

**Propagation of Narrowleaf Cottonwood by Stem Cuttings I:  
Media Density and Auxin Formulation.**

**by:**

**John T. Harrington, David R. Dreesen and Stephen Hine**

**Summary**

Several factors can influence the ability of a stem cutting to produce adventitious roots. Some species, such as many members of the genus *Populus*, will generate roots readily. Even though a species may be genetically fit to produce adventitious roots, other factors such as source plant physiology, post cutting treatments, and rooting environment can inhibit rooting and the subsequent growth of the cutting. The interaction of two or more of these factors can be more influential than their effects individually. For instance, elevated exogenous auxin levels and warm rooting media temperatures can be used to overcome internal restraints in mature cuttings. However, applying these factors individually would not be sufficient to achieve successful rooting. In this study and the two following it, various aspects of an asexual propagation program are evaluated.

**Introduction**

Stock plant physiology and vigor, strongly impact rooting success and cutting growth (Dirr and Heuser 1987). In *Populus*, 3 - 10 year old stock plants produce the most vigorous cuttings and the highest rooting percentages (Phipps et al. 1977). Frequently nurseries establish stooling blocks of desirable clones. This allows the nurseries to manipulate stock plant vigor through irrigation, fertilization and pest management. In some situations this may not be feasible and post harvest treatment of cuttings must be employed to obtain satisfactory rooting and growth.

One technique commonly used to induce rooting in difficult to root material is through exogenous auxin applications. Auxin, a plant growth regulator, can be obtained in many formulations. The three most common formulations are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalenacetic acid (NAA). The latter two formulations, IBA and NAA are the most common formulations used in rooting of cuttings. Many species respond differently to the two formulations while other species

do not differ in their responses. When initiating a vegetative propagation program it is recommended to test various formulations at various concentrations (Phipps et al. 1977).

The objectives of this study were to evaluate the effect of auxin formulation and media density on rooting and cutting growth of narrowleaf cottonwood (*Populus angustifolia* James) cuttings; and, to examine the influence of stock plant vigor (source) on these responses.

### Methods and Materials

Stem (branch) cuttings used in this study originated from five distinct stands (ecotypes) of narrowleaf cottonwood growing at the Molycorp Inc. molybdenum mine in Questa, New Mexico. This area is located in the Red River canyon approximately five miles east of the town of Questa, New Mexico. Ecotype locations are described in Table 1. Ecotypes were separated by no less than 1,000 meters. Stem cuttings originally taken from these ecotypes in 1992 were used to establish stooling blocks at the Los Lunas Plant Materials Center in Los Lunas, NM. The stooling blocks were established in the spring of 1993. Source identification of the stooling block material was maintained to the ecotype level, but not to the individual tree.

Table 1. Ecotype locations and elevations:

Ecotype	Elevation
Capulin	9,800
Raspberry Ridge	9,850
Pinon Knob	9,300
Neutral	8,600
River	8,100

The stem cuttings used in this study were harvested from both the original

stands at the mine as well as from the 3-year-old stooling block material growing in Los Lunas, NM. The source plants at the mine site ranged in age from 3 years to 15 years. When possible branches were harvested from young trees or younger materials from older trees. Branches were harvested in the last week of February 1996 for the Los Lunas source material and the first week of March 1996 for the mine source material. Branches were then transported to the Mora Research Center and stored in coolers (2 - 4°C) until used in the study (less than 2 weeks). Individual branches were subdivided into stem cuttings immediately prior to treatment imposition. Stem cutting length ranged from 10 cm to 15 cm in length and contained a minimum of 3 buds. Subdivision of the stem cuttings was done using a hand pruner.

The rooting hormones evaluated in this study were indole-3-butyric acid (IBA) and naphthalenacetic acid (NAA). Stock solutions of 1000 ppm IBA and 1000 ppm NAA were prepared. Through dilutions the test concentrations of 250 ppm IBA, 250 ppm NAA and 125 ppm IBA + 125 ppm NAA were prepared. A distilled, de-ionized water control was also used. Rooting hormone application was a 5 sec dip into the appropriate rooting hormone treatment immediately followed by sticking the cutting into the container.

Two rooting media were evaluated in this study. The rooting media were a 1:1:1 and a 1:2:1 ratio of peat:perlite:vermiculite (v:v:v). Media components were mixed using a large paddle mortar mixer. Slow release fertilizer (Osmocote 14-14-14; 3 month) was incorporated into the media at the rate of 4kg/m<sup>3</sup> during the mixing process.

Once cuttings were treated they were stuck in 164 cm<sup>3</sup> Ray-Leach Super Cells. Stem cuttings were placed in a greenhouse on a propagation bench with bottom heat which kept root zone temperature at 24°C. Greenhouse temperatures were 20 - 22°C days and 16 - 18°C nights. Photoperiod was a 10-hr light/14-hr dark with the dark cycle being interrupted twice at 5 hours and 10 hours with 30 minute light periods. Artificial light used to extend the ambient light period and light interruptions was provided by 1000-watt high pressure sodium vapor lamps suspended 3 meters above the stem cuttings.

Cuttings were misted 4 times daily until the majority of cuttings had significant bud break (approximately 3 weeks). Following budbreak, cuttings were irrigated as

needed, increasing from once every three days at the beginning of the study to once every day at week 20. Fertilizer applications consisted of foliar feed applications of a 25 ppm N solution of Peter's Foliar Feed following every second irrigation from week 4 through week 12. At week 13, this fertilization was increased to applications of 100 ppm N of Peter's Conifer Grower (20:7:19) every other irrigation. This fertilization rate was retained until the end of the study. At week 20, cuttings were non-destructively analyzed for rooting response, seedling height and number of leaves.

The treatment structure for this study was a factorial design with two source plant locations, five ecotypes, two media types, and the four levels of rooting hormone applications. The planting design was a randomized complete block design. Due to limited plant material availability, a variable number of trees (2 - 8) were used to represent each ecotype by site combination. Each tree/ecotype/site was represented in each of the 8 media X hormone treatments by 14 stem cuttings. Data was analyzed using analysis of variance procedures.

### **Results**

Stock plant location (site), ecotype and rooting hormone formulation all strongly impacted rooting percentage (Table 2). Cuttings from Los Lunas were unaffected by auxin applications. Cuttings collected from the mine were unaffected by the IBA treatment but had reduced rooting percentages in the presence of NAA alone or in combination with IBA (Figure 1). Individual ecotypes responded differently to the two auxin formulations. The Pinon Knob and River ecotypes were negatively affected by NAA (Figure 2). Rooting in the other three sources was unaffected by NAA. IBA promoted rooting in two of the three ecotypes, but had no effect on the other three ecotypes. The Los Lunas derived material showed greater rooting percentages, averaging 95% (Figure 3). The differences between the two source locations was greatest for the rooted cuttings derived from the higher elevation ecotypes. Cuttings obtained from the Capulin ecotype growing at the mine, had over 25% less rooted cuttings than those derived from the Capulin source growing in Los Lunas. This difference in rooting response by source was less evident in the River ecotype, where the reduction in total rooting percent was less than 7% (Figure 3).

Shoot growth of the cuttings was strongly influenced by site, ecotype, media and

Table 2. Analysis of variance table for the effects of rooting media, auxin formulation, stock plant location (Site) and ecotype on the rooting percentage of narrowleaf cottonwood cuttings.

Source	df	MS	Pr > F
Model	79	7.03	0.0001
Media (M)	1	7.03	0.7688
Formulation (F)	3	337.18	0.0072
M x F	3	67.01	0.4809
Site (S)	1	22650.31	0.0001
M x S	1	10.35	0.7213
F x S	3	433.70	0.0015
M x F x S	3	52.54	0.5852
Ecotype (E)	4	684.05	0.0001
M x E	4	118.98	0.2143
F x E	12	212.49	0.0032
M x F x E	12	57.78	0.7376
S x E	4	683.02	0.0001
M x S x E	4	58.47	0.5785
F x S x E	12	270.87	0.0002
M x F x S x E	12	119.61	0.1379
Error	165	81.06	
Total	244		

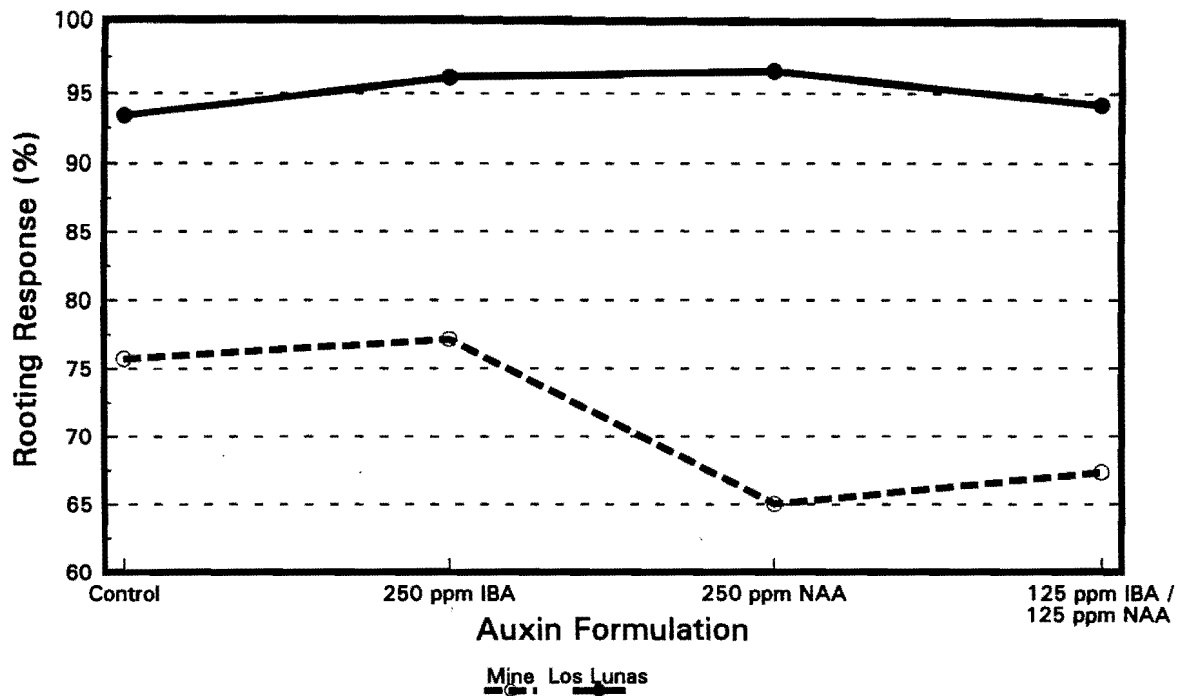


Figure 1. Influence of auxin formulation on the percentage of narrowleaf cottonwood cuttings rooting from cuttings collected from the mine and from stooling blocks growing in Los Lunas, NM.

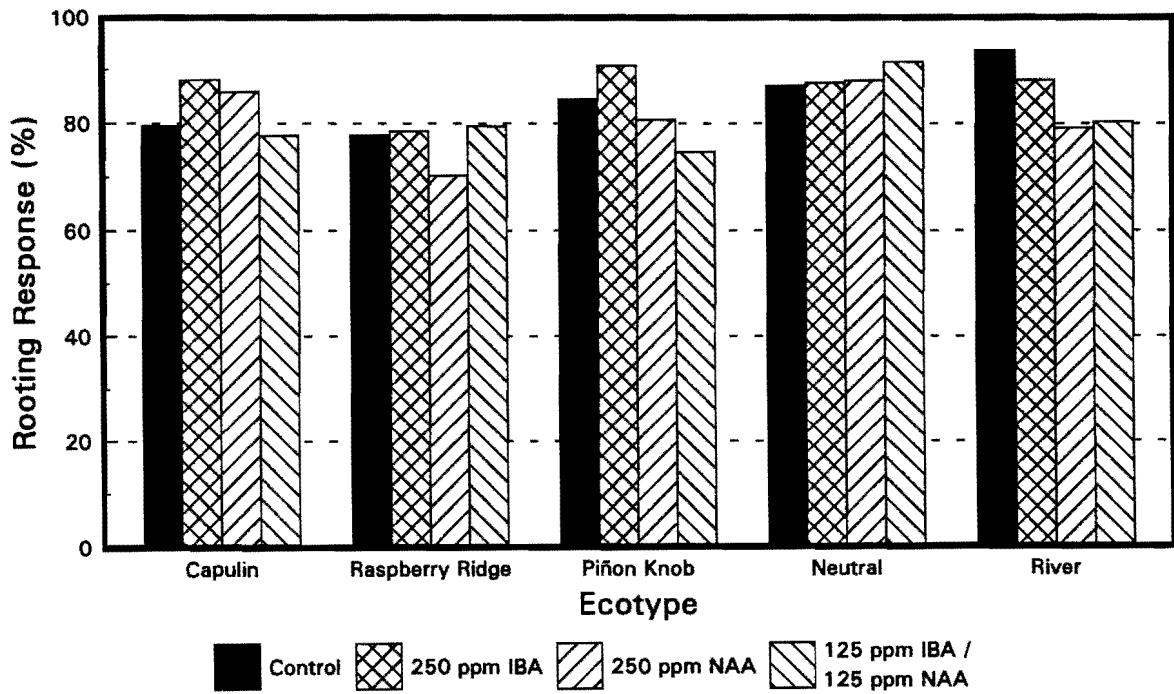


Figure 2. Influence of auxin formulation on the percentage of narrowleaf cottonwood cuttings rooting from five ecotypes of the species.

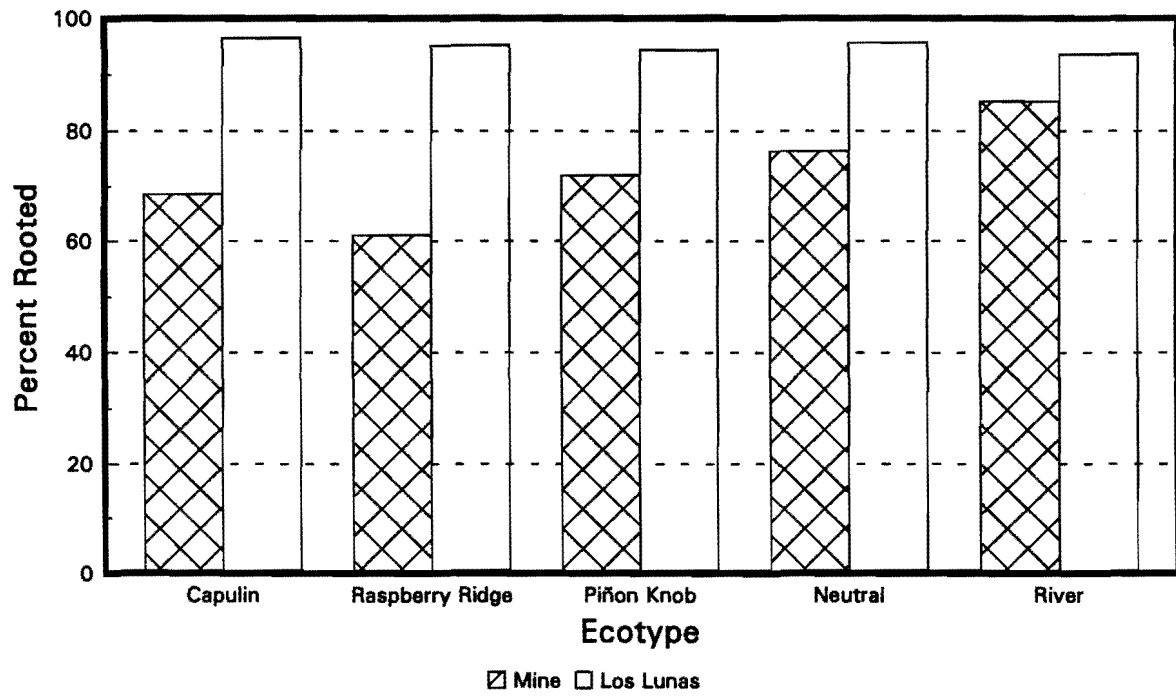


Figure 3. Influence of stock plant location and ecotype on the percentage of narrowleaf cottonwood cuttings rooting in this study.

their interactions (Table 3). Materials derived from the Los Lunas site had much better shoot growth than did those rooted cuttings from the Questa site (Figure 4). The magnitude of this effect was more pronounced in materials from the higher elevation ecotypes. There was over a two-fold increase in shoot growth of Los Lunas originated Capulin stem cuttings when compared to Capulin stem cuttings from the actual mine site in Questa. The difference in growth based on site was less than 5 cm for the two lower elevation ecotypes (Figure 4). For mine derived materials, the lower elevational sources did much better than the upper elevational sources.

Shoot growth was better on the slightly less porous media (1:1:1) regardless of the source of the plant material (Figure 5). The influence of media became much more pronounced in the lower elevational ecotypes: Pinon Knob, Neutral, and River. Shoot growth differences between the two media types was less than 1 cm for the two upper elevation sources and more than 6 cm for the cuttings derived from the Neutral ecotype.

Stock plant location, ecotype, and their interaction had the greatest influence on the number of leaves produced in this study (Table 4). The Los Lunas derived materials consistently had more leaves than the Questa derived materials (Figure 6). The difference between the two sites was greatest for the higher elevational sources averaging about 5 leaves per plant. At the two lower elevation ecotypes, the Neutral and River ecotypes, the difference in the number of leaves between the two sites was less than 2 leaves. Regardless of the ecotype, the Los Lunas derived rooted cuttings produced between 12 and 14 leaves per rooted cutting. In the Questa derived stem cuttings, the number of leaves increased from the higher elevational ecotypes which had an average of 8 leaves per stem to 11.5 leaves per stem for the lower elevation ecotypes.



Table 3. Analysis of variance table for the effects of rooting media, auxin formulation, stock plant location (site) and ecotype on the shoot growth of narrowleaf cottonwood cuttings.

Source	df	MS	Pr > F
Model	79	2161.72	0.0001
Media (M)	1	8605.33	0.0001
Formulation (F)	3	273.75	0.3587
M x F	3	270.11	0.3649
Site (S)	1	49596.98	0.0001
M x S	1	78.12	0.5798
F x S	3	1317.86	0.0014
M x F x S	3	206.59	0.4878
Ecotype (E)	4	2105.82	0.0001
M x E	4	1124.68	0.0015
F x E	12	360.46	0.1512
M x F x E	12	434.28	0.0596
S x E	4	4579.63	0.0001
M x S x E	4	768.69	0.0170
F x S x E	12	733.59	0.0006
M x F x S x E	12	388.09	0.1082
Error	3392	254.83	
Total	3471		

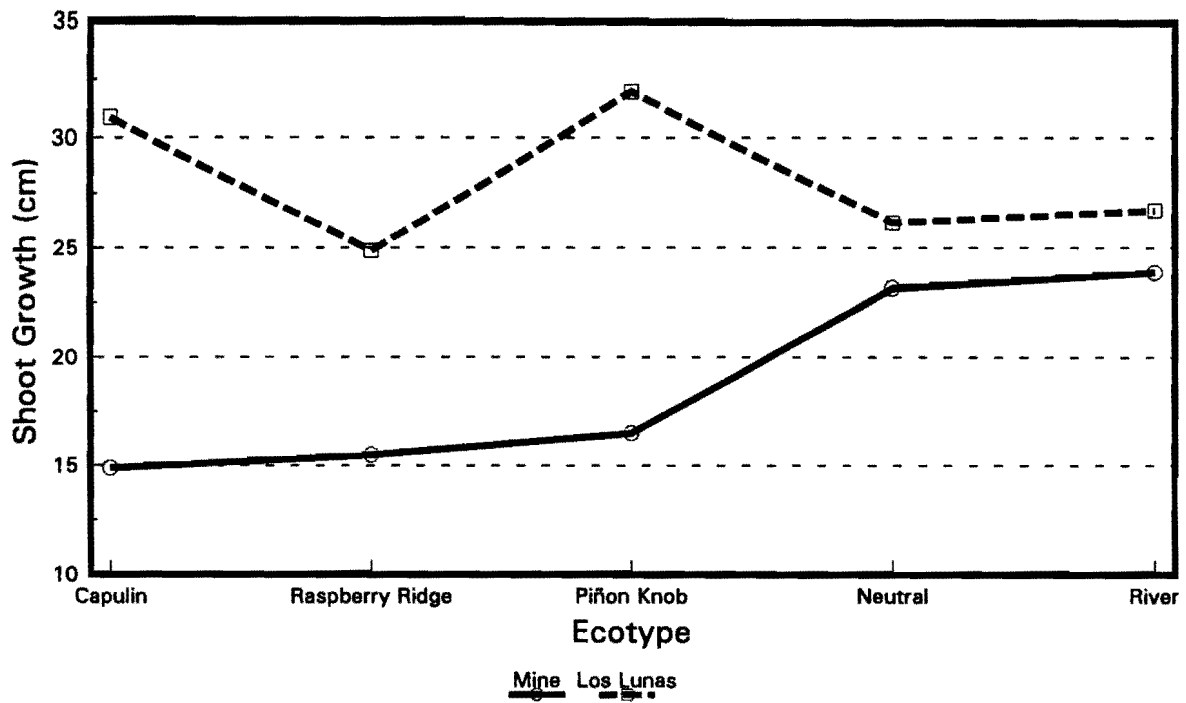
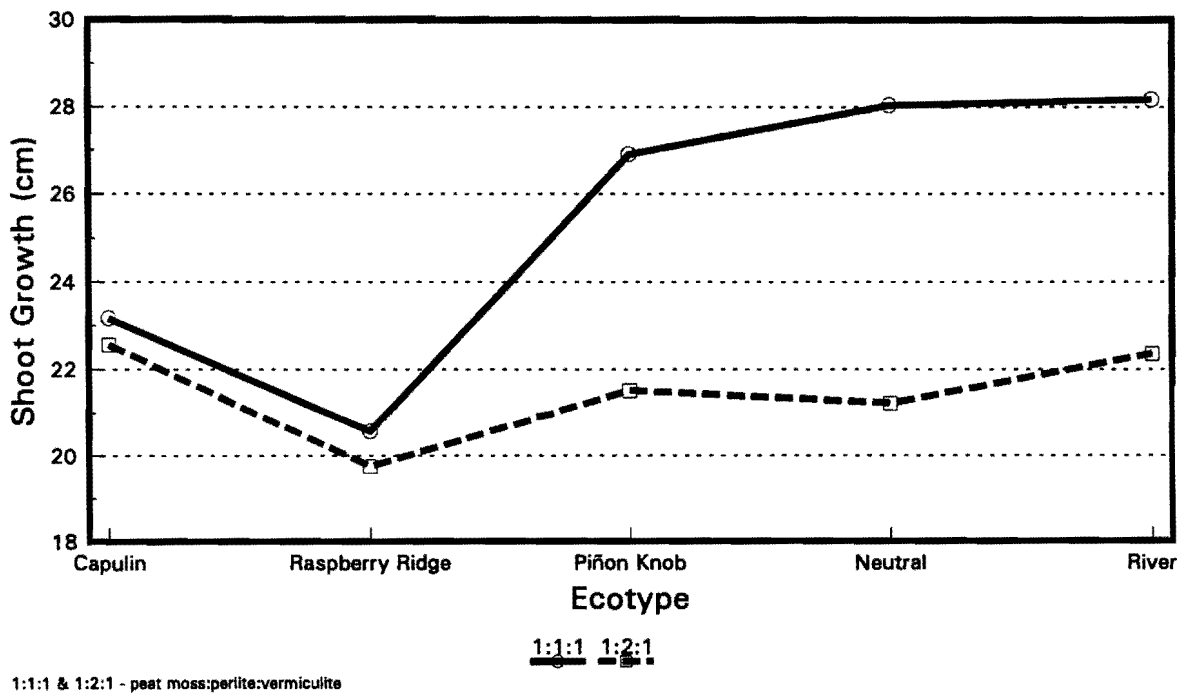


Figure 4. Influence of ecotype on shoot growth of rooted narrowleaf cottonwood cuttings collected from the mine and from stoling blocks growing in Los Lunas, NM.



1:1:1 & 1:2:1 - peat moss:perlite:vermiculite

Figure 5. Influence of media density on shoot growth of rooted narrowleaf cottonwood cuttings from five ecotypes of the species.

Table 4. Analysis of variance table for the effects of rooting media, auxin formulation, stock plant location (site) and ecotype on the number of leaves produced on rooted narrowleaf cottonwood cuttings.

Source	df	MS	Pr > F
Model	79	292.28	0.0001
Media (M)	1	14.55	0.4622
Formulation (F)	3	67.80	0.0576
M x F	3	54.69	0.1072
Site (S)	1	8880.83	0.0001
M x S	1	0.06	0.9613
F x S	3	179.60	0.0002
M x F x S	3	12.65	0.7032
Ecotype (E)	4	653.29	0.0001
M x E	4	151.91	0.0002
F x E	12	57.69	0.0120
M x F x E	12	43.71	0.0780
S x E	4	415.00	0.0001
M x S x E	4	127.98	0.0008
F x S x E	12	88.92	0.0001
M x F x S x E	12	51.26	0.0293
Error	3392	26.91	
Total	3471		

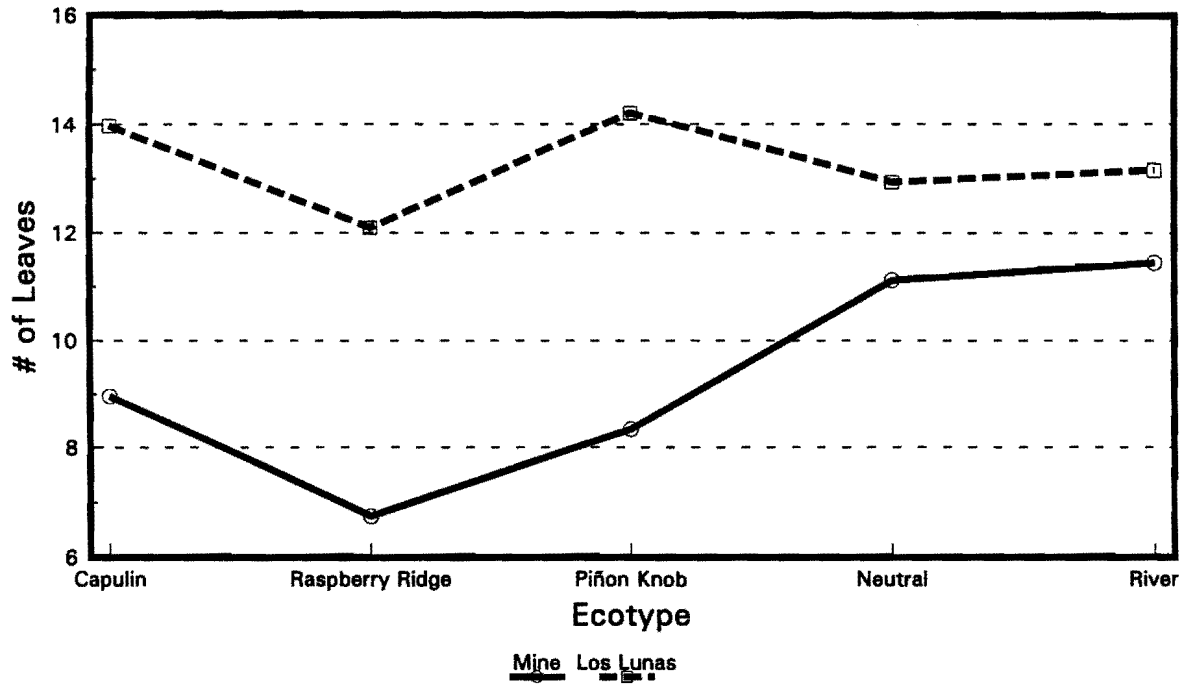


Figure 6. Influence of stock plant location (vigor) on the number of leaves produced on rooted narrowleaf cottonwood cuttings for five ecotypes of the species.

### **Applications**

Stock plant vigor (stock plant location) and ecotype had the greatest impacts of rooting success. The younger, more vigorous stock plants from Los Lunas had much greater rooting success. The river ecotype located at the mine was growing in a less stressful, riparian site relative to the mine site ecotypes growing on upland sites, had a similar rooting performance to materials growing in Los Lunas. However, some ecotypes (genetics) consistently rooted better than others. There appears to be some sensitivity of the material collected directly from the mine to NAA on the rooting process. Individual (ecotype) responses indicate some differential sensitivity to auxin formulation in rooting. The influence of stock plant vigor was also evident in shoot growth. The more vigorous stock plants growing in Los Lunas produced larger more vigorous seedlings.

### **Literature Cited**

Dirr, M.A. and C.W. Heuser Jr. 1987. The Reference Manual of Woody Plant Propagation, From Seed to Tissue Culture. Varsity Press, Inc. Athens, GA 239pp.

Phipps, H.M., D.A. Belton, and D.A. Netzer 1977. Propagating cuttings of some *Populus* clones for tree plantations. *The Plant Propagator* 23(4):8 - 11.