Propagation

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Propagation of *Anemone* x *hybrida* by Root Cuttings

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Index Words: Anemone, Perennials, Propagation, Root Cuttings, Mineral Nutrition, K-IBA.

Nature of Work: Fall flowering anemones (*Anemone* x *hybrida*) are robust, adaptable plants within the limits of USDA cold hardiness zones 5 to 8. Their foliage remains attractive from its emergence in spring, to the first hard frost, and their elegant flowers are produced in profusion at a time of year when few other perennials are in flower (5). In a survey of perennials propagation firms across the United States conducted by the authors, they were the perennial most often cited as needing improvement in propagation. At present, most plants of *A.* x *hybrida* sold in the United States are grown from bareroot field divisions produced in The Netherlands. They are normally propagated vegetatively, by division or root cuttings, with very low multiplication rates in either case. Published information regarding this topic is limited to general recommendations on handling of root cuttings, based on scant research involving woody species (2).

Several studies have shown a strong positive correlation between high nitrogen (N) levels in the growing substrate of stock plants, and both the number of shoot buds produced on roots of *Chondrilla juncea* L. (skeleton weed) and *Euphorbia esula* L. (leafy spurge), and their ability to regenerate whole plants (3, 4). In stem cuttings, however, adventitious root formation has been reported to be affected negatively by high N nutrition of stock plants (1). Therefore, the objective of this study was to determine the effect of N nutrition of stock plants and K-IBA treatment of root cuttings on the development of complete plants from those root cuttings.

Sixty four #1 field divisions each of cultivars ‘Honorine Jobert’ and ‘Richard Arhens’ anemone were grown for 30 weeks, beginning in April, in 3.8 L (#1) containers filled with a substrate of 8 composted pine bark: 1 sand (by vol.), amended with 2.4 kg/m^3 (4 lb/yd^3) dolomitic limestone and 0.9 kg/m^3 (1.5 lb/yd^3) MicroMax (The Scotts Co., Maryville, OH.). Plants were fertigated once a day via pressure-compensated spray stakes with a nutrient solution providing 10, 40, 80, or 150 mg/L (ppm) N in a constant ratio of 1 ammonium: 2 nitrate, in addition to P, K, Ca, Mg, and S at constant concentrations.

At the end of 30 weeks (November), root cuttings 4 cm in length were harvested from the stock plants and treated with the potassium salt of indolebutyric acid (K-IBA) at 0, 100, 500, or 1000 mg/L (ppm), then placed in bedding plant containers (9x4 cells, #1020 flat) containing a pine bark-based medium, one cutting per cell [cell vol. = 160 cm^3 (9.8 in^3)] and covered with 1.5 cm (0.6 in) medium. Containers were placed under intermittent mist in a heated greenhouse under natural photoperiod and irradiance with days/nights of 24° ± 1.7° C (75° ± 3° F) / 20° ± 1° C (68° ± 2° F). Mist was applied via a gantry mounted travelling spray boom (ITS, McConkey Co., Sumner, WA) with continuous adjustment of frequency as a function of relative humidity, and travelling speed set at 15 m/min (50 ft/min). This setting resulted in the medium surface just reaching dryness before being misted again. The experiment was in a randomized complete block design with a factorial arrangement of treatments: 2 cultivars, 4 rates of N applied to the stock plants, and 4 rates of K-IBA applied to the root cuttings. There were 6 replications with 6 cuttings per replication.

After 8 weeks, misting was discontinued, and plantlets were irrigated overhead every 3 days. Twelve weeks after initiation of the experiment, roots of the resulting plantlets were washed free of substrate, and separated from the shoots. Roots and shoots were dried 96 hr at 70° C (160° F), and weighed. Data were subjected to analysis of variance and regression analysis where appropriate. Effects of cutting weight were analyzed by analysis of covariance, with cutting weight as the covariate.

Results and Discussion: Overall, 84% of the root cuttings of ‘Honorine Jobert’, and 98% of the cuttings of ‘Richard Ahrens’ regenerated a complete plantlet. Weight of the root cuttings and rate of N applied to the stock plants did not affect percent regeneration in either cultivar, while response to K-IBA was quadratic in ‘Honorine Jobert’, with a maximum of 90% regeneration predicted at 240 mg/L (ppm) K-IBA. Percent regeneration was unaffected by K-IBA in ‘Richard Ahrens’.

Cutting fresh weight had a strong positive influence on size of the plantlet produced. Within the range used in this study, each unit increase in cutting fresh weight resulted in approximately one unit increase in the dry weight of the plantlet produced. Plantlet dry weight increased linearily with increasing N in ‘Honorine Jobert’, and responded quadratically in ‘Richard Ahrens’, with maximum plantlet weight predicted at 115 mg/L (ppm) N for the latter. The largest mean dry weight by rate of N was 358 mg (0.013 oz.) for ‘Honorine Jobert’, observed at 150 mg/L (ppm) N, and 469 mg (0.016 oz.) for ‘Richard Ahrens’, at 80 mg/L (ppm)N. At those respective rates of N, plantlet dry weight responded to K-IBA in a quadratic manner in both cultivars. However there was no interaction be-

tween rate of N and K-IBA. Maximum response is predicted to occur at 459 mg/L (ppm) K-IBA in 'Honorine Jobert', and at 425 mg/L (ppm) in 'Richard Ahrens'. At these concentrations, a 37% increase in plantlet dry weight would be expected in 'Honorine Jobert', as compared to not using any K-IBA. In 'Richard Ahrens', the increase would be expected to reach 13%.

Significance to Industry: Results demonstrated that production of A. (hybrida transplants in bedding-plant containers can be accomplished from root cuttings, with percentage regeneration ≥ 90%. Weight of the root cutting does not influence whether or not a plantlet can be regenerated, but heavier cuttings will yield larger plantlets. Optimal N nutrition of the stock plant will increase size of the plantlets, but the optimal rate of N varies by cultivar, and does not influence percentage regeneration. Treatment of the root cuttings with K-IBA is helpful in increasing the size of plantlets in particular cultivars. As K-IBA concentration increases, however, both percent regeneration and time to production of a shoot may be adversely affected, again cultivar dependent.

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Effects of Jiffy^{R} Forestry Peat Pellets on Rooting Stem Cuttings of Loblolly Pine

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Index Words: Loblolly Pine, *Pinus taeda* L., Vegetative Propagation, Jiffy Forestry Peat Pellets

Nature of Work: Vegetative propagation of loblolly pine (*Pinus taeda* L.) by stem cuttings can be used to multiply full-sib families and clones (Frampton and Hodges, 1989). However, propagation protocols for large-scale production of rooted stem cuttings of loblolly pine need further improvement (Zobel, 1992) . One aspect of this research is to study effects of different types of containers on rooting, root quality, and subsequent field performance of rooted cuttings. Therefore, the objective of this research was to compare rooting and first year field growth of stem cuttings of loblolly pine rooted in Jiffy^{R} forestry peat pellets (Jiffy Products, Shippagan, New Brunswick, Canada) and Ray-Leach Super Cells™, the current industry standard (Stuewe and Sons, Inc., Corvallis, Ore.).

Experiments were conducted in January 1998 (hardwood cuttings) and June 1998 (softwood cuttings) to evaluate rooting of stem cuttings of three full-sib families of loblolly pine in Jiffy forestry peat pellets of varying sizes. The peat pellets differed in dry diameters and expanded heights and are referred to by the sizes in Table 1. Both studies utilized the same size pellets with the exception of the June study which did not include the 36-75 mm (1.4-3.0 in) size pellet. Ray-Leach Super Cells [(vol. = 162 cm^3)(9.9 cm^3)], containing a medium of 2 peat moss:3 perlite (v/v), served as the control for both experiments.

Cuttings were taken from hedged stock plants and rooted under mist on raised greenhouse benches in a humidity-controlled greenhouse. Mist was delivered by a traveling gantry (boom) system (ITS, McConkey Co., Sumner, Wash.). The experimental design was a split plot with eight (or seven in June) pellet sizes (including the control), three families, 10 cuttings per family per treatment, and eight replications.

Twelve weeks after the cuttings were stuck, various data were recorded which included rooting percentage, number of primary roots > 1 mm (0.04 in) in length, root system symmetry (at least two primary roots 130°

apart], and total root dry weight [dried at 70C (158F) for 48 hr]. All rooting data, excluding rooting percentage, were based on cuttings that rooted.

Data from each experiment were subjected to analysis of variance procedures (ANOVA) and regression procedures. Means were separated using LSMEANS (SAS Inst., Inc., 1985) (due to unequal sample sizes) when the ANOVA was significant at $P<0.05$.

Results and Discussion: Rooting percentages in January for hardwood cuttings rooted in pellet sizes 42-80 mm (1.7-3.1 in) (36%) and 50-95 mm (2.0-3.7 in) (57%) were less than the control (83%) (Fig. 1). Cuttings rooted in pellet size 30-65 mm (1.2-2.6 in) had more roots per cutting than the control (6.6 vs. 4.4, respectively), whereas cuttings rooted in pellet size 42-80 mm (1.7-3.1 in) had fewer roots than the control (2.2 vs. 4.4). The percentage of symmetrical root systems for cuttings rooted in pellet size 42-80 mm (1.7-3.1 in) (36%), was less than the control (71%). Total root dry weight for each pellet size was less than the control (Fig. 2).

Rooting percentage in June for softwood cuttings rooted in pellet size 36-65 mm (1.4-2.6 in) (77%) was higher than the control (64%) whereas rooting percentages for cuttings rooted in pellet sizes 42-80 mm (1.7-3.1 in) and 50-95 mm (2.0-3.7 in) (50% and 52%, respectively) were less than the control (Fig. 1). There was no significant difference between pellet size and the control for root number or symmetry (data not presented). Total root dry weights for cuttings in pellet sizes 25-65 (1.0-2.6 in) (0.05 g), 30-65 (1.2-2.6 in) (0.06 g), 36-65 (1.4-2.6 in) (0.05 g), and 42-65 mm (1.7-3.1 in) (0.07 g) were less than the control (0.1 g)(Fig. 2). Total root dry weight, excluding control data, increased linearly with pellet diameter (r^2 =.83; data not presented). Total root dry weights for all treatments in June were less than in January (Fig. 2).

In both experiments, cuttings in the smaller sized pellets rooted quickly, and with time, the roots elongated into adjacent pellet treatments. Roots extending beyond the pellet were severed when recording data, resulting in a reduction of total root dry weight. Cuttings in larger sized pellets [42-80 (1.7-3.1 in) and 50-95 mm (2-3.7 in)] rooted poorly. Due to increased diameter of larger sized pellets, cuttings were spaced farther apart in their respective pellet treatments. As a result, foliage density, which is important for intercepting mist and increasing relative humidity surrounding the cuttings, was less for cuttings in larger sized pellets compared to foliage density of cuttings in smaller sized pellets. Additionally, more of the mist delivered to the larger sized pellet treatments reached the surface of the pellet saturating the medium. The combined effects of

lower relative humidity surrounding the cuttings (Tukey, 1978) and increased media saturation (Geisler, 1965) might explain some of the decreased rooting of cuttings in larger pellet sizes. However, there was no significant differences regarding rooting percentages of the different pellet sizes. Approximately 400 rooted cuttings in pellets and Ray Leach Super Cells, from the June 1998 experiment, were planted in a field test in eastern Georgia in December 1998 to study the effect of pellet size and cutting development on first-year field growth.

Significance to Industry: In both experiments cuttings in the four smallest pellet sizes rooted well but had similar or lower total root dry weights than the control, whereas cuttings in the two largest pellet sizes rooted poorly yet had large total root dry weights. Therefore, an ideal use of Jiffy forestry peat pellets might involve rooting cuttings for a limited time at high density in smaller sized pellets, followed by transplanting to containers or the field. This method could use greenhouse space efficiently and decrease root growth into adjacent pellets. Additionally, this method would reduce transplant shock of cuttings being removed from rooting beds. Genera with larger leaf areas (e.g., *Rhododendron* L. and *Magnolia* L.), however, may benefit from increased spacing of the larger sized pellets.

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Table 1. Sizes of Jiffy forestry peat pellets used for rooting stem cuttings of loblolly pine.

Peat pellet size	Dry diam. (mm)	Expanded diameter (mm)	Expanded height (mm)	Expanded volume (cm^3)
25-65	25	28	65	35
30-65	30	33	65	60
36-65	36	39	65	80
36-75	36	39	75	90
42-65	42	46	65	110
42-80	42	46	80	140
50-95	50	55	95	250

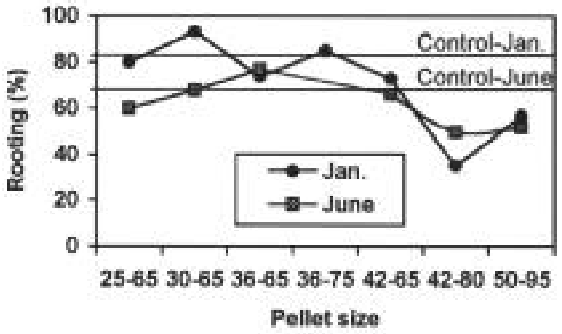

Fig. 1. Rooting percentage of stem cuttings of loblolly pine rooted in Jiffy forestry peat pellets. Legend also applies to Fig. 2. Symbols represent means, n=240.

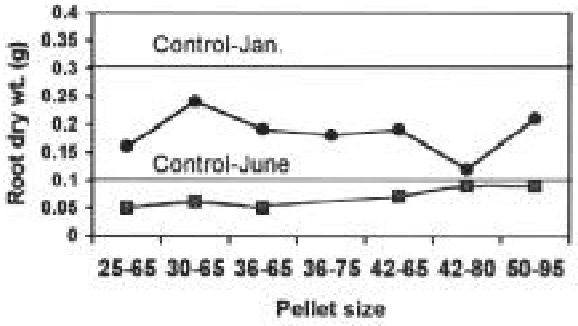

Fig. 2. Root dry weight for stem cuttings of loblolly pine rooted in Jiffy forestry peat pellets. Symbols represent means, n=240 for Jan., and n=120 for June.

Secondary Seed Dormancy of *Rhododendron catawbiense* and *Rhododendron maxiumum*

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Index Words: *Rhododendron catawbiense*, *Rhododendron maximum*, Seed Germination, Sexual Propagation, Seeds

Nature of Work: Seeds of *Rhododendron catawbiense* Michx. (Catawba rhododendron) and *Rhododendron maximum* L. (rosebay rhododendron) were germinated at 25°C (77°F) or an 8/16 hr thermoperiod of 25°/15°C (77°/59°F) with constant light after imbibed seeds were maintained in total darkness for 0, 9, 18, 27, 36, 45, 54, or 63 days at the same temperatures.

Results and Discussion: Maintenance of imbibed seeds of *R. catawbiense* in darkness at 25°C (77°F) for up to 63 days caused no induction of secondary dormancy while induction occurred for seeds in darkness at 25/15°C (77°/59°F). When imbibed seeds of *R. catawbiense* were subjected immediately to light following imbibtion, 30-day germination at 25°C (77°F) was 98% compared to 95% for imbibed seeds maintained in darkness for 63 days and then exposed to Glenn et al. 2 light. If germinated at 25°/15°C (77°/59°F), immediate light exposure resulted in 99% germination which decreased significantly to 76% after 63 days of dark treatment. Seeds of R. maximum maintained in darkness developed secondary dormancy at both temperatures. Thirty day germination of seeds subjected immediately to light following imbibition at 25°C (77°F) was 82% which decreased to 29% after dark treatment for 9 days. Further reductions in germination continued as the length of dark treatment increased with <10% germination after maintenance in darkness for 27 days. At 25/15°C (77°/59°F) induction of secondary dormancy was not as dramatic as that at 25°C (77°F). Without dark treatment, 30-day germination at 25/15°C (77°/59°F) was 99% which decreased significantly to 88% after dark treatment for 18 days. Reductions in germination continued up to 63 days with 67% germination. Partial removal of secondary dormancy in seeds of *R. maximum* was achieved by subjecting seeds to moist-chilling.

Significance to Industry: Seeds of *R. catawbiense* and *R. maximum* can be germinated when mature without any pretreatment but they require light for germination. Our research demonstrates, however, that when germinating seeds of these species, the seeds must be subjected

immediately to light following imbibition. If imbibed seeds are not exposed immediately to light or if exposure is delayed, secondary dormancy may be induced. Such dormancy will prevent germination and will require treatment for removal.

Influence of scarification treatments on the germination of Hairy Wild Indigo

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Index Words: Hairy Wild Indigo, *Batisia hirsuta*, *Baptisia calycosa* var. *villosa, Baptisia calycosa* var. *hirsuta*, Scarification

Nature of Work: Hairy wild indigo is a herbaceous perennial endemic to the sandhills and scrub of Florida with a range encompassing an area from Santa Rosa to Holmes counties (1). The species is considered threatened in Florida because of the limited number of individuals occurring in its natural range and the threat of native habitat loss (3). The taxonomy of this species has undergone revision and nomenclature may be somewhat confusing because different scientific names are used for this plant by Florida Natural Areas Inventory (FNAI) and the Florida Department of Agriculture and Consumer Services (FDACS). The species is presently known as Hairy wild indigo, *Baptisia hirsuta* Small (1) and additionally by the synonyms *Baptisia calycosa* var. *villosa* (pineland wild indigo; hairy wild indigo) (4) and *Baptisia calycosa* var. *hirsuta* (pineland wild indigo; hairy wild indigo) (2,5).

The plant has yellow pea-like flowers similar to the common wild blue indigo (*Baptisia australis*) but is unique to the fire-dependent community of the longleaf pine wiregrass ecosystem in the panhandle of florida. The attractive gray-green foliage, yellow flowers and tolerance of sandy soils of low moisture make this plant an excellent candidate as a garden perennial for low input landscapes. The species is distinguished by trifoliate leaves with cuneate to broadly oblanceolate leaflets less than 4 cm long, stems that are hirsute, a yellow to cream-colored corolla, and calyx lobes that are spatulate, exceeding the tube (1). Information on the germination requirements of this species is unavailable thereby limiting the potential for commercial production of the species for ecological restoration, preservation or landscape installation.

Seeds of hairy wild indigo were collected from roadsides throughout north Santa Rosa County, Florida in Nov. 1997. Five to fifteen seeds were collected per plant and a total of 1505 seeds were collected. Seeds were graded to remove deformed, damaged, or undersized seed and placed in an incubator at 90°F for 1 week (Nov. 14-20, 1997) prior to dark, cold storage in a sealed glass bottle for 10 weeks (Nov. 21, 1997-

Jan. 28, 1998) at 40°F. Stratification requirements for this species are presently unknown, however, maximum germination percentage was reported for wild blue indigo after stratification at 40°F for 10 weeks (6). On Jan. 29, 1998, seed were allocated to lots of 50 seeds, weighed and subjected to one of the following scarification treatments (4 replications of 50 seed; 200 seed per treatment). Treatments included mechanical scarification (scarification by hand utilizing a metal file to disrupt the seed coat at the micropilar end), hot water soak (seeds placed in boiling tap water, removed from heat source and allowed to remain in the water at room temperature for 24 hours, and chemical scarification (15, 20, or 25 min. soak in concentrated sulfuric acid followed by a tap water rinse and soak at room temperature for 24 hours).

Seeds were planted on 30 Jan. 1998 in 72 cell flat inserts with a single seed planted per cell. The germination medium was Metromix 360 Growing Medium and the seed were covered to a $^{1}/_{4}$ inch depth with Vermiculite No. 3. Flats were placed on a bench equipped with bottom heat and watered daily. Medium temperature was maintained from 82 to 84°F. The number of germinated seeds was recorded daily for a period of nine weeks. Treatment differences were determined using analysis of variance and repeated measures analysis and mean separation within a single date was determined using an LSD with a 95% confidence interval.

Results and Discussion: After cleaning and grading, 82% of woodland-collected hairy wild indigo seed remained for the germination experiment. Based on the weight of cleaned and graded seeds the number of seeds per pound for this species was estimated to be approximately 27,898 seeds/lb. Mechanical scarification was the best overall scarification treatment with greater than 50 % germination by week 2 and 78% germination by week 8 (Table 1., Figure 1.). Seeds subjected to hot water scarification were slower to germinate than mechanically scarified seed. Germination of 50% of the hot water scarified seed did not occur until 7 weeks after sowing. Percent germination did not differ between mechanical and hot water scarified seeds after week 7. Sulfuric acid treatments of 15, 20, or 25 minutes were not effective scarification treatments when compared to hot water or mechanical scarification.

Significance to Industry: This work provides an estimate for the number of hairy wild indigo seeds per pound and demonstrates the effectiveness of mechanical and hot water scarification treatments. Nursery professionals may expect germination results for mechanical and hot water scarified seeds to ultimately be similar but hot water scarified seeds may require up to 5 additional weeks to achieve germination percentages similar to mechanically scarified seed. Sulfuric acid

treatment for a period of time greater than 25 minutes may be required to adequately scarify hairy wild indigo seed, however, a maximum exposure time should be determined to prevent injury to the seed.

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HortScience 28:899-901.

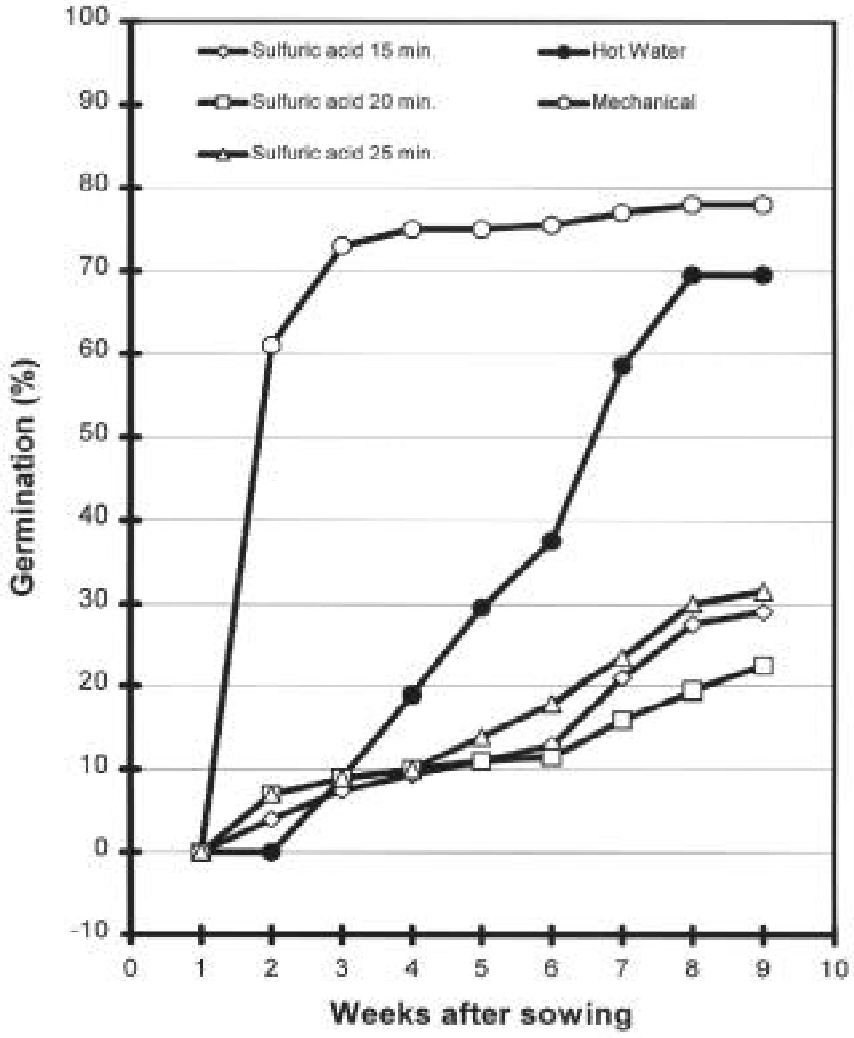
Table 1. Percent germination of *Baptisia hirsuta* following scarification.

	Weeks after sowing								
Scarification treatment[z]	1	2	3	4	5	6	7	8	9
Sulfuric acid 15 min.	0.0a	4.0b[y]	7.5b	9.5b	11.0c	13.0c	21.0c	27.5b	29.0b
Sulfuric acid 20 min.	0.0a	7.0b	9.0b	10.0b	11.0c	11.5c	16.0c	19.5b	22.5b
Sulfuric acid 25 min.	0.0a	7.0b	9.0b	10.0b	14.0c	18.0c	23.5c	30.0b	31.5b
Hot water	0.0a	0.0b	9.0b	19.0b	29.5b	37.5b	58.5b	69.5a	69.5a
Mechanical	0.0a	61.0a	73.0a	75.0a	75.0a	75.5a	77.0a	78.0a	78.0a

[z] Sulfuric acid = 15, 20, or 25 min. soak in concentrated sulfuric acid followed by a tap water rinse and soak at room temperature for 24 hours; Hot water = seeds placed in boiling tap water, removed from heat source and allowed to remain in the water at room temperature for 24 hours, and Mechanical = scarification by hand utilizing a metal file to disrupt the seed coat at the micropilar end.

[y] Means separation within rows by LSD alpha = 0.05. Means followed by the same letter do not differ.

Figure 1. Germination of Hairy Wild Indigo following scarification.



Chlorophyll Fluorescence and Rooting Stem Cuttings of *Taxus*

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Index Words: Propagation, Taxus, Chlorophyll Fluorescense

Nature of Work: Chlorophyll fluorescence is the small portion of light that is re-emitted from chlorophyll during the processes of photosynthesis. It is an estimation of photosynthetic efficiency providing an indirect measure of plant stress, potentially prior to human detection. Current applications include the detection/evaluation of environmental stresses such as cold tolerance (1), heat stress (2), and water stress (3), as well as nutrient deficiencies, irradiance levels, and air pollution (ozone). It has been used in micropropagation of Transvaal daisy (*Gerbera jamesonii*) (4), but there are no reported studies involving conventional propagation by stem cuttings.

If a quick, reliable method of determining potential rooting of cuttings based on the condition of a specific stock plant was available for propagators, then rooting success could be predicted prior to an investment in time, labor, and resources. The objective of this study was to examine chlorophyll fluorescence readings of ten cultivars of *Taxus* over the course of propagation and compare initial fluorescence measurements with subsequent rooting percentages.

Ten cultivars of *Taxus* were selected for the study: Brownii, Dark Green Pyramidalis, Dark Green Spreader, Densiformis, Densiformis Gem, Hicksii, L.C. Bobbink, Runyan, Tauntoni, and Wardii. Cuttings were taken in mid-October from field grown plants at Zelenka Nursery, Grand Haven, MI and were placed in cold storage at 2.5 °C (36 °F) for five weeks. Following storage, they were re-cut to a uniform length of 4.5 inches, treated with Woods Rooting Hormone (IBA 1.03%; NAA 0.66%) at 2800 PPM (5:1 ratio), and placed into a 100% perlite medium. The experiment followed a randomized complete block design consisting of six blocks and ten cultivars. There were 10 cuttings within each block/cultivar combination for a total of 600 cuttings. Periodic chlorophyll fluorescence measurements were taken with a Morgan CF-1000 Chlorophyll Fluorescence Measurement System throughout storage and rooting. Readings consisted of the ratio of variable fluorescence to

maximum fluorescence (F_v/F_m). Following 188 days in the rooting beds, cuttings were evaluated and rooting percentages were determined. Means for each group of ten cuttings were subjected to analysis of variance (ANOVA) procedures to determine significant effects and means were separated by an LSD test.

Results and Discussion: Fluorescence measurements exhibited a sharp decrease following severance from the stock plant which continued through storage and into the first two weeks in the propagation bed (Fig. 1). This decline in fluorescence measurements quantifies the increase in stress that the cuttings are enduring during the propagation period. Rooting occurred about 75 days after the original cutting was taken, at which point an increase in F_v/F_m can be seen. This increase continues as a general trend until harvest, when there is a slight drop in F_v/F_m. Changes in chlorophyll fluorescence over time differed among cultivars.

Differences among cultivars were also found in initial field chlorophyll fluorescence levels and final rooting percentages (Tables 1 and 2). Rooting percentages varied from a low L.C. Bobbink at 31.7% to Densiformis at 96.7%. F_v/F_m ratios ranged from .731 to .873. An overall correlation between the two was not found, indicating that chlorophyll fluorescence values need to be examined at the individual cultivar level.

Significance to Industry: Although there is some scientific value to our results, we do not feel confident that initial stock plant chlorophyll fluorescence measurements are a practical means of predicting rooting in *Taxus*. Initial values were not statistically correlated with subsequent rooting and we did not find a general threshold value that growers could use as a standard. Chlorophyll fluorescence threshold values would need to be determined for each individual cultivar. However, measurements did identify periods of stress during the propagation period.

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Table 1 ROOTING PERCENTAGE

Cultivar	Rooting %	
Densiformis	96.7	a b c
Wardii	88.3	a b c d
Densiformis Gem	85.0	a b c d e
Dark Green Pyramidalis	76.3	b c d e f
Runyan	70.0	c d e f
Hicksii	65.0	d e f
Dark Green Spreader	61.7	d e f g
Tauntoni	56.7	e f g
Brownii	46.7	f g
L.C. Bobbink	31.7	g

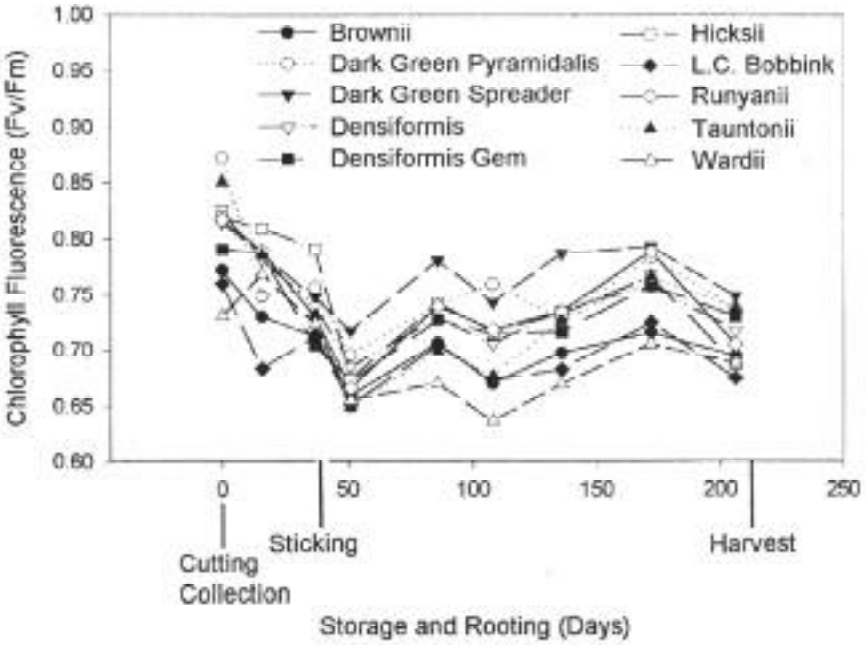
Mean separation among cultivars by LSD, P<= 0.05

Table 2 INITIAL CHLOROPHYLL FLUORESCENCE MEASUREMENTS

Cultivar	F_v / F_m*	
Dark Green Pyramidalis	0.873	a
Tauntoni	0.851	a b
Densiformis	0.826	b c
Hicksii	0.819	b c
Runyan	0.817	b c
Dark Green Spreader	0.815	b c
Densiformis Gem	0.791	c d
Brownii	0.772	d
L.C. Bobbink	0.760	d e
Wardii	0.731	e

*Ratio of variable fluorescence to maximum fluorescence
Mean separation among cultivars by LSD, P<= 0.05.

Figure 1. CHLOROPHYLL FLUORESCENCE IN *TAXUS* OVER THE COURSE OF PROPAGATION



Effect of Cell Pack Size During Propagation of Strawberry

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Index Words: Strawberry, Plug Plant Production, Bedding Plants, Propagation

Nature of Work: Due to ease of rooting, vegetative means are the preferred method of strawberry (*Fragaria* x *ananassa* Duch.) propagation (1). Propagation of plug plants offers advantages to greenhouse growers over bare-root plant production, as well as providing farmers with quicker field establishment. Although plug plant production is not a new concept, advantages of plug plants in annual hill plasticulture in recent years has prompted research to improve methods of plug plant production (9).

Literature Review: Limited information is available on strawberry plug plant production (1,9), however there are a number of studies of effects of container size on floral and vegetable transplants. Larger containers are important for retailers of flower bedding plants due to extension of plant shelf life (3). Root restriction from smaller containers reduces lateral branching and leaf expansion and delays flowering in salvia bedding plants (10). Larger containers increase yields of many vegetables (4,6,7,8,11,12).

Increased yields from plants which were grown in larger cell trays/packs are attributed to increased physical size or vigor of transplants from larger cells (2,4,11), absence of root volume restrictions (6,7,8,12), or the greater availability of soil volume or nutrients (6,8). Vegetables whose production is a measure of fruit produced versus those where yield is derived from vegetative or floral structures also seem to respond to increased transplant container size. Increased yields were found with pepper (11) and tomato (6) when transplants were grown in larger-sized containers. Kemble (5) found tomato transplants grown in larger containers and then field planted had fewer days until early bloom while others (12) reported increased early yield without an increased overall yield. Plug plant propagators generally use 60-cell rigid plastic trays (9), but it is not known if cell size used in production of the transplant affects subsequent yields of strawberry.

Materials and Methods: The purpose of this study was to determine the effect of cell size used in strawberry plantlet propagation and production on fruit yield. Strawberry plantlets were rooted in cell packs of four

different volumes commonly available for bedding plant production: 32-cell (143 cm^3), 48-cell (88 cm^3), 60-cell (70 cm^3) and 72-cell (53 cm^3). Strawberry plantlets were collected from mother plants of 'Chandler' (*Fragaria* x *ananassa* Duch.) that had fruited the previous spring at the Auburn University Chilton Area Horticultural Substation (CAHS) in Thorsby, Ala. (32_ 51'lat. x 85_ 38' long.). Collected plantlets were inserted in cell packs of the various sizes containing Pro Mix BX media (Premier Horticultural Products, Red Hill, PA) on 16 Sept., 1992 and 21 Sept., 1993. Plantlets were propagated in Auburn, Ala. for eventual field planting at the Gulf Coast Substation (GCS), Fairhope, Ala. (30° 33' lat. x 87° 53' long.). Also, on 27 Aug., 1993, plantlets of 'Chandler' grown in Canada obtained from Strawberry Hill, Inc., Mooresville, NC were inserted in 3 sizes of cell pack containers: 48-cell, 60-cell and 72-cell-containing Pro Mix BX media for field planting at CAHS.

Plantlets were placed under intermittent mist with an on/off cycle of 5 sec/2 min under natural light conditions for one week until root formation and then placed on a greenhouse bench receiving manual watering twice daily, weekly fertilization, and fungicidal sprays twice weekly for approximately 3 weeks.

The field planting was arranged in a complete randomized block planting with 6 replications of 14 plant plots for each treatment. Plants were field planted at GCS on 23 Oct., 1992 and 13 Oct., 1993; and at CAHS on 4 Oct., 1992 on raised beds covered with black plastic mulch. At CAHS field plots received 50 lbs/a of N, P, and K preplant fertilizer. Soil tests indicated no need for lime (pH = 6.0). Methyl bromide was applied under the plastic mulch at a rate of 150 lbs/a. Plots also received fertilizer applied through irrigation water at a rate of 1 lb/a N and K2O per day beginning 13 April, 1994 through 13 May 1994. At GCS plots received 2 t/a dolomite, 500 lb/a of 13-13-13 fertilizer based on soil tests prior to planting and were also treated with methyl bromide under black plastic mulch at 150 lbs/a. Plants were irrigated by subsurface drip irrigation and received weekly fertilization of 10 gal/a of 7-0-7 (7 lb/a) N and K_2O per application in irrigation water from 16 Feb., 1994 through 18 May, 1994.

At GCS the two outside plants on either end of each plot served as guard plants and were not harvested, leaving 10 interior plants for harvest. In 1993, harvest began 15 Feb. continuing at 3-5 day intervals until 31 May. In 1994 harvest began 21 March and continued at 3-5 day intervals until 23 May. At CAHS all plants in each plot were harvested at 3-5 day intervals beginning 13 April and ending 26 May 1994.

Results and Discussion: In the trial at CAHS involving 48-cell, 60-cell and 72-cell packs there were no differences in any of the yield factors (Table 1). Mixed results in total yield were found at the GCS (Table 2). In 1993, all container sizes produced greater total yield than the industry "standard" 60-cell pack, but there were no differences in total yields between 32-cell, 48-cell and 72-cell packs. In 1994 no differences in total yields were found among the 4 treatments, and no differences were found for early yields or berry weight at GCS for in 1993 or 1994 (Table 2).

Significance to Industry: Because there were no differences between 60 and 72-cell packs, growers might consider using the smaller cell packs. Using 72-cell over 60-cell packs offers an advantage of producing more plug plants in the same greenhouse area without compromising total yields, early yields or berry size (Table 1; Table 2). While this study suggests that greater efficiency in greenhouse space may be achieved through utilizing 72-cell (53 cm^3) inserts, it is possible that even greater efficiencies could be achieved if even smaller container sizes do not negatively affect production.

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Table 1. Effect of cell pack size on yield and berry weight of 'Chandler' plug plants, Chilton Area Horticultural Substation, 1994

	Total yield per plant (g)			Average
Treatment	Early Harvest	Late Harvest	Total Harvest	Berry Weight
48 cell	324.70a[z]	206.63a	531.33a	14.57a
60 cell	292.62a	236.44a	529.06a	14.19a
72 cell	250.36a	235.91a	486.27a	14.27a

[z]Mean separation by Duncan's Multiple Range Test, considered significant at P=0.05.

Table 2. Effect of cell pack size on yield and berry weight of 'Chandler' plug plants, Gulf Coast Substation, 1993 and 1994.

	1993		1993		1994			1994
	Total yield per plant (g)			Avg. Berry	Total yield per plant (g)			Avg. Berry
Treatment	Early Harvest	Late Harvest	Total Harvest	Weight	Early Harvest	Late Harvest	Total Harvest	Weight
32 cell	133.67a[z]	366.35a	500.02a	14.81a	538.47a	192.27a	730.74a	6.00a
48 cell	124.57a	430.68a	555.25a	14.47a	47.32a	181.45a	651.77a	5.40a
60 cell	100.31a	315.19	415.50b	14.31a	461.71a	208.12	669.83a	5.28a
72 cell	128.61a	404.35a	532.96a	14.32a	498.74a	190.90a	689.64a	5.56a

[z]Mean separation by Duncan's Multiple Range Test, considered significant at P=0.05.

Propagation of Prickly-Pear (*Opuntia amyclaea* Tenore) Cactus Cladodes: Influence of Auxin and Wounding

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Index Words: Propagation Prickly-Pear, *Opunita amyclaea* Tenore, Auxin Influence, Wounding Influence

Nature of Work: Prickly-pear cactus (*Opuntia amyclaea* Tenore) is native to the American hemisphere and is cultivated in other areas of the world, including Brazil (300,000 ha), Italy (100,000 ha), and Chile (50,000 ha) [Bravo, 1978; Pimienta, 1994]. Prickly-pear cactus is primarily cultivated for its fruit production, which includes a wide variety of fruit flavors and colors (Pimienta, 1994). Young cladodes are also used as spring vegetables or consumed as relish, while older cladodes are used for animal consumption. Due to its low water requirements and high adaptability, prickly-pear cactus has become important as an alternative fruit and vegetable crop for other world regions (Pimienta, 1994). Valuable byproducts include wine, candies, and the pigment, acetocarmine (Barbera, 1995).

Prickly-pear cactus can be propagated from seed, cladodes and other asexual methods such as grafting and tissue culture (Estrada-Luna *et al*., 1994). Whole cladodes are most commonly used for orchard establishment. However, other alternative methods include the division of the cladodes into small sections, which increases propagule numbers and reduces production costs (Barrientos and Brauer, 1964). Suberization of the wounded area where the cladodes are detached from the stock plants is a common practice to prevent fungal infections.

There is an increased need to integrate studies on prickly-pear cactus growth systems and improve the efficiency of the propagation process (Fabbri *et al*., 1996). Indole-3-butyric acid has been reported to produce more roots on *Opuntia ficus-indica* (L.) Mill (the Indian fig or tuna) under *in vitro* conditions (Escobar *et a*l., 1986; Mohamed-Yasseen *et al*., 1995). Naphthalene acetic acid (NAA) has been reported to increase rooting of young shoots and increase cladode weight of *Opuntia ficus-indica* (Mulas *et al*., 1992). Though not commercially done, treatment with auxins may be an advantage for enhancing rooting of prickly-pear cladodes over a shorter propagation period to improve field establishment.

The objective of this current study was to evaluate the effect of auxin, different wounding methods and suberization on the rooting of cladodes of prickly pear-cactus. These findings are important for developing techniques that enhance propagation efficiency and lead to faster orchard establishment. Because asexual propagation practices vary widely among growers, traditional and nontraditional methods of wounding were analyzed.

Results and Discussion: Mature cladodes of prickly-pear cactus (*Opuntia amyclaea* Tenore. cv. Reina) were treated with five wounding methods and four concentrations of potassium salt indole-3-butyric acid (K-IBA) to stimulate adventitious root formation. The wounding method and K-IBA had highly significant effects on root number and root dry mass of cladodes. Interaction between K-IBA and wounding methods showed that greater root number was obtained at the higher auxin concentrations and with wounding methods that had the greatest cut surface area. Indole-3-butyric acid (potassium salt) concentrations from 4,144 to 41,442 (M (1,000 to 10,000 $mg.L^{-1}$) increased root dry mass. Only the wounding method affected rotting of cladodes. Treatments allowing suberization had a higher percentage of non-rotted cladodes. This research validates the commercial practice of allowing cladodes to suberize early in the propagation cycle. Indole-3-butyric acid (potassium salt) altered rooting polarity and stimulated adventitious root formation along the wounded cladode surfaces. The vertical non-suberized wounding methods and auxin treatments are an excellent classroom demonstration for manipulating rooting polarity. Auxin application and wounding could be of commercial benefit for enhanced rooting in the clonal regeneration of new selections for prickly-pear cactus orchards.

Significance to Industry: Our findings show a positive effect of K-IBA at higher rates (4,144 to 41,442 µM) and wounding methods on the root number and root dry mass of prickly-pear cactus cladodes. We conclude that increasing K-IBA concentrations linearly increased root number in all wounding methods. Root length decreased with increasing K-IBA concentration. Treatments with 4,144, and 12,433 µM of K-IBA in basal suberized and pre-suberized wounding methods had the best root system in terms of root number and length (Fig. 2a and 2b). Basal suberized and pre-suberized treatments had the higher root dry mass and K-IBA application increased root dry mass per cladode of prickly pear cactus.

Suberization for at least three days increased the percentage of non-rotted cladodes. Increasing K-IBA concentrations with the vertical non-suberized wounding method makes an excellent classroom demonstra-

tion for manipulating rooting polarity. Treatment with 4,144 µM K-IBA and wounding at the basal surface followed by suberization are alternative techniques for enhancing root system development that should be commercially tested for the establishment of prickly pear cactus orchards.

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Testing Parentage of Flowering Dogwood (*Cornus florida*) Seedlings

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Index Words: DAF, DNA Fingerprinting, Cultivars of *Cornus florida*

Nature of Work: New cultivars of flowering dogwood (*Cornus florida*, L.) are usuallly selected as sports from existing cultivars or developed from seedlings that show new and interesting horticultural traits. Although there have been relatively few efforts to develop dogwood improvement programs, breeding historically has been accomplished by tedious hand pollination, which is labor intensive and expensive (4, 7). Insect-mediated pollination has been attempted with some success (5, 7). In other trials that compared open-pollinated with hand-pollinated dogwood flowers, results have been mixed (2, 4). We tested open-pollinated seeds from *C. florida* 'Cherokee Chief' using molecular marker techniques to identify pollen sources (1, 7). Our hypothesis was that pollen was donated only from nearby trees.

In fall 1997, berries were harvested from a *C. florida* 'Cherokee Chief' tree growing in close proximity to two other mature *C. florida* trees varieties, 'Cloud 9' and 'Cherokee Brave'. Since dogwoods are considered self-sterile (2, 3), we assumed that pollination occurred by either pollen donated by the nearby dogwood trees or by rogue pollen. Approximately one hundred berries from 'Cherokee Chief' were soaked in tap water for 2 days, depulped, and seeds cold-stratified in moist sand and peat moss (1:1) in a zip lock bag at 4 C (40 F) for 4 months. In the greenhouse, seeds were planted in composted pine bark and Pro Mix BS (3.5 :1), and after germination, seedlings were fertilized weekly with Peters' 21- 7-7 acid special at 200 ppm N. In about eight weeks, the second set of young, not fully expanded leaves of twenty-eight seedlings, along with young leaves from 'Cherokee Chief', 'Cherokee Brave' and 'Cloud 9' were harvested. Leaves were frozen in liquid nitrogen and stored at -80 C (-112F). Subsequently, DNA was extracted from the leaves with a PureGene kit (Gentra, Minneapolis, MN). As an outgroup, we also isolated DNA from leaves of the following C. florida cultivars: 'Pygmy', 'Fragrant Cloud', 'Springtime', 'Cherokee Princess', 'Cherokee Daybreak', and 'Appalachian Spring'. DNA Amplification Fingerprinting (DAF) was performed on genomic DNA of each of the cultivars and seedlings, according to the method described by Trigiano and Caetano-Anolles (6). Seven octomer primers were used in DAF. Data (finger-

prints) were recorded as a binary code (1 for band present, 0 for absent bands) and subjected to cluster analysis and principal component analysis using NTSYS version 2.01 (Exeter Software, Setauket, NY) (1, 6) to determine parentage of the seedlings.

Results and Discussion: A total of 191 loci (bands) (average 27.3 per primer) were compared among the progeny, putative parents, and the outgroup. According to the statistical analysis, each seedling was more closely related to other seedlings and to 'Cherokee Chief' and 'Cherokee Brave' than to any other cultivar tested (Figure 1). Although in the field 'Cloud 9' was in close proximity to 'Cherokee Chief,' none of the seedlings was very closely related to that cultivar. Since 'Cloud 9' flowered slightly earlier than 'Cherokee Brave' and 'Cherokee Chief,' it is possible that 'Cloud 9' pollen was not available (5). In this case, crosses were carried out simply by having flowering trees grouped closely together, rather than pollinating by hand or supplying honey bees (4, 5, 7).

Significance to Industry: Breeding dogwood trees for various disease and pest resistance is a practical solution to the problems faced by many nurserymen and homeowners. However, pollinating trees by hand has been an arduous task, which necessitated supplying controlled environments (cold storage). According to our results, desired crosses can be achieved with open pollination in carefully chosen locations that minimize nearby sources of rogue pollen. This almost labor-free method of cross pollination can accomplish the same result as meticulous hand pollinations or using honey bees (4, 5, 7). Furthermore, no matter how seeds are produced, seedlings can be tested using PCR and DAF to identify the pollen donator in about a week (1, 7).

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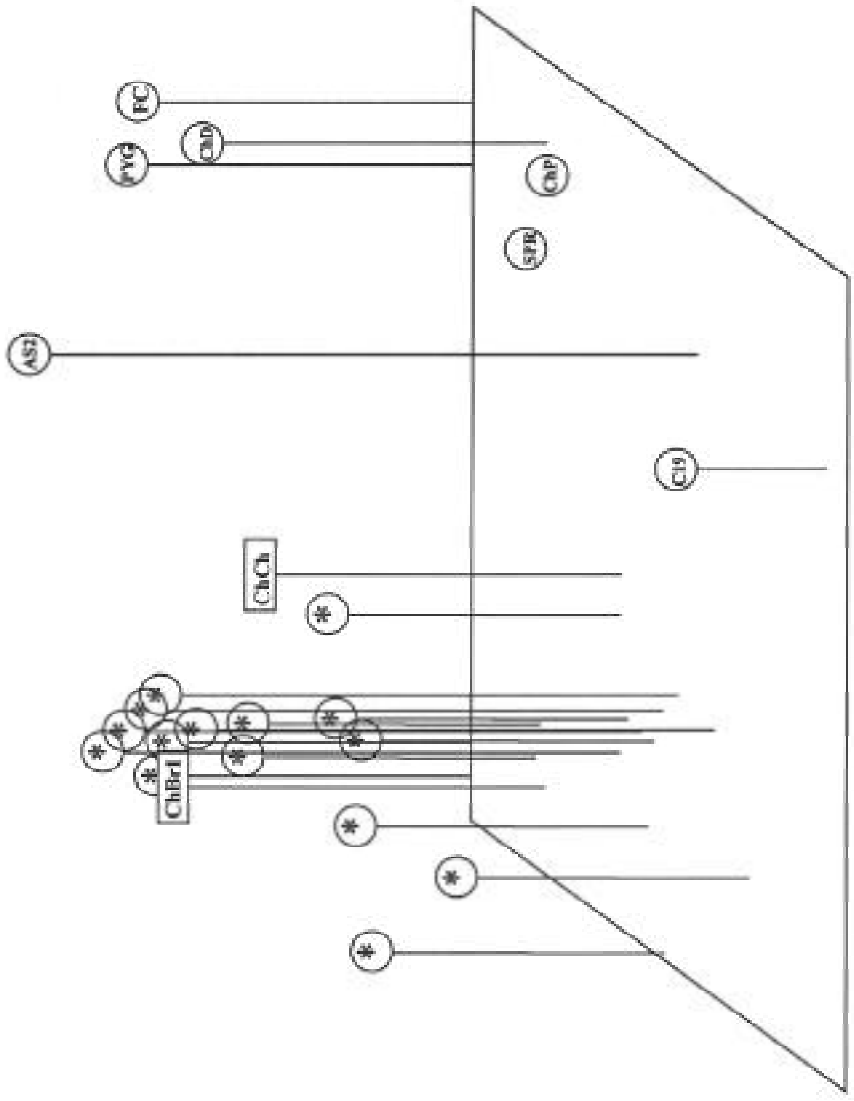

Figure 1. Principal Component Analysis of data from DAF, using seven octomer primers, 191 loci (bands). Distance between points represents genetic similarity. *-hybrid seedlings, ChCh- 'Cherokee Chief', ChBr1- 'Cherokee Brave', Cl9-'Cloud 9', AS2-'Appalachian Spring', SPR-'Spring-time', ChP- 'Cherokee Princess', ChD-'Cherokee Daybreak', PYG- 'Pygmy', FC-'Fragrant Cloud'

Germination of Woody Plants Using Coir as a Peat Alternative

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Index Words: Seed Germination, Peat, *Magnolia grandiflora*, *Rhamnus caroliniana*, *Callicarpa dichotoma*, *Cornus florida,* Coir

Nature of Work: Peat moss is currently a major component of container mixes for commercial plant production. However, ecologists say peat is harvested from fragile wetlands at rates which are non-sustainable (1). The peat industry maintains that they can manage the use and preservation of the bogs. They also understand that peat alternatives must be considered to meet environmental concerns of consumers. Conservationists continue to suggest the reduction of peat moss use, but adequate substitutes are hard to find. Rice hulls, wool and fiberglass materials have been tried (1), but none of these have met the quality and satisfaction received from peat-based mixes.

One recent alternative is coir, the thick, fibrous mesocarp of *Cocos nucifera*, coconut fruit, and it is showing promising results. The long fibers are extracted from the coconut husk and used in manufacturing of rope, floor mats and rugs. The remaining short fibers and dust are used for growing media. Until the early 1980's, the waste product of the above mentioned manufacturing had no significant use. It is now marketed as a peat moss alternative to the horticulture industry (5).

Coir has several characteristics, which make it a suitable alternative to peat. Coir is similar in appearance. It has a rust or reddish brown color and consists primarily of lignin and cellulose fibers. Coir does not contain sticks or weeds. Water-holding capacity of coir is similar to that of peat (4). Coir has acceptable pH, cation exchange capacity, and easier wetability (4). Coir usually has a higher electrical conductivity. Although these levels are considerably higher than peat moss, excess salts are easily leached under irrigation (3). The objective of this experiment is to compare the germination of seed from four woody plant species in coir- and peat-based growing media.

Seeds of *Magnolia grandiflora*, *Cornus florida*, *Rhamnus caroliniana* and *Callicarpa dichotoma* were collected, cleaned and visually inspected. Seeds were sorted and placed in moist, sand filled 6.5 inch x 5.5 inch poly bags for stratification. *C. dichotoma* was not stratified as it needs no

stratification for germination (2). All other seeds were then placed in a laboratory cooler. The seeds were stratified for 93 days at 37F. Following stratification, samples were cleaned and re-inspected to remove any discolored seeds prior to planting.

Three growing media were mixed and used for the experiment. Media treatments were 100 % coir (The Crystal Company, St. Louis, MO), 70% coir :15% vermiculite : 15% perlite (CVP), and Premier's Pro-mix BX. Eight 128 cell plug trays were used for each media treatment and the four species were placed in a random complete block design (RCBD) in each tray (32 seeds of each species per tray).

On 6 Feb 99, all seeded flats were placed in a misting greenhouse to start germination tests. Germination was checked on 22, 31, 40, and 55 days after placement in the misting greenhouse. Germination was determined to be when cotyledons emerged from the media. All data was tested using analysis of variance (ANOVA). If differences were found, data was further tested using Least Significant Difference (LSD) at $P = 0.05$.

Results and Discussion: *Magnolia grandiflora* and *Rhamnus caroliniana* germination in the 100% coir-based media was significantly greater than those germinated in the peat-based media. Germination in the 100% coir was 41.75% for *Magnolia* and 57.5% for *Rhamnus* 55 DAT (Table 1). Germination of in Promix and CVP media was significantly less. *Callicarpa dichotoma* also showed results similar to the *M. grandiflora* and *R. caroliniana* germination data. Germination increased from 12.5% to 60.5% on 22 DAT to 55 DAT. There was little germination of seeds sown in Pro-mix and CVP media. *Cornus florida* germination was very minimal. The low germination may be due to low viability of the seed lot or not meeting stratification requirements. *C. florida* needs a minimum of 100 to 130 days of cold stratification (2). Seeds were stratified for only 93 days.

Coir is beginning to be marketed throughout the horticulture industry. Coir appears to be an acceptable substitute for peat moss as a germination media. Three species had higher germination in the 100% coir media. When amendments, such as perlite and vermiculite, were added to the coir, germination percentages declined with similar results in Promix media. Increasing the media's air space may have reduced the germination percentages in these media. Additional study is needed to determine if coir has consistent germination enhancing qualities. The primary decisions on whether or not to use coir instead of sphagnum peat moss will most likely be due to accessibility and economic comparison. Coir is cost competitive with sphagnum peat moss. The primary

advantage of coir over sphagnum peat, if all other aspects were presumed equivalent, would be that coir is a renewable resource.

Significance to Industry: The results of our work indicate that using coir as a stand alone media increases germination percentage or woody plant seeds. This suggests that coir may have properties that would be beneficial in germinating seeds having low germination percentages or seeds with decreased viability.

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Table 1. Germination of three woody ornamental species in coir and peat germination media.

	Magnolia grandiflora				*Rhamnus caroliniana*				*Callicarpa dichotoma*			
	Days After Treatment											
Media	22	31	40	55	22	31	40	55	22	31	40	55
100% Coir	8 a[z]	24 a	37 a	42 a	12 a	30 a	54 a	57 a	12 a	27 a	47 a	66 a
CVP	0 b	0 b	0 b	0 b	0 b	0 b	12 c	12 c	0 b	0 b	0 b	0 b
Promix	0 b	1 b	4 b	5 b	0 b	0 b	29 b	33 b	0 b	0 b	1 b	4 b

[z]Mean separation within columns by least significant difference (LSD), P=0.05.

Tissue Culture of Medicinal Plants

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Index Words: Tissue Culture, St. John's Wort, Goldenseal, Purple Coneflower, Mullein

Nature of Work: In vitro culture of plant material enables the manipulation of growth and morphological development, biochemical metabolism and the production of secondary plant products from which most pharmaceuticals are derived. Several medicinally important plants are being studied with the objective to establish tissue culture protocols. Extracts of various coneflowers, *Echinacea purpurea* L., *E. angustifolia* DC., *E. pallida* Nutt. are used in pharmaceutical preparations known for their immunostimulating properties (Schulthess et al., 1991) and are used to treat chronic and recurrent infections of respiratory and urogenital organs, chronic inflamation/allergies, tonsillitis and sinusitis, infected wounds, chronic bronchitis, and malignant diseases (Bauer and Wagner,1991). St. John's Wort (*Hypericum perforatum* L.) is used as a sedative, as an astringent, to relieve anxiety, and currently as an antidepressive. Goldenseal (*Hydrastis canadensis* L.) was used for eye and skin ailments, and as a diuretic, a stimulant, and a treatment for ulcers and dropsy (edema) (Duke, 1989). Common mullein (*Verbascum thapsus* L.) has been historically used as a treatment for inflammatory disease, asthma, coughs and migraine headaches (Millspaugh, 1974).

In vitro cultures were initiated using seedlings grown from disinfested seeds, or from plants grown either in the field or in the greenhouse. Explants tested included hypocotyl, shoot and root sections, petiole segments and leaf discs. Plant materials were disinfested with a clorox solution containing Tween 20 (2-3 drops/100ml) followed by several rinses in distilled, sterile water. Basal media consisted of Murashige and Skoog Miminal Organics Medium (MSMO; Murashige and Skoog,1962), except studies with Goldenseal which used Gamborg's B-5 (Gamborg,1968), and with 30 g/l sucrose and 8 g/l agar at pH 5.7. Hormones included in media trials included the auxins IAA (indoleacetic acid), NAA (naphthalene acetic acid) and, 2,4-D (2,4-dichlorophenoxyacetic acid), and the cytokinins BA (benzyladenine) and kinetin. Cultures were incubated at 25 C under a 16-hr photoperiod (22-28 µmole/m^2/s). Morphological characteristics evaluated were: formation of callus, and presence and numbers of shoots and/or roots.

Results and Discussion: Tissue culture protocols have been established for St. John's Wort, Goldenseal, Purple Coneflower and Common Mullein. Depending on hormone regime, callus, or shoots and roots can be induced (Table 1). Micropropagation was achieved with all four plants and plants were transplanted to pots in the greenhouse or growth chamber. Plant form and flower color were true to form of the native plants. Multiple shoots per explant were obtained with St. John's Wort, Goldenseal and Mullein, whereas a single plant per explant was obtained with Purple Coneflower.

Significance to Industry: The in vitro culture of medicinally important plants could provide an alternate approach to the traditional ethnobotanical search for plants that produce pharmaceuticals (Cox and Balick, 1994), or to the production of plant products. In vitro culture of plants known to produce pharmaceuticals would allow investigations into the metabolic pathways, the optimization of product production in an in vitro culture (possibly including cell suspensions with a fermentation approach), and the propagation of medicinally important plants for specific large scale production. In vitro production of difficult-to-propagate plants or endangered species, some of which may grow in remote areas of the world, would facilitate isolation and production of their pharmaceutical components. As the demand for medicinal (herbal) plants continues to increase and wild crafting (harvest of native plants from the wild) becomes limiting, there will be greater opportunities for commercial growth of these plants.

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Table 1. Tissue culture of medicinal plants: hormonal regime, and morphological characteristics observed.

Plant/Hormone Regime	Explant	Characteristic
St. John's Wort (*Hypericum perforatum* L.)		
IAA (0.1-1 mg/l) + BA (0.5-2 mg/l)	nodal segments	callus, shoots, roots
IAA (0.1-1 mg/l) + BA (0.5-2 mg/l)	axillary shoots	callus, shoots, roots
IAA (0.1-1 mg/l) + BA (0.5-2 mg/l)	hypocotyl	callus, shoots, roots
IAA (0.1-1 mg/l) + BA (0.5-2 mg/l)	leaf discs	callus, shoots, roots
Purple Coneflower (*Echinacea purpurea* L.)		
NAA (1-3mg/l) + kinetin (1-2 mg/l)	hypocotyl	callus, shoots, roots
2,4-D (0.5-1 mg/l) + 1-2 kinetin (1-2 mg/l)	hypocotyl	callus, shoots, roots
Goldenseal (*Hydrastis canadensis* L.)		
NAA (0-1 M) + BA (0-10 M)	leaf	callus, shoots, roots
NAA (0-1 M) + BA (0-10 M)	root segments	callus, shoots, roots
Common Mullein (*Verbascum thapsus* L.)		
BA (1-5 mg/l)	leaf discs	shoots
BA (1-5 mg/l)	petiole segments	shoots
BA (1-5 mg/l)	root segments	shoots
BA (3 mg/l) + 2,4-D (0.1, 0.5, 1 mg/l) or BA (3 mg/l) + NAA (1,3, 5 mg/l)	leaf discs	shoots

Induction and Breaking of Bud Dormancy in American Elms

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Index Words: Elm, *Ulmus americana* L, Dormancy, Propagation, Chilling

Nature of Work: American elm (*Ulmus americana* L.) was at one time one of most favored trees for planting in urban areas because of its graceful vase-shaped crown, rapid growth habit, adaptability to wide range of climate and soil conditions, and tolerance to air pollution. It was an important component of landscapes throughout the United States. However, since its introduction to the North America in late 1 920's, Dutch elm disease (DED) has caused losses of millions of American elm trees. Today, American elms are rarely used in landscaping because of their susceptibility to DED. Development of American elms resistant to DED is of primary importance to the forest ecosystems, forest recreational industries and the nursery industry. The breeding of DED-resistant cultivars will increase the production and sale of American elms, and benefit the America's nursery industry.

Ai Tennessee State University, we are anempting to incorporate disease resistance genes into American elms. While young leaves are generally desirable materials for use in genetic transformation research using *Agrobacterium* infection, they are only available for a relatively short period of time during the spring season. In order to make young leaves available all the times for our research, we have developed techniques for inducing bud-break in American elms. This paper describes the techniques and reports the results from our studies.

Two-year-old American elm seedlings were used in the present study. They were grown in a greenhouse in 5-gallon pots filled with a mixture of 1:1:1 topsoilCanadian sphagnum peat-perlite (v:v:v). The temperature in the greenhouse was not controlled. Five plants were used for each treatment in all three experiments.

The objective of the Experiment 1 was to promote new growth on plants with mature foliage. In late July 1995, American elm plants were either pruned, sprayed with 500 ppm BA, or subject to chilling at 5C° for 30, 60, or 90 days. For the pruning treatment, shoots were cut back approximately 114 to 1/3 from the apical buds. After pruning, plants were maintained in the greenhouse, as were plants that had been sprayed with 500

ppm BA once a week for 8 weeks. Another group of plants was defoliated by hand and placed in a growth chamber. The temperature of the chamber was first set to 15C°, and then gradually decreased to 5C° over a week. After the trees were acclimated to the growth chamber, they were maintained for an additional 30, 60 or 90 days before they were returned to the greenhouse. The number of growing buds on each tree was recorded every five days for two months following the treatments.

Experiment 2 was conducted in iate July 1996. The objective of this study was to compare the effects of hand and chemical defoliation on bud-break prior to chilling. Trees were defoliated either by hand or by foliage application of ethyphon. For each treatment, five plants were sprayed once to run-off with 100, 500 or 1000 ppm ethyphon. After treatment, trees were then transferred into the growth chamber at 5C°. Trees with intact leaves were also stored as controls. After 60 days, the plants were removed from the growth chamber and maintained in the greenhouse. Data collection was performed as the Experiment 1.

Experiment 3 was designed to evaluate the effects of long term storage of dormant trees at 5C°. Ten trees were removed from the greenhouse and placed outside in early November 1997 to induce dormancy under natural conditions. Five of these plants were transferred into the growth chamber in early February 1998 and stored until almost all buds initiated new growth. The rest of plants were kept outside as controls. The number of growing buds was recorded every two weeks starting when bud break began.

Results and Discussion: Results of Experiment 1 are illustrated in Fig. 1. Neither pruning nor BA application promoted new growth in the elm seedlings. Bud-break was observed beginning 15 days after plants were removed from cold storage in all chilling treatments. The accumulative percentage of bud-break increased dramatically after 15 days in the greenhouse for plants that were in cold storage for 60 and 90 days. After 35 days in the greenhouse, approximately 90 percent of all buds initiated new growth in seedlings that were maintained in cold storage for 60 or 90 days, as compared to 38 percent for the 30 days chilling treatment. From this study, it is estimated that the American elms require a minimum of 1,500 chilling units, which is less than typical for plants native to the temperate zone (1).

Leaves of the control plants and plants that were sprayed with 100 ppm ethyphon dropped off after approximately 30 days after in the growth chamber, while defoliation occurred within 10 days in plants that were sprayed with 500 or 1000 ppm ethyphon. The application of 100 ppm ethyphon did not have any effect on bud-break (Fig. 2). Plants that had

been defoliated by hand or with the use of 500 - 1000 ppm ethyphon had a significantly higher percentage of bud-break than the control plants. However, increasing the concentration of ethyphon above 500 ppm resulted in no additional increase in bud-break.

Based on the observations in 1998, American elm seedlings initiated new growth by the end of March under the natural environment (Fig. 3). The percentage of bud-break increased dramatically during the month of April, and eventually 92% of the buds grew into shoots. When dormant plants were maintained in 5C° cold storage, they initiated new growth in mid-July. It is surprising that dormant elm trees could grow at temperatures as low as 5C°. Nonetheless, the growth of dormant plants could be delayed for 16 weeks by cold storage in a growth chamber.

In the present study, we developed techniques to induce or delay bud break in American elms. These techniques not only allow us to make plant materials available at all times for plant tissue culture research, but also can be adopted by nursery growers to expand their propagation and shipping seasons.

Significance to Industry: The techniques for the induction and breaking of bud dormancy described herein will enable nursery growers to produce new softwood growth year-round. This growth can be used either as cuttings for rooting, or as explant materials for micropropagation. Cold storage of dormant elm plants allows nursery growers to increase the duration of shipping season of bare-root plants.

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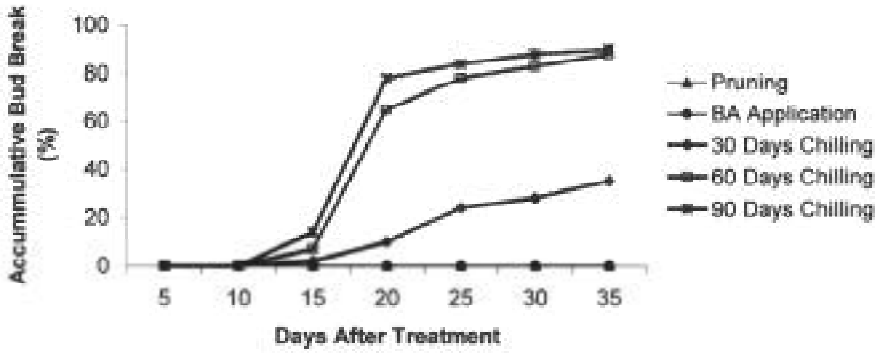

Fig. 1. Effect of chilling treatment on new growth in American elm seedlings.

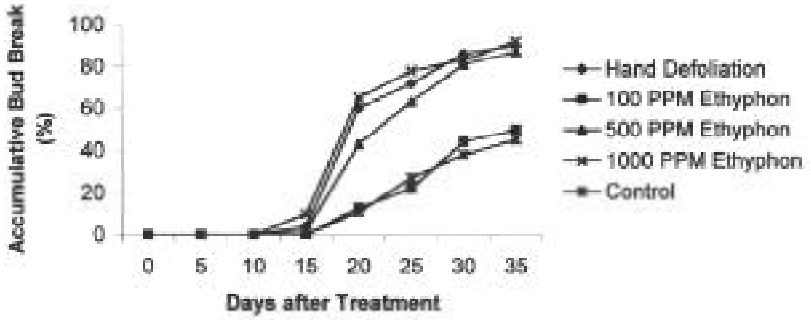

Fig. 2. Effect of leaf dropping methods on new bud breaking in American elm seedlings.

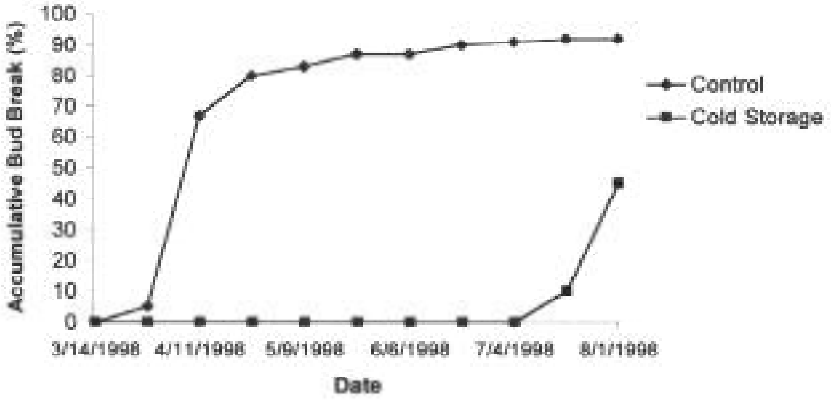

Fig. 3. Delay of bud break in American elm seedlings by cold storage.

Embryo Rescue in *Hydrangea*

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Index words: *Hydrangea macrophylla,* Embryo Rescue, Tissue Culture, Interspecific Hybridization

Nature of Work: *Hydrangea macrophylla is* a popular summer-flowering shrub that is valued for its large inflorescences of bright blue or pink flowers. Due to the fact that its flower buds are formed on old wood, a severe winter or late spring freeze can result in a lack of flowering the following summer. It is not reliably winter hardy north of USDA cold-hardiness zone 6b or 7, depending on cultivar. *Hydrangea paniculafa,* on the other hand, forms flowers on new wood and is rated as cold hardy to zone 4. The white to pale pink panicles of *H. paniculata* do not have the visual appeal of the intensely colored *H. macrophylla* inflorescences. Creating a hybrid between these species, for the purpose of combining cold-hardiness and flower color, is the goal of this research project.

Numerous hybridizations between *H. macrophylla* and H. *paniculata* have been attempted (1). Seeds are produced only if H. *macrophylla is* used as the female. These seed do not germinate; lack of germination appears to be the result of

embryo abortion during seed development. The objective of this study was to develop an in vitro procedure that could be used to rescue these hybrid embryos. The first part of the work, which is reported herein, utilized intraspecific (i.e. *H. macrophylla* x *H. macrophylla)* crosses for developing a media that would support growth of immature *Hydrangea* embryos. Intraspecific crosses were used to insure that sufficient viable embryos would be present to adequately evaluate media formations and different stages of embryo maturity. Subsequent studies will involve applying the results of this work to interspecific hybrids.

H. macrophylla plants were moved into the greenhouse in early summer 1998. Sterile flowers were removed from the inflorescences and the fertile flowers were emasculated. Pollen was applied 1 to 3 days after emasculation. Inflorescences were covered with breathable plastic bags both after emasculation and after pollination. Ovaries were collected 3, 4, 5, and 6 weeks after the pollinations were made. Ovaries were surface-sterilized and sliced opened to reveal the enclosed ovules. The

ovules were extracted and placed onto culture media in 100 x 15 mm petri plates. The plates were sealed and placed in a 25C incubator for 3 months, at which time they were scored for number of plants. Total number of ovules cultured was also determined, and data was expressed as percentage of ovules cultured producing plants.

Six media, involving two basal media and three sucrose concentrations, were tested. The basal media utilized were Murashige and Skoog (MS), and Gamborg's B-5 (B-5). Three sucrose concentrations, 2%, 8%, and 12%, were tested with each of the basal media. The ovules from 8 ovaries were cultured on each media on each of the four collection dates.

Results and Discussion: Both collection date and culture media had a significant effect on ovules producing plants. Plants were obtained from ovules cultured at all of the collection dates, however, the largest number of plants was obtained from ovules cultured 5 and 6 weeks after pollination (Fig. 1). B-5 media with 2% sucrose was the best medium tested for supporting growth of immature *H. macrophylla* ovules (Fig. 2). Some of the plants obtained from these cultures were transferred to soil, and all grew normally.

Significance to Industry: Development of a hydrangea with the flower coloration of *H. macrophylla,* but with increased cold-tolerance would extend the range in which brightly colored hydrangeas could be grown and marketed. This study is the first step in developing the embryo rescue procedure that appears necessary for producing hybrids between *H. macrophylla* and *H. paniculata.*

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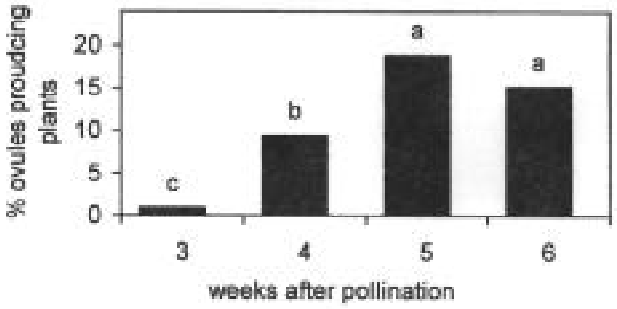

Figure 1. Effect of date of collection (weeks after pollination) on percentage of *H. macrophylla* cultured ovules producing plants. Mean separation by LSD, P=0.05.

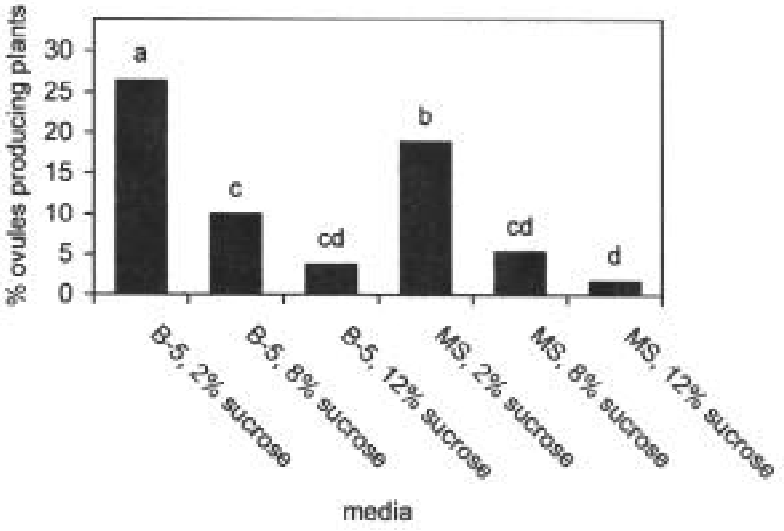

Figure 2. Effect of media on percentage of *H. macrophylla* ovules producing plants. Mean separation by LSD, P=0.05.

Rooting Flowering Dogwood (*Cornus florida*) Microshoots

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Index Words: Acetylsalicylic Acid, Indolebutyric Acid, Locust Diffusate, Root Stimulating Bacterium, Salicylic Acid, Tissue Culture, Willow Diffusate

Nature of Work: Flowering dogwood (*Cornus florida* L.) is a common tree found in the eastern United States. It is a valuable tree for the nursery and landscape industry, especially in Tennessee, where it provides significant income for nursery producers. In the last few years much damage has been caused to *C. florida* trees in both landscape and natural environment due to powdery mildew and dogwood anthracnose. Flowering dogwoods are commercially propagated from seeds and by vegetative methods such as grafting or rooting cuttings, which may be unpredictable. If a reliable method of producing flowering dogwoods through tissue culture is developed, it can be used to rapidly produce many copies of an important cultivar (genotype) that may have extraordinary horticultural attributes or resistance to diseases. Low rooting percentage of microshoots has been a major problem in tissue culture of *C. florida*. This study was conducted to increase rooting efficiency of flowering dogwoods microshoots over that previously achieved (3).

Five- to six- week old *C. florida* microshoots originating from acclimatized axillary and nodal bud stock cultures maintained on Woody Plant Medium (WPM) (4) amended with 4.4 µM 6-benzylaminopurine (BA) were used as explants. Microshoots were excised and treated with various root promoting substances and a recently discovered Root Stimulating Bacterium (RSB) (2). In the first experiment, microshoots were dipped for 24 h in aqueous diffusates of black locust (*Robinia pseudoacacia* L.) or contorted willow (*Salix x erythroflexuosa Rag.*) that were prepared by leaching stem cuttings (1). Microshoots were transferred to WPM or WPM amended with 4.9 µM indolebutyric acid (IBA) after 24 h. The second experiment consisted of a continuous exposure or 24 h pulse treatment with salicylic acid (SA) and acetylsalicylic acid (ASA). In the continuous treatment microshoots were directly placed in test tubes containing WPM amended with 4.9 µM IBA and different concentrations of SA or ASA (50, 100 or 200 µM). After 24 h exposure to SA or ASA (10,

25, 50 µM), the pulse-treated microshoots were transferred to test tubes containing WPM + 4.9 FM IBA. In the third experiment, microshoots were placed in medium amended with different volumes of bacterial extract obtained at pH 3 (12.5 ml, 2.5 ml and 0.5 ml) and pH 7 (12.5 ml, 5.0 ml, 2.5 ml, 1.0 ml, 0.5 ml and 0.25 ml) as previous attempts at co-culturing with bacterial cells did not stimulate any significant rooting.

Test tubes containing explants were incubated at 26 C under 25 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and 16/8 h light-dark photoperiod for 5 weeks. Rooting efficiencies of microshoots grown on WPM and treated with these root-promoting substances and bacterial extracts were compared to those obtained with only 4.9 µM IBA. Randomized incomplete block designs were used for all the experiments. Data collection consisted of counting the number of roots formed on each microshoot and data were analyzed using a mixed model analysis of variance.

Results and Discussion: The willow and locust diffusates slightly inhibited rooting of C. florida microshoots. Microshoots exposed continuously to SA (100 µM) and ASA (50 and 100 µM) generated more roots than those exposed to other concentrations. Explants pulse treated with 10 µM SA and 25 or 50 µM ASA formed more roots than those treated with other concentrations. Seventy and ninety percent rooting were obtained when microshoots were transferred to WPM amended with 2.5 ml and 0.5 ml of pH7 RSB extract respectively. Bacterial extract obtained at pH 3 did not stimulate any rooting in flowering dogwood microshoots. Among various treatments tested on C. florida microshoots, maximum rooting of ≥70% was achieved with 4.9 µM IBA. This is significantly greater than that previously achieved by Kaveriappa et al. (3). Microshoots that had turned reddish-brown (presumably more mature) appeared to root more easily than those that were green.

Significance to Industry: WPM amended with 4.9 µM IBA can be used to obtain ≥70% rooting of *C. florida* microshoots. Propagation by this method can be used to easily and rapidly produce many copies of important flowering dogwood cultivars.

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Breeding Disease Resistant Flowering Dogwood (*Cornus florida*)

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Index Words: Dogwood, Honey Bees, Molecular Markers, Pollination

Nature of Work: In the past decade flowering dogwood (*Cornus florida* L.) has been subjected to new disease pressures. Two diseases that have severely affected flowering dogwoods are dogwood anthracnose, caused by *Discula destructiva* (4) and powdery mildew, caused by *Microsphera pulchra* (3). Dogwood anthracnose was first reported on the east coast of the US from near New York City in 1978 and since then has spread through the Appalachian highlands to Alabama and Georgia (7). Some symptoms of dogwood anthracnose include leaf spots and blights, profusion of water spouts and annual cankers along trunks and limbs, and ultimately, death of trees (2). This disease has devastated dogwood populations in some natural settings. For example, in Catoctin Mountain Park, Maryland, dogwood anthracnose killed over 97% of the trees (6). The second disease, powdery mildew increased dramatically on dogwood around 1994 (5). Symptoms of powdery mildew include curling and increased reddish pigment of leaves, stunted growth, and a grayish powdery film on foliage. This disease has made it nearly impossible grow seedlings to a size suitable for budding, the primary commercial nursery production technique for cultivars, without extensive use of fungicides (9). The broad objective of this project is to breed a tree that is resistant to both powdery mildew and dogwood anthracnose.

The breeding stock for this project included cultivars ‘Appalachian Spring’, ‘Cloud 9’, ‘Cherokee Brave’, and trees from six powdery mildew resistant lines (11). ‘Appalachian Spring’, a recent release by the Tennessee Agriculture Experiment Station (TAES), was developed from a tree found in 1994 in Catoctin Mountain Park, Maryland, and has outstanding resistance to dogwood anthracnose (10). ‘Cherokee Brave’ has demonstrated resistance to powdery mildew in greenhouse and field tests (9). Unnamed powdery mildew resistant lines were developed from seedlings found in a middle Tennessee nursery (11).

In the spring of 1999, flowering dogwood trees were placed in four screened cages. Honey bees were introduced in three of the cages,

whereas bees were omitted from the fourth cage. One 'Appalachian Spring' tree and some combination of trees from powdery mildew resistant lines were placed in the cages with honey bees. To enhance visitation of bees to flowers, a small drop of a 3:1 sugar/Fruitboost (queen mandibular pheromone) aqueous solution was placed at the base of the bracts each morning and evenings for three consecutive weeks. Trees of 'Cloud 9' and Cherokee Brave' were placed inside the fourth cage which had an extra layer of mesh screen to exclude all potential insect pollinators.

DNA from each of the potential parents was characterized using DNA Amplification Fingerprinting (DAF). Genomic DNA was isolated from young leaves using the Puregene kit (Gentra systems, Minneapolis, MN). All named cultivars and powdery mildew resistant lines have been characterized with eleven octomer primers, using DAF according to the method described by Trigiano and Caetano-Anollés (1998).

Results and Discussion: Honey bees were attracted to the sugar/ pheromone solution placed at the base of bracts and they were observed flying between inflorescences of both dogwood anthracnose and powdery mildew resistant trees. Although we did not examine honey bees for dogwood pollen, berries/seeds were set on trees within the screen cages that excluded outside potential pollinators. Trees in cages with the honey bees set 116 seeds, which will be tested for parentage in the spring of 2000. In the double screened cages without honey bees, berries/seeds did not develop, supporting the contention that an insect pollinator is critical for flowering dogwoods to complete sexual reproduction. Furthermore, our study demonstrates that honey bees, which may be a natural pollinator (1), can effectively and efficiently pollinate dogwood.

DNA was easily amplified and revealed relatively homogeneous banding patterns for most of the trees used in the study. However, there was sufficient genomic diversity to permit visualization of unique bands that identified individual trees in breeding pairs of trees (Figure 1). Future efforts will be directed towards isolating, cloning and sequencing (Sequence Characterized Amplified Regions-SCARS) these markers for use as molecular probes identifying parentage of putative disease resistant seedlings. Development of probes will permit rapid screening of large number of crosses for parentage.

Significance to Industry: The development of pollination and parentage screening techniques will allow us to pursue the objective ofcombining resistance to dogwood anthracnose and powdery mildew in a single cultivar. A cultivar resistant to both diseases should alleviate production and sales concerns of flowering dogwood.

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