB Swiss, from Hamilton Bell Company (30 Craig Rd., Montvale, NJ 07645).
9. Colored 2.5 cm wide polyethylene tape.
10. A permanent black marker pen.

Emasculation Method: Floral selections and emasculations are best if made between 1-3 hours before dawn, when inflorescences have opened but have not yet shed pollen. Choose only "healthy" inflorescences to emasculate (see discussion in Hutton and Gray, 1959) with an abundance of thick turgid styles and having minimal damage by moth caterpillars (e.g., *Ithome lassula* in Australia [Beattie, 1981] and a probable *Ithome* species in Hawaii [personal communication with Hawaii State Entomologist Bernard Kanashiro, 1988]). Petroleum distillates are lethal to leaf and floral tissue, and their presence in most insecticide sprays precludes the use of such sprays.
The subtending leaf is cut off just above the leaf gland with scissors. The petiole provides resistance to the wind revolving the bag windmill-fashion around the branch, and cutting the petiole just above the petiolar nectary gland delays the dehiscence of the petiole. If inflorescences or pods are present at the same node, these are removed by hand or cut off with scissors just below the capitulum.

The branch and peduncle are held lightly with one hand and all the anthers on an inflorescence are removed with tweezers, leaving the best 10-15 styles. Filaments are cut off just above the sepals to help distinguish them from styles. Anthers that have not lengthened normally are also dug out and removed. We prefer to excise the styles and anthers from unutilized florets over removing the whole floret as this minimizes floral damage; also the florets provide structural support to the florets that will be pollinated. Florets damaged by *Ithome* are completely removed, along with the caterpillars. Florets damaged by caterpillars usually do not open and wilt quickly.

The wax paper pollination bag is carefully opened and placed over the inflorescence and leaf petiole. The bag is adjusted so the subtending branch runs through the vertical 3.5 cm cut in the bag. The edges of the bag are tightly folded in at 45 degree angles around the branch and the bag is stapled shut to keep small insects from entering. By not folding the bag along its old creases, we prevent the bag from closing and possibly damaging the emasculated inflorescence. Pollination bags are cut with pinking shears to produce a jagged edge, which helps keep the bag properly attached. The bag is marked ("E" for emasculated) to indicate successful emasculations.

Pollination Method: Inflorescences may be bagged the day before anthesis for inflorescences with sepals that have changed from green to a greenish yellow. This is essential if rain is expected during the night or early morning, or if *L. retusa* or *L. greggii* are to be used as females because their florets can open asynchronously throughout a 24-hour period.

Predawn bagging is efficient as we can bag just the best inflorescences for use as females. This is especially important in species which frequently produce female-sterile inflorescences (FSI). FSI have apparently normal anthers, but abortive styles. FSI occur most frequently in young trees, or in species with over 200 florets per inflorescence (Table 1). A representative "female-fertile" and "female-sterile" flower of *L. lanceolata* K393 was dissected in July 1988. The percentage of florets per inflorescence with healthy styles was 37 and 0 percent, respectively (the inflorescences had 378 and 386 florets per inflorescence, respectively).

Pollination bags are cut off of inflorescences to be used as pollen sources by cutting through the paper between the staple and the branch. The inflorescences are slowly pried off and then twirled quickly back and forth between the thumb and index finger inside an open ziploc bag. Allowing the anthers to lightly contact the walls of the bag helps to dislodge pollen. The ziploc bag is marked with the tree number and other information.

Pollen is collected from most species within a 60-90 minute period, usually 3.5-4.5 hours after dawn. Pollen of diploid *L. diversifolia*, *L. macrophylla*, and *L. trichodes* is shed quickly, often within a 30 minute period, so pollen collection is timed accordingly. *L. diversifolia* sheds pollen 30-60 minutes earlier than most other species.

To transfer pollen the nail file blade of the fingernail clipper is sterilized in alcohol and dried with a tissue. The tip of the reverse side of the nail file (which is a smooth side) is then scraped through the pollen and the pollen is pressed into a very thin smear. The inflorescence is lightly held and the blade is moved across a stigma at a 45 degree angle to force the pollen into the stigmatic pore. When the pollen is smeared properly, this leaves tiny clean furrows across the pollen smear.
Table 1. Florets per inflorescence, relative frequency of female-sterile inflorescences (FSI), and relative heaviness of pollen production of selected leucaena accessions.

<table>
<thead>
<tr>
<th>Species</th>
<th>K Number</th>
<th>Florets/Head</th>
<th>Frequency of FSI</th>
<th>Relative Pollen Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. collinsii</td>
<td>K740</td>
<td>52.4 ± 8.4</td>
<td>none</td>
<td>med</td>
</tr>
<tr>
<td>L. collinsii</td>
<td>K185</td>
<td>218.2 ± 12.6</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>L. diversifolia</td>
<td>K409</td>
<td>91.4 ± 13.3</td>
<td>none</td>
<td>low</td>
</tr>
<tr>
<td>L. diversifolia</td>
<td>K483</td>
<td>138.0 ± 11.5</td>
<td>low</td>
<td>med</td>
</tr>
<tr>
<td>L. diversifolia</td>
<td>K156</td>
<td>78.2 ± 5.3</td>
<td>low</td>
<td>med</td>
</tr>
<tr>
<td>L. esculenta</td>
<td>K138</td>
<td>107.0 ± 9.5</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>L. greggii</td>
<td>K859</td>
<td>175.0 ± 15.6</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>L. lanceolata</td>
<td>K10</td>
<td>222.8 ± 3.7</td>
<td>med</td>
<td>high</td>
</tr>
<tr>
<td>L. lanceolata</td>
<td>K385</td>
<td>484.0 ± 5.7</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>L. macrophylla</td>
<td>K158</td>
<td>229.6 ± 9.7</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>L. pallida</td>
<td>K748</td>
<td>133.8 ± 6.7</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>L. pulverulenta</td>
<td>K340</td>
<td>42.4 ± 7.3</td>
<td>none</td>
<td>med</td>
</tr>
<tr>
<td>L. retusa</td>
<td>K280</td>
<td>195.8 ± 19.0</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>L. salvadorensis</td>
<td>K746</td>
<td>123.6 ± 12.9</td>
<td>med</td>
<td>high</td>
</tr>
<tr>
<td>L. shannoni</td>
<td>K465</td>
<td>171.6 ± 12.7</td>
<td>med</td>
<td>high</td>
</tr>
<tr>
<td>L. trichodes</td>
<td>K738</td>
<td>117.0 ± 4.3</td>
<td>med</td>
<td>low</td>
</tr>
</tbody>
</table>

* Derived from counts of 5-10 inflorescences in July 1987.

After pollination the following information is written on the bag: 1) Date and number of the cross on that date, 2) Trees used as parents, 3) Whether emasculation was involved, 4) # of styles pollinated, 5) Whether the cross was accomplished perfectly (high quality), accomplished with minor difficulties (medium quality), or is for observational purposes only (low quality).

Checking Crosses for Pod Set: Bags are checked for successful pod set in 3-4 days by which time most species will have begun producing tiny pods. Unsuccessful crosses will usually have dehisced by this time; if not, the cross should be treated as a successful cross until it is known the cross has failed. The bags are carefully removed to prevent any part of the bag from catching on and breaking off the inflorescence. Leaving the bag on the inflorescence longer than four days has sometimes caused severe damage from psyllids, Heteropsylla cubana, to the developing pods.

If the cross has failed the date and "failed" is written on the pollination bag, and the bag kept for recording the data on the computer. If the cross is successful, a 20 cm colored polyethylene ribbon is tied to the branch-tip side of the node and cross, cross date and other information is written on the ribbon. Again, the observation date and number of developing pods is written on the pollination bag and saved for recording the data on the computer.

Miscellaneous Notes

1. We believe there is a positive correlation between how early pollen is shed and increases in night/morning temperature. Hot nights speed up pollen shed significantly and vice-versa. L. leucocephala has shed pollen as early as 4:30 AM following an unusually hot night, and L. pallida has shed pollen as late as 1 p.m. (dawn at 6 a.m.) after an unusually cool morning.

2. Some L. shannoni are difficult to use as females because they only carry 1-2 pods per inflorescence, on the average, to maturity, regardless how many pods were originally forming. Also, since L. shannoni (and L. macrophylla) generally produce
inflorescences at nodes lacking leaves, the petioles can not be used to stabilize the bag against wind action.

3. Pollen explode if they come in contact with water. Neither wet pollen nor wet inflorescences should be used.

4. Pollen should normally be used within 30 minutes after collection. Leucaena pollen must be desiccated and kept cool if it not used the day of collection. Our experiments in storing pollen storage showed L. leucocephala pollen maintained viability for 2-3 weeks at room temperature under desiccation and vacuum.

5. Once pollen lose its stickiness (the rate is species dependent) it does not attach well to stigmas. We have used glycerin successfully, but a sterile 15-20% sugar-water solution of 15-20% would probably be best. Dab the solution to the stigmas and then touch the pollen to it.

6. L. macrophylla pollen is shed as clumps. These aggregations of pollen do not usually spread easily, but otherwise it can be pollinated in a the same manner as other species.

Comparison to Other Emasculation Methods: We have not yet tested extensively the soap-emasculation technique of Hutton and Gray (1959), and the bud emasculation technique of Gonzalez (1966 unpublished) and Gupta and Patil (1984). It appears that our method is superior in effectiveness and repeatability, however. Gonzalez unsuccessfully tried Hutton’s soap technique in 1965, and we have not retried the method, although Dr. Hutton recently maintained the technique was valid and useful (personal communication, 1986). Inflorescences must be dipped in soap at just the right time, and bad timing may have prevented Gonzalez from being successful.

The early emasculation technique of Gonzalez (1966, unpublished) and Gupta and Patil (1984) was tried in 1984 with poor success. This technique involves prematurely opening florets and teasing out and removing all anthers. Even when only the anthers of 10-15 florets (100-150 anthers) were removed and the remaining florets were totally removed, this method was slow and delicate, and resulted in unacceptably high levels of missed anthers and damaged styles. The technique is difficult to use on L. diversifolia because it has smaller florets than L. leucocephala. Gonzalez’s emasculations using this method resulted in high (about 40%) contamination from selfs.

References:


