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# Clonal propagation of *Leucaena* by single bud splice grafting with a new grafting tool, and by modified veneer crown grafting

ERIC B. BRENNAN* and KENNETH W. MUDGE
*Department of Floriculture and Ornamental Horticulture, 20 Plant Science Building, Cornell University, Ithaca, NY, USA (*Present address: Department of Pomology, University of California, Davis, CA 95616, USA)*

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**Application.** A new tool called a *graft guide* was developed to perform single bud splice grafting for clonal propagation of the tropical woody leguminous genus *Leucaena*. The technique is useful for grafting small diameter shoots and for maximizing available scion wood. Using single bud splice grafting and a modification of veneer crown grafting, a high degree of intraspecific graft compatibility within the genus *Leucaena* was demonstrated. Single bud splice grafting should be useful for cloning self incompatible parental lines of *Leucaena* to be used in orchard production of interspecific hybrid seed and for cloning selected seedless interspecific hybrids.

**Abstract.** A simple tool called the graft guide, and its use with a new grafting technique called the single-bud splice (SBS) graft is described. The graft guide assures precise complementary rootstock and scion cuts, and thus ensures good cambial contact necessary for graft union formation. The use of this tool to graft small (3–15 mm) vegetative shoots of *Leucaena* species and hybrids is illustrated. An overall grafting success rate of 72% was achieved using this technique for several leucaenas. An additional modified veneer grafting technique for use when rootstock is larger in diameter than the scion, achieved 73% success. Graft compatibility results from 961 grafts between scions of 15 *Leucaena* species and three interspecific hybrids, and rootstocks of two species are presented. Of the 48 scion/rootstock combinations attempted, 43 (90%) were successful.

## Introduction

Grafting small vegetative shoots can be difficult for both experienced and novice grafters. It is especially challenging to make straight, accurate cuts and ensure that the scion and rootstock are in adequate contact to form a successful union. This paper describes a unique and simple tool called the *graft guide*,[1] that allows the grafter to make precise complementary cuts on

[1] Graft guides may be purchased from the non-profit Petchaboon Scholarship Fund of Thailand. Contact E.B. Brennan for more information.

rootstocks and scions with stem diameters as small as 3 mm. The graft guide is used to perform a single bud splice (SBS) graft which ensures continuous scion/rootstock contact, thus increasing the likelihood of successfully grafting small-diameter shoots.

Why graft (or otherwise clonally propagate) leucaenas, since most species in the genus are easily grown from seed? There are two major reasons. Firstly, it allows for clonal selection and multiplication of elite seedless triploid (3n) hybrids for use in plantation and agroforestry systems. Such seedless hybrids are attractive because they are not prone to weediness which is a serious problem with some other leucaenas (Hughes and Styles 1987, Brennan 1990), and because several of these 3n hybrids exhibit rapid growth (Sorensson et al. 1994) and psyllid resistance (Wheeler and Brewbaker 1990). Brewbaker and coworkers at the University of Hawaii have reported vegetative propagation of one triploid seedless hybrid from cuttings, but indicate that cuttings rooted from horizontal lateral branches remain strongly plagiotropic (grow horizontally) (Shelton and Pottinger 1995). Secondly, vegetative propagation can be used to multiply a single clone of a self-incompatible (SI) species (e.g., tetraploid *L. pallida* Britton & Rose, and all diploid species) for use in the large-scale production of interspecific F1 hybrid seed (Bray and Fulloon 1987). Individuals of a single clone of a SI species would theoretically set only interspecific hybrid seed when interplanted with another species. Such interspecific seed orchards would allow for the production of both F1 fertile tetraploid progeny (e.g., 4n *L. pallida* × 4n *L. leucocephala* (Lam.) de Wit) and sterile triploid interspecific hybrids (e.g., 2n *L. diversifolia* (Schlecht.) × 4n *L. leucocephala*).

Despite previous reports on tissue culture (Datta and Datta 1984, Goyal et al. 1985, Toruan-Mathius 1992), cuttage (Hu and Liu 1981, Bristow 1983), and grafting (Bray and Fulloon 1987, Brennan 1992, Brewbaker 1988, Versace 1982), the genus *Leucaena* is considered difficult to clone (Litzow and Shelton 1991). Of all these vegetative propagation methods, grafting is the simplest method in that it can be carried out with the least amount of equipment and materials, and most easily be adopted on small-scale farms.

The graft guide tool was developed and it used to perform the SBS graft in order to take advantage of the intraclonal self-incompatibility required for use in an experimenatl hybrid seed orchard system. It was necessary to produce over 1000 grafted plants from a limited number of scion donor plants using small seedling rootstocks as early as two months after germination. None of the previously reported grafting techniques for leucaena, including cleft inarching (Brennan 1992), budding (Brewbaker 1988), or whip and tongue grafting (Versace 1982), met all these criteria. Although splice grafting of small scions from mature ortets onto seedling rootstocks has previously been reported for *Pinus pinaster* (Tranvan and David 1985) and *Sequoia sempervirens* (Tranvan et al. 1991), they were micrografts performed in vitro for the purpose of scion rejuvenation, and hence would not be well suited for the Leucaena seed orchard production system for which the grafted plants produced in this study were intended. Niether would in vitro micrografting be suitable for use on small farms or nurseries which is another potential application of the method described here.

SBS grafting was used in this study for grafting small diameter material (<3 mm), whereas a modified veneer (MV) grafting method was used for larger material. Data are presented on the use of these techniques to determine interspecific graft compatibility between scions of 15 *Leucaena* species and three interspecific hybrids, on two different rootstock species.

## Materials and methods

### *The graft guide*

The graft guide, as shown in Figure 1, A and B, is constructed of plexiglass (or plywood). It includes a handle (10 × 3 cm) with a slotted block (1.8 × 3 cm) on one end that guides the grafter's razor blade while cutting. The slot allows the rootstock and scion to be cut at the same reproducible angle (56°) facilitating a snug fit. Use of the graft guide also avoids wavy or jagged diagonal cuts on the scion and stock. A new razor blade was used after each 10 grafts to faciltiate clean cuts and avoid crushing pressure.

### *Rootstock propagation*

Seeds for rootstock seedlings were scarified with boiling water and then soaked for 12 hours. The scarified seeds were either sown directly in the potting medium, or pre germinated on moist paper towels in the dark prior to sowing. All rootstocks were inoculated with the *Rhizobium* peat mixture TAL 1145 (Agroforester Tropical Seeds Company, Kona, HI) when sown. For *Rhizobium* inoculation moist pre germinated seeds were placed in a bag of inoculum and gently shaken until completely coated. The seeds were immediately sown in either 3.8 cm diameter × 14 cm deep Super Stubby Cells (Stuewe and Sons, Inc., Corvallis, OR), or 6 cm diameter × 25 cm long D40 Deepots (Stuewe and Sons, Inc.) filled with Metro-Mix 360 (Grace Sierra Horticultural Products Company, Milpitas, CA). Seedling rootstocks were grown in greenhouses under 400 watt, high pressure sodium lamps with a 13-hour photoperiod, and a day and night temperature of 23 to 27 °C and 21 to 23 °C, respectively. The three tetraploid rootstocks included giant *L. leucocephala* subsp. *glabrata* (University of Hawaii Accession No. K636), shrubby

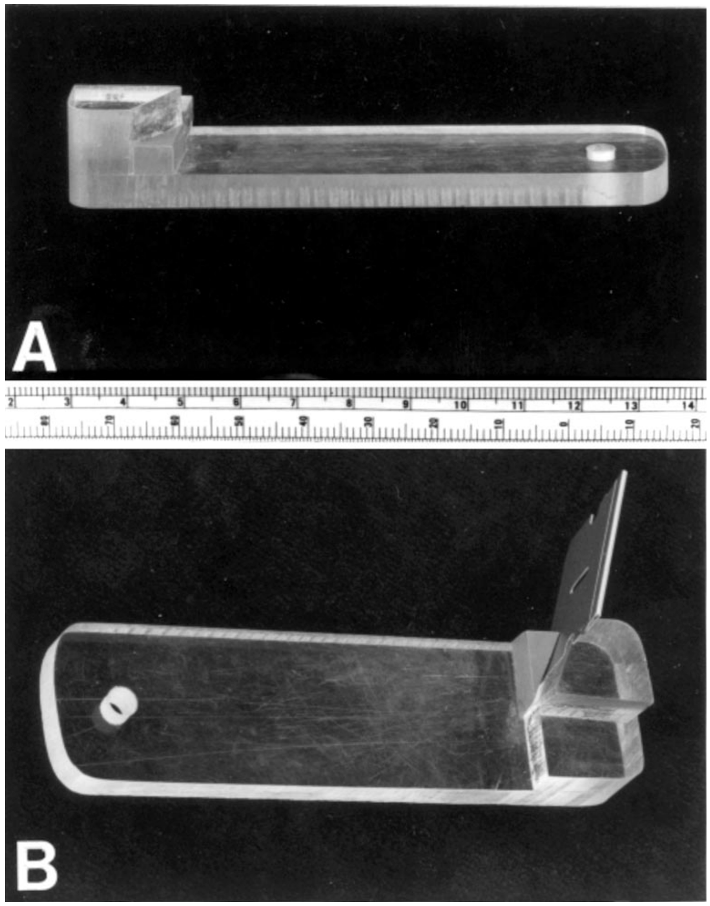

*Figure 1*. Graft guide, constructed of Plexiglas, for use in Single Bud Scion grafting. (*A*) Side view, (*B*) Top view.

*L. leucocephala* subsp. *leucocephala*, and *L. diversifolia* subsp. *diversifolia* (K156). These lines were evaluated as rootstocks since they are among the most widely available of any *Leucaena* species, and we were interested in their effect on scion growth (Brennan 1995).

*Scion propagation*

Scion material for the diploid (2n) *L. diversifolia* (K907), the triploid (3n) hybrids *L. leucocephala* × *L. esculenta* (Moc. & Sesse ex DC.) Benth. (K636 × K838) and *L. pulverulenta* (Schlecht.) × *L. diversifolia* (K877), and the 4n *L. leucocephala* (including giant types K420, K636, and the intermediate type K481) and *L. pallida* (K804) were obtained from reproductively mature branches of field grown trees at the University of Hawaii's Waimanalo Research Station. Scions from reproductively mature branches were grafted in Hawaii using either SBS or MV procedures described in this paper or cleft inarch grafting (Brennan 1992). These were transported to a greenhouse in Ithaca, NY where they served as scion donor plants for subsequent grafting. Scion donors included the 4n species *L. diversifolia* (K156) and *L. pallida* (K953), and the 2n species *L. collinsii* Britton & Rose (K912, K905), *L. greggii* S. Watson (K956), *L. lanceolata* S. Watson (K952), *L. macrophylla* Benth. (OFI 39/89), *L. multicapitula* Schery (K955), *L. pulverulenta* (K957), *L. retusa* Benth. (OFI 23/86), *L. shannonii* Smith (K954), *L. salvadorensis* Standley ex B. & R. (OFI 7/91), and *L. trichoides* (Jacq.) Benth. (OFI 61/88).

*Single-bud splice graft*

This technique was generally used with younger rootstock seedlings (3–6 months old), and when the scion and rootstock were about the same diameter; approx. 3–6 mm.

*Cutting the rootstock.* For a right-handed grafter, the graft guide was held in the left hand with the slotted block side facing the grafter. The rootstock was grasped and held firmly between slotted block and thumb of the left hand, about 3–5 cm above the soil line. The shoot of the rootstock was held squarely against the block, with the thumb holding the shoot near the top, as shown the Figure 2A. A single sided razor blade (held in the right hand) was inserted into the slot and firmly pushed against the shoot with a gentle front to back rocking motion to cut it cleanly at the preset angle (Figures 2B and 2C). A drop of clean water was placed on the cut surface to prevent desiccation. The end of an approximately 10 cm long Parafilm strip (Parafilm M Laboratory Film, American National Can, Neehan, WI), was loosely bent into a J shape and placed around the rootstock cut with the short end of the J facing towards the grafter (Figure 2D). With the thumbnail, the bottom of the short end of the Parafilm J was lightly pinched against the longer end so that it formed a cylindrical collar that was self supported and surrounded the angled cut. The cut surface was centered in the cylinder.

*Cutting the scion.* Scion shoots with a visible but dormant axillary bud and a fully expanded leaf attached at the node (Figure 3A) were chosen to approximate the diameter of the rootstock. Single node scion pieces were cut perpendicular to the main axis, about 5 mm above the chosen axillary bud. Similarly the petiole was severed about 1.5 cm from the bud. To make the diagonal cut, the scion piece was held between the thumb and slotted block as was done previously when cutting the rootstock ( Figure 2A), with the trimmed

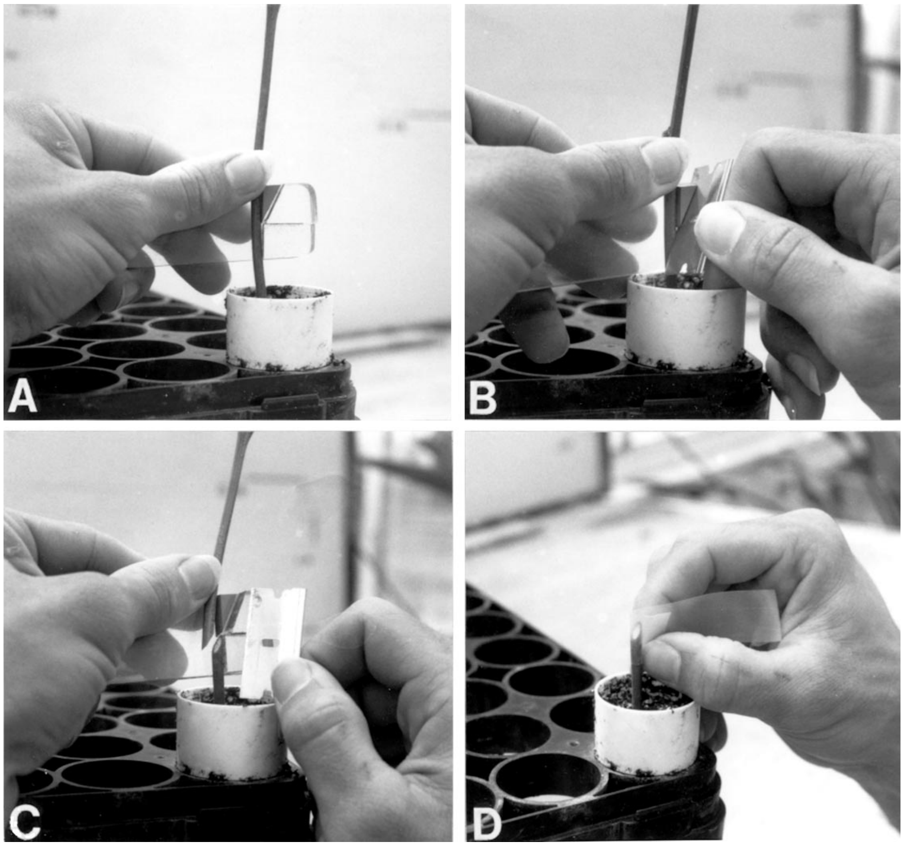


*Figure 2*. Use of the graft guide to perform a Single Bud Scion graft of *Leucaena*. (*A*) Positioning the graft guide to make a rootstock cut. (*B*) Making the angled rootstock cut. (*C*) The resulting angled rootstock cut. (*D*) Placing the Parafilm strip on the rootstock cut.

petiole pointing in the direction of the graft guide handle (Figure 3A). The scion shoot was recut about 1.5 cm below the axillary bud using the slotted block to guide the blade.

*Joining scion and rootstock.* The scion was carefully inserted (Figure 3B) into the Parafilm tube such that the scion and rootstock surfaces were as complementary as possible. The top of the Parafilm J was pinched shut and then carefully but firmly, the union was wrapped with with the remainder of the Parafilm J (Figure 3C).

*Moisture management.* To prevent desiccation a clear plastic bag was placed over the entire grafted plant, maintaining 5–7 of head space, and secured around the top of the pot. The label identified each graft and supported the

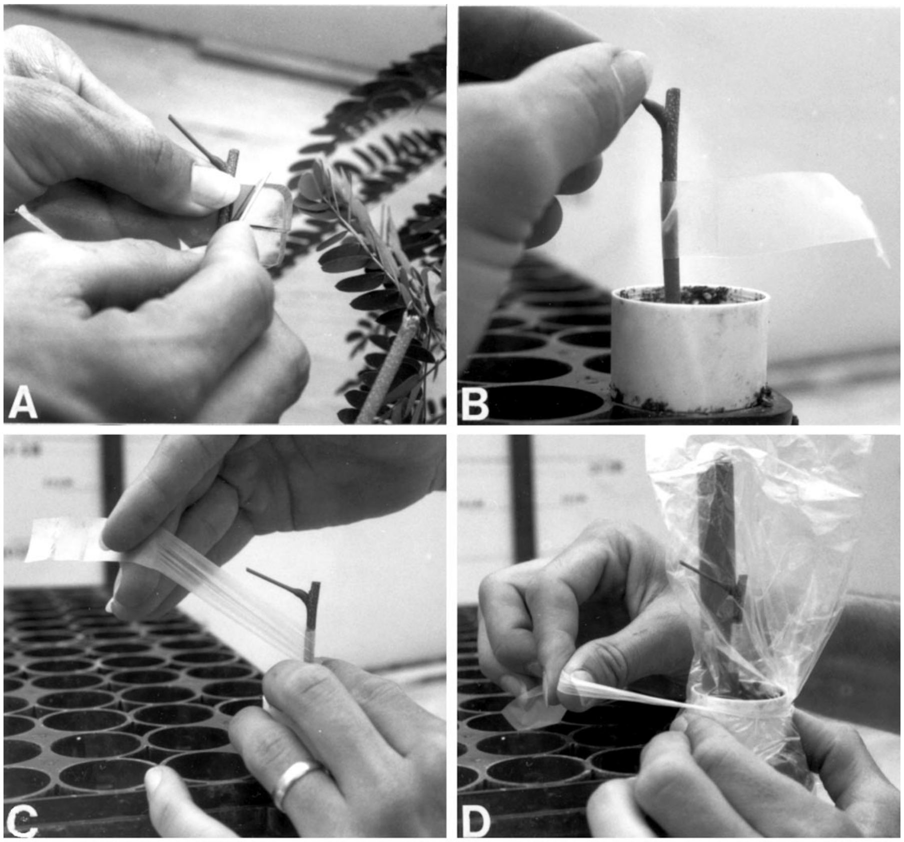


*Figure 3*. Use of the graft guide to perform a Single Bud Scion graft of *Leucaena*, cont. (*A*) Preparing the single-bud scion. (*B*) Inserting the scion piece into the Parafilm tube. (*C*) Wrapping the scion/rootstock graft union. (*D*) Using Parafilm to secure the plastic bag to the pot.

top of the plastic bag. Grafted (bagged) plants were sub irrigated weekly by placing them in 1 cm of standing water. Under high light conditions (sunny days), the grafted seedling were placed under a single layer of 40% shade cloth.

*Post grafting care.* About 6–7 days after grafting bud expansion was visible on successful grafts. At this time any rootstock sprouts were carefully removed. After 2.5 weeks, a successful graft had a single rapidly growing shoot that had elongated to the point of pushing against the plastic bag. At this time a 1 cm ventilation cut was made in the plastic bag to begin acclimating the shoot to ambient conditions. Every 4–5 days the bag was cut another 1 cm until eventually it was completely opened up.

*Variations.* As an alternative to separately bagging each seedling, successful grafts were also obtained by placing grafted seedlings in a fogging chamber. Furthermore, the SBS grafting technique was successful for top working small shoots on larger trees. All of the methods and dimensions also apply to top working, except that the plastic bag was attached to the rootstock shoot.

*Modified veneer graft*

In cases where the rootstock stem diameter was larger than that of the scion (between 0.5–1.5 cm), a modified veneer (MV) graft was used. The MV graft was similar to the veneer crown graft described by Garner (1988).

*Cutting the Rootstocks.* The shoot of the rootstock was severed perpendicular to the main axis, approximately 2–5 cm above the soil level. A drop of water was placed on the cut surface to prevent desiccation. A flap of rind (bark), approximately as wide as the scion (3–6 mm), under which the scion was to be inserted, was cut into the rootstock by a single vertical slit, approx. 1.5 cm long and 1.5 to 2 mm in from the outer surface of the bark. The cut which created the flap was a secant to the circular outer perimeter of the stock. Hence, the flap for the MV graft was made from a single vertical cut, in contrast to the veneer crown graft (Garner 1988) where it is made with 2 parallel vertical cuts and consists of rind only.

*Scion Preparation.* Scion shoots were selected and prepared similarly to the SBS technique except that the basal diagonal cut was several times longer (approx. 1 cm) and was made freehand (without the use of the graft guide slot). This angled (diagonal) cut was accomplished by pushing the razor blade against the scion shoot at approximately the same angle as that made by the graft guide, although it was made freehand. Finally, the basal tip of the scion was trimmed to a wedge shape by making a second shorter (approx. 3 mm long) diagonal cut on the petiole side to expose cambial tissue.

*Joining the Scion and Rootstock.* The scion was when carefully inserted between the rind flap and central portion of the rootstock such that the petiole faced outward and the cut surfaces of both stock and scion were in close contact (Figure 4). Parafilm was then wrapped around the scion and rootstock to secure the graft and the grafted section was bagged for moisture management as described for the SBS technique. Up to 4 scion shoots could be grafted around the perimeter of a single rootstock with stem diameter of 8–10 mm.

*Figure 4*. Modified Veneer Graft used when the rootstock stem diameter (between 0.5–1.5 cm) was larger than that of the scion.

### *Evaluation of graft union formation (success) and graft compatibility*

Between January 1992 and March 1995, 961 grafts were performed in a greenhouse at Cornell University using both the SBS and MV grafting techniques. Success or failure of individual grafts was determined within several weeks of grafting, as indicated by active growth of the scion bud, or scion desiccation and death respectively. Short term graft failure was merely recorded as such and no attempt was made to determine its cause. After a graft was deemed successful the plant was further observed in the greenhouse for up to 3 months for signs of delayed incompatibility which was defined as the appearance of bulging at the graft union, scion dieback, and chlorotic scion foliage. In May, many of those grafted plants which survived and grew in the greenhouse were transported to and planted in field sites in Hawaii for use in hybrid seed orchard experiments, or in an agroforestry system. Plants in the field sites were observed for incompatibility symptoms for up to one year after grafting.

Scion clones evaluated in both greenhouse and subsequently in seed orchards included accessions of *L. leucocephala* (K420, K481, K636), *L. pallida* (K804, K953), *L. esculenta* (K948) and 2n *L. diversifolia* (K907), and the interspecific hybrid KX3 (*L. leucocephala* (K636) × *L. diversifolia* (K156)). The triploid hybrids including *L. leucocephala* × *L. esculenta* (K636 × K838) and *L. pulverulenta* × 4n *L. diversifolia* (K877) were evaluated in

*Table 1*. Summary of interspecific graft success by rootstock and by grafting method.

| | Rootstock | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Shrubby LEU (K997) | | Giant LEU (K636) | | DIV 4 (K156) | | All rootstocks | |
| Grafting method | Success/ Total | Percent Success | Success/ Total | Percent Success | Success/ Total | Percent Success | Success/ Total | Percent Success |
| SBS | 182/269 | 68 | 221/282 | 78 | 178/255 | 70 | 581/806 | 72 |
| MV | — | — | 83/105 | 79 | 30/50 | 60 | 11/155 | 73 |
| Combined | — | — | 304/387 | 79 | 208/305 | 68 | 694/961 | 72 |

the (field) agroforestry system. Field experiments are described elsewhere (Brennan 1995, and manuscripts in preperation). In addition, several other scion species were evaluated in the greenhouse only. These included 4n *L. diversifolia* (K156), and 2n species including: *L. collinsii* (K905, K912), *L. greggii* (K956), *L. lanceolata* (K952), *L. macrophylla* (OFI39/89), *L. multicapitula* (K955), *L. pulverulenta* (K957), *L. retusa* (OFI 23/86), *L. shannonii* (K954), *L. salvadorensis* (OFI 7/91), and *L. trichoides* (OFI 61/88).

Rootstock genotypes evaluated for most scion species (specified below) included the giant type *L. leucocephala* (K636), the shrubby type *L. leucocephala* (K997), and the 4n *L. diversifolia* (K156).

## Results and discussion

### *Grafting success*

Across all the scions and rootstocks, the overall grafting success rate in the greenhouse averaged 75% for the 23 scion/stock combinations where more than 15 grafts were attempted (Table 1), ranging from 25 to 100% for individual combinations (Table 2). There was no apparent difference in grafting success between the two grafting methods (Table 1). The variation in percent grafting success amongst the various stock/scion combinations could be due to several factors including grafting skill, grafting method, environmental variation, genetic incompatibility, and their interactions. The effect of genetic variation within a specific scion/rootstocks combination was minimized by using a single clone of each scion variety, and by growing rootstocks from seed of species that are noted to be highly self-compatible and self-pollinating (Sorensson and Brewbaker 1994) and thus highly homozygous.

In contrast with grafting success in the greenhouse in the temperate zone, grafting success under field conditions in Hawaii, during the collection of scion donor plants (prior to greenhouse grafting), using both the MV and SBS techniques was generally low, at 20 to 50% (data not shown).

Although there was no apparent difference in success rate for the two grafting methods (Table 1), the SBS was preferred to the MV technique because (1) it resulted in a more stable/smooth graft union, (2) it did not require rewrapping of the graft union as did the MV grafted trees, (3) it required fewer cuts to both the scion and rootstock, and (4) it could be done on younger rootstocks. Of the three rootstocks, the giant type *L. leucocephala* (K636) and *L. diversifolia* (K156) were the easiest to graft since they have straight stems and do not branch near the soil as is the case with the shrubby type, *L. leucocephala* (K997). Furthermore, the two former rootstocks were large enough to graft as soon as two months after sowing, while the latter required three months.

*Field survival*

Successfully greenhouse-grafted trees were transplanted to the field 2–3 months after grafting when they had approximately 15 to 30 cm of scion growth. Of the 427 grafted trees evaluated in field experiments in Hawaii, over 95% survived and were growing well as of January 1995 (7 months after transplanting); several of the scion/rootstock combinations grew several meters in the first 6 months of growth. The field growth and performance of the scions in seed orchards and the rootstock effects on the growth of the sterile interspecific hybrids in an agroforestry system were reported by Brennan (1995).

*Interspecific graft compatibility*

Table 2 shows all interspecific grafts and grafts involving species and interspecific hybrid scions, and indicates the apparent compatibility or delayed incompatibility of each scion/rootstock combination. Keeping in mind that the primary goal of greenhouse grafting was to generate plant material for the field experiments in Hawaii, the grafting success and compatibility data amongst the scion/rootstock combinations presented in Table 2, was not compared statistically because grafts were performed at the different times and because two different grafting methods were used. Incompatibility symptoms on stock/scion combinations evaluated in both the greenhouse and field (GI) or in the greenhouse only (gi) were usually apparent in the greenhouse as soon as 1.5 months after grafting. The only scion species exhibiting delayed incompatibility were *L. esculenta* and *L. shannonii* grafted onto all three rootstocks, with the former evaluated in greenhouse and field, and the latter only in the greenhouse only. Twenty-four of the 28 interspecific scion/rootstock combi-

*Table 2.* Grafting success and compatibility of various scion/rootstock combinations for Leucaena species[1] and interspecific hybrids[2]

| | Rootstock | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shrubby LEU (K997) | | | | Giant LEU (K636) | | | | DIV4 (K156) | | | |
| Scion | Method | Success/ Total | Percent Success | Comp. Status[3] | Method | Success/ Total | Percent Success | Comp. Status | Method | Success/ Total | Percent Success | Comp. Status |
| COL | — | — | — | — | MV | 7/7 | 100 | gc | MV | 1/1 | 100 | gc |
| DIV2 | SBSG | 21/34 | 62 | GC | SBSG | 30/44 | 68 | GC | SBSG | 22/37 | 60 | GC |
| DIV4 | — | — | — | — | SBSG | 12/12 | 100 | gc | — | — | — | — |
| ESC | SBSG | 7/10 | 70 | GI | SBSG | 7/9 | 78 | GI | SGSG | 7/9 | 78 | GI |
| GRE | — | — | — | — | MV | 1/1 | 100 | gc | MV | 1/1 | 100 | gc |
| LAN | — | — | — | — | MV | 7/7 | 100 | gc | MV | 6/7 | 86 | gc |
| LEU K420 | SBSG | 28/33 | 85 | GC | SBSG | 29/32 | 91 | GC | SBSG | 30/35 | 86 | GC |
| LEU K481 | SBSG | 18/29 | 62 | GC | SBSG | 20/26 | 77 | GC | SBSG | 19/33 | 58 | GC |
| LEU K636 | SBSG | 30/35 | 86 | GC | SBSG | 30/32 | 94 | GC | SBSG | 25/32 | 78 | GC |
| MAC | — | — | — | — | MV | 7/7 | 100 | gc | MV | 3/7 | 43 | gc |
| MUL | — | — | — | — | MV | 1/1 | 100 | gc | MV | 1/1 | 100 | gc |
| PAL K804 | SBSG | 5/11 | 46 | GC | SBSG | 4/13 | 31 | GC | MV | 4/11 | 36 | GC |
| | | | | | MV | 16/25 | 64 | GC | | | | |
| PAL K953 | — | — | — | — | MV | 25/28 | 89 | GC | — | — | — | — |
| PUL | — | — | — | — | MV | 5/5 | 100 | gc | MV | 1/1 | 100 | GC |
| RET | — | — | — | — | MV | 2/2 | 100 | gc | MV | 2/2 | 100 | gc |
| SAL | — | — | — | — | MV | 5/6 | 83 | gc | MV | 5/7 | 71 | gc |

*Table 2*. Continued

| | Rootstock | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shrubby LEU (K997) | | | | Giant LEU (K636) | | | | DIV4 (K156) | | | |
| Scion | Method | Success/ Total | Percent Success | Comp. Status[3] | Method | Success/ Total | Percent Success | Comp. Status | Method | Success/ Total | Percent Success | Comp. Status |
| SHA | — | — | — | — | MV | 2/8 | 25 | gi | MV | 2/5 | 40 | gi |
| TRI | — | — | — | — | MV | 5/8 | 63 | gc | MV | 4/7 | 57 | gc |
| LEU × DIV4 | SBSG | 26/40 | 65 | GC | SBSG | 33/38 | 87 | GC | SBSG | 29/37 | 78 | GC |
| LEU × ESC | SBSG | 34/54 | 63 | GC | SBSG | 34/53 | 64 | GC | SBSG | 31/52 | 60 | GC |
| PUL × DIV4 | SBSG | 13/23 | 57 | GC | SBSG | 22/23 | 96 | GC | SBSG | 15/20 | 75 | GC |

[1]Rootstock and scion abbreviations: Diploids – COL = L. collinsii, DIV2 = L. diversifolia, ESC = L. esculenta, GRE = L. greggii, LAN = L. lanceolata, MAC = L. macrophylla, MUL = L. multicapitula, PUL = L. pulverulenta, RET = L. retusa, SHA= L. shannonii, SAL = L. salvadorensis, TRI = L. trichoides; and tetraploids – DIV4 = L. diversifolia subsp. diversifolia, LEU = L. leucocephala.
[2]For interspecific hybrids, the maternal parent is written first, followed by the paternal parent.
[3]Graft compatibility status: GC = a compatible combination as assessed in both the green house and field, gc = a compatible combination as accessed in the green house only, GI = an (delayed) incompatible combination as accessed in the greenhouse and field, gi = an (delayed) incompatible combination as accessed in the greenhouse only.

nations evaluated were successful, indicating a high level of graft compatibility within the genus *Leucaena*. Note that these 24 successful combinations include only interspecific grafts, and do not include LEU/LEU, DIV4/DIV4 and the interspecific hybrid scions on any rootstocks.

Some researchers have suggested that graft compatibility is correlated with sexual compatibility (Addison and Tarares 1952, Evans 1960). However, there does not appear to be a relationship between graft compatibility and sexual compatibility for *Leucaena*, since 5 of the 24 compatible interspecific scion/rootstock combinations (GRE/DIV4, MAC/DIV4, MAC/LEU, MUL/LEU, TRI/DIV4), were found by Sorensson and Brewbaker (1994) not to be sexually compatible.

Most interploidal graft combinations succeeded (i.e., triploid hybrids grafted on tetraploid species and diploids grafted onto tetraploids), as did all grafts where interspecific hybrids were used as scion varieties (Table 2). Although ploidy differences between two species are an obvious barrier to sexual compatibility (due to chromosomal pairing during meiosis), it does not necessarily follow that ploidy would affect graft compatibility since graft union formation is at the cellular, rather than chromosomal level.

In conclusion, we anticipate that these grafting techniques could be used widely on a small or large scale to propagate leucaena parental lines in hybrid seed orchards, to maintain and exchange important clones for breeding purposes, and to multiply seedless hybrids for use in plantations and agroforestry systems. The graft guide also may be useful for grafting vegetative shoots of other herbaceous and woody species in agroforestry, horticultural, and forestry settings.

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## References

Addison, G. and Tavares, R. 1952. Hybridization and grafting in species of *Theobroma* that occur in Amazonia. Evolution 6: 380–386.

Bray, R. A. and Fulloon, M. G. 1987. Producing F1 seed of *Leucaena leucocephala* and *Leucaena pulverulenta* as potential forage plants. Leucaena Rsch. Reports 8: 19–20.

Brennan, E. B. 1990. Using farmers and their ideas for effective extension work, pp. 94–97. In: Haugen, C., Medema, L. and Lantican, C. B. (Eds.) Multipurpose Tree Species Research for Small Farms: Strategies and Methods. Winrock International Institute for Agricultural Development and the International Development Research Center of Canada.

Brennan, E. B. 1992. A new grafting technique for *Erythrina*, *Leucaena*, and possibly other nitrogen fixing tree species. Nitrogen Fixing Tree Research Reports 10: 85–88.

Brennan, E. B. 1995. Vegetative and seed propagation of *Inga and Leucaena*. M. S. Thesis, Cornell University, Ithaca, NY.

Brewbaker, J. L. 1988. Cloning of seedless Leucaenas for plantation use. Leucaena Rsch. Reports 9: 111–112.

Bristow, S. 1983. Propagation of *Leucaena leucocephala* by cuttings. Nitrogen Fixing Tree Research Reports 1: 28–29.

Datta, S. K. and Datta, K. 1984. Clonal multiplication of ‘elite’ trees – *Leucaena leucocephala* through tissue culture. Leucaena Rsch. Reports 5: 22–23.

Evans, A. M. 1960. Relationships between vegetative and sexual compatibility in *Trifolium*. Welsh Plant Breeding Station Reports 1959: 81–87.

Garner, R. J. 1988. The Grafter’s Handbook. Oxford University Press, New York, NY, p. 258.

Goyal, Y., Bingham, R. L. and Felker, P. 1985. Propagation of the tropical tree, *Leucaena leucocephala* K67 by in vitro bud culture. Plant Cell Tissue and Organ Culture 4: 3–10.

Hu, T.Wei and Liu, Chih-Cheng. 1981. Vegetative propagation of Leucaena by leafy cuttings under mist spray. Leucaena Research Reports 2: 50.

Hughes, C. E. and Styles, B. T. 1987. The benefits and potential risks of woody legume introductions. The International Tree Crops Journal 4: 209–248.

Litzow, D. R. and Shelton, H. M. 1991 Establishing *Leucaena leucocephala* and *Gliricidia sepium* from stem cuttings. Leucaena Research Reports 12: 3–6.

Nitrogen Fixing Tree Association. 1986. Erythrinas provide beauty and more. Nitrogen Fixing Tree Highlights 86-02.

Rotar, P. P., Joy, R. J. and Weissich, P. R. 1986. ‘Tropic Coral’ tall Erythrina. Research Extension Series 072. Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, HI.

Shelton, M. and Pottinger, A. (Eds.). 1995. New seedless triploid from Hawaii, Leuc-net News 1(1), Oxford Forestry Institute, Oxford, UK (World Wide Web URL: http://ifs.plants.ox.ac.uk/ofi/leucaena.htm#top).

Skolman, R. G. 1977. Clonal propagation of *Acacia koa* Gray by tissue culture and conventional methods. Ph.D. Dissertation, University of Hawaii, Honolulu, HI.

Sorensson, C. T., Shelton, H. M. and Austin, M. T. 1994. Seedling vigor of some *Leucaena* spp. and their hybrids. Tropical Grasslands 28: 182–190.

Sorensson, C. T. and Brewbaker, J. L. 1994. Interspecific compatibility among 15 *Leucaena* species (Leguminoseae: Mimosoideae via artificial hybridization). Amer. Jour. of Bot. 8: 240–247.

Toruan-Mathius, N. 1992. Micropropagation of *Leucaena* species by tissue culture techniques. Leucaena Rsch. Reports 13: 44–52.

Tanavan, H. and David, A. 1985. Greffage in vitro du pin maritime (*Pinus pinaster*). Can. J. Bot. 63: 1017–1020.

Tranvan, H., Bardat, F., Jacques, M., and Arnaud, Y. 1991. Rajeunissement chez *le Sequoia semperivirens*: effets du microgreffage in vitro. Can. J. Bot. 69: 1772–1779.

Versace, G. 1982. Propagating leucaena by grafting. Leucaena Rsch. Reports 3: 3.

Wheeler, R. A. and Brewbaker, J. L. 1990. An evaluation of the results from the leucaena psyllid trial network. Leucaena Rsch. Reports 11: 23–31.