

SUCROSE SYNTHASE LOCALIZATION DURING SEED AND HAIR DEVELOPMENT: COTTON OVULES AS A MODEL SYSTEM

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Immunolocalization of sucrose synthase in cotton ovules was conducted to clarify the relationship between this enzyme and sucrose import during seed and hair development. Although rapidly growing plant organs are known to have high activities of sucrose synthase, information on specific localization has been minimal at the cell level. Cotton ovules were examined in the present work as representatives of developing dicot seeds, and because their expanding hairs would have a locally high demand for cell wall synthesis. Initial analyses focused on ovules immediately before and after anthesis/pollination. Heavy immunolabel was observed solely in the nucellar tissue 1 day before anthesis. Immediately following anthesis/pollination, however, sucrose synthase became evident in cells of the external (but not internal) integument, expanding from the chalazal to micropylar end of this tissue. Little sucrose synthase was directly associated with very young epidermal hairs prior to their most rapid expansion. Immunolabel appeared to be localized in cells associated with the vascular bundle of this external integument. The shift in spatial/temporal localization of sucrose synthase in cotton ovules following pollination indicates a close association between sucrose synthase and probable sites of sucrose import at a cellular level during early seed development.

DAYLILY RESPONSE TO PLANT BIOSTIMULANTS

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Application of plant biostimulants to various crops has enhanced yields and increased growth. This study was conducted to determine the effect of plant biostimulants on growth of *Hemerocallis* x 'Aztec Gold'. Roots of single-plant divisions were immersed for 5 min in solutions of 520 ppm KELPAK (seaweed concentrate), 390 ppm PGR IV (hormone concentrate containing nutrients), 250 ppm AGRI-GRO (contains dormant nitrogen-fixing bacteria) + 250 ppm molasses, or water prior to planting on 20 April 1992. Plants were grown in 3.8-liter containers under 31% shade. Foliar sprays (to wet) of these biostimulants (same concentrations as before) were applied on 20 May, 19 June, and 13 August 1992. Plants were harvested on 21 October 1992. None of the biostimulants affected final plant height, number of divisions, or shoot and root fresh and dry weights. However, plants treated with KELPAK had higher shoot to root ratios (fresh and dry weight) compared to the control.

COMPETITIVENESS OF SIXTEEN WILDFLOWERS WITH BUFFALOGRASS AND BLUE GRAMA TURF.

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Sixteen species of herbaceous perennial flowering plants were planted with five different treatments (three buffalograss lines, one blue grama line, and no turf) to determine the growth and flowering characteristics of these herbaceous plants with the turf treatments. Plants were measured for survival, time of emergence, percent of plants flowering, initial flowering, number of flower stalks, plant height, and pod number/plant one-year after planting. There were highly significant differences for all traits for plots, species, and plot x species interaction. Individual plant vigor was highest where there was no grass competition. Species that performed well in all turf treatments included *Echinacea pallida*, *Linum perenne*, *Salvia azurea*, *Solidago rigida*, and *Ratibida pinnata*.

DELAYING BUDBREAK OF BARE-ROOT CHINESE CHESTNUT SEEDLINGS WITH FOLIAR APPLICATIONS OF PACLOBUTRAZOL

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Foliar applications of paclobutrazol (PAC) at rates of 0, 400, 800, and 1200 mg/liter were applied to one-year-old liners of *Castanea mollissima* Blume in the field on August 27 and September 27, 1991. In January, 1992, the seedlings were lifted from the field and placed in 4 C storage. At transplanting to the field (May, 1992) an additional set of seedlings received the same PAC treatments. The objective was to determine if PAC treatments could be used to delay budbreak (BB, green visible between bud scales) and/or 1st leaf expansion (LE, 1 cm length) until significant amounts of new roots were regenerated to enhance initial transplant establishment. PAC applications prior to fall lifting delayed BB by 1 to 3 days and LE by 1 to 3 days compared to controls. Numbers of

new small (< 1mm diameter) roots present at BB and new large (\geq 1 mm diameter) roots at LE were greater on PAC treated seedlings, but were insufficient to improve 1st year growth or survival. PAC at 800 and 1200 mg/liter applied at transplanting reduced internode length by approximately 20 percent. End of season new shoot dry weights, total leaf area per plant and average area per leaf were not affected by PAC level or time of application.

FACTORS AFFECTING PROPAGATION OF MAACKIA AMURENSIS FROM SOFTWOOD CUTTINGS

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Little is known about asexual propagation of *Maackia amurensis* Rupr. & Maxim. (Amur maackia), a N₂-fixing tree species with potential for increased use in the landscape. Terminal cuttings were collected from 20 trees (10 at the Minnesota Landscape Arboretum and 10 in the Washington, D.C., area) at two times in 1992. Cuttings were treated with either 0 or 2500 mg·kg⁻¹ IBA and held in a humidified greenhouse for 12 weeks. Primary and secondary roots were counted, and a subjective rooting score was assigned at harvest. The rooting percentage of the genotypes ranged from 19 to 92, and the number of roots and the rooting scores of the genotypes also varied significantly. Collection date and IBA had relatively minor effects on rooting. We conclude that *M. amurensis* can be propagated from softwood cuttings, but the usefulness of this method varies with genotype.

FACTORS INFLUENCING AXILLARY BUD BREAK AND SHOOT PROLIFERATION OF ROSA HYBRIDA L.

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The effect of node position of axillary buds of greenhouse-grown Hybrid tea rose cultivars 'Carefree' and 'Adelaide Hoodless' on shoot induction and proliferation was investigated. Explants were cultured on a Murashige and Skoog (MS) medium containing 2 mg/l 6-benzyladenine (BA) and 0.1 mg/l α -naphthaleneacetic acid (NAA). Axillary buds collected closest to the shoot apex gave rise to the highest shoot proliferation; whereas, those collected from the second, third, and fourth nodes yielded longer shoots and higher leaf number/explant. The influence of varying levels of BA concentrations in the culture medium was also investigated. It was observed that high BA concentrations, 1-3 mg/l, stimulated shoot proliferation. The effect of carbohydrate source, fructose versus sucrose, on shoot induction was also investigated. A high shoot number/explant was observed on media containing fructose; however, longer shoots (>1 cm) were observed on media containing sucrose as a source of carbon.

EVALUATION OF SEED GERANIUM CULTIVARS FOR THE LANDSCAPE

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Seed geranium (*Pelargonium* x *hortorum*) cultivars were evaluated based on days to flower, flower and umbel diameter, flower color, plant dimensions, uniformity, appearance, longevity and floriferousness during the spring and fall of 1992. **Spring:** Days from sowing to flower for 60 cultivars ranged from 96 to 115 days. 'Tetra Scarlet' was significantly later to flower than all other cultivars except 'Orange Appeal' which flowered at 112 days. Plant height ranged from 24.4 to 38.4 cm. 'Pinto White' was taller than all other cultivars except 'Tetra Scarlet' and 'Pinto Bicolor'. Umbel diameter ranged from 9.4 cm to 14.0 cm. 'Freckles' had the largest umbel diameter. At 111 days after sowing, umbel number ranged from 1.0-11.5, with 'Multibloom Salmon' and 'Multibloom Pink' producing the most. **Fall:** Days from sowing to flower for 62 cultivars ranged from 85 to 136 days. 'Orange Appeal' flowered later than any other cultivar, followed by 'Tetra Scarlet' at 119 days; also significantly different than any other cultivar. Mature plant height ranged from 25.5 to 42.3 cm. 'Pinto White' was taller than 41 other cultivars. Umbel diameter ranged from 8.3 to 13.2 cm.

620 (PS 3)

GENETIC ANALYSIS OF ISOENZYME LOCI IN *RHODODENDRON*

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The genus *Rhododendron* is widely distributed and diverse, consisting of over 900 species. Although this diversity has been exploited by hybridizers for over 100 years, few single gene traits have been genetically characterized. In order to develop genetic markers useful for biological, breeding, and commercial applications, we are investigating allelic variability and mode of inheritance at enzyme loci using starch gel electrophoresis. Thus far, allozyme segregation data at 4 enzyme loci (*Pgi-2*, *Idh-1*, *6Pgd-2*, *Est-1*) fit the Mendelian model for single gene inheritance at the diploid level. An additional 4 polymorphic loci are presently being characterized: *Pgm-2*, *Mdh-1*, *Mdh-2*, and *6Pgd-1*. Disomic inheritance appears to be consistent across a wide array of genetic backgrounds in these crosses, most involving parents with interspecific pedigrees. Variability at these loci is fairly abundant within the Leach breeding population. This should enable us, as an initial application, to genetically 'ID' many of the Leach hybrids on the basis of their electrophoretic phenotypes.

621 (PS 3)

EFFECT OF CONTRASTING PRODUCTION LOCATION ON LEAF MORPHOLOGY OF TWO LANDSCAPE SHRUBS

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Same-source rooted cuttings of *Feijoa Sellowiana* Hort. and *Ligustrum japonicum* L. were grown under identical production systems in Tempe, AZ, USA or Tifton, GA, USA during the Spring and Summer 1992. Leaf area was largest and specific leaf weight was lowest for all Georgia-grown plants. Scanning electron microscopy revealed that Arizona *Feijoa* leaves had trichomes on both the abaxial and adaxial leaf surfaces, whereas, leaves of Georgia *Feijoa* had trichomes on the abaxial surface only. Arizona *Feijoa* leaves also had increased abaxial surface pubescence compared to leaves of Georgia *Feijoa*. Both *Feijoa* and *Ligustrum* grown in Arizona had a higher density of palisade and spongy mesophyll cell layers compared to their Georgia counterparts. When placed under the same irradiance source, adaxial leaf surface temperatures of Georgia-grown *Ligustrum* and *Feijoa* were approximately 4.2 and 0.2°C, respectively, higher than for those grown in Arizona. Higher leaf temperatures of Georgia-grown plants were correlated with darker leaf color compared to the Arizona-grown plants.

622 (PS 3)

STUDY ON THE PRODUCTION OF TREES GROWN IN CONTAINERS

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The growth potential was observed for young trees of *Tilia cordata* 'Glenleven', of *Malus floribunda* 'Red Splendor' and of *Fraxinus pensylvanica* 'Patmore' when produced in field or in containers. The three species were submitted to five treatments: field planting, above or sunk below ground containers with or without a copper coated fabric container. The 15 "species-treatments" combinations were randomly distributed in a block and repeated 4 times. Results demonstrate that the mean trunk diameter is greater by 18% for apple trees and by 6% for ash trees grown in containers compared to the other trees grown in field. Moreover, the trunk diameter of linden is 9% greater for trees in sunk below ground containers; the root dry mass is also more important in above ground containers. It is our opinion that the superior yields obtained with containers are due mainly to the better care given to the trees in terms of fertilization and irrigation. The greater quantity of dry mass obtained with trees produced in fabric containers could on the one hand be due to root ramification caused by copper and on the other hand to an increased to apical tissue volume also caused by copper. Visual observations have permitted to detect the effect of temperature on root development in the zone adjacent to the side of the container; it may have a regulator effect on the development of the root system and seems to be combined to the effect of copper.

623 (PS 3)

GROWTH AND NUTRIENT DYNAMICS IN *ILEX CRENATA* THUNB. 'HELLERI' ACROSS AN ALUMINUM GRADIENT

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Ilex crenata 'Helleri' (Helleri holly) can experience landscape establishment problems in the Southeast. Since aluminum toxicity is a major problem in acid soils of the Southeast, this experiment studied the effects of aluminum on Helleri holly grown in solution culture. A modified Hoagland's solution contained low phosphorus concentrations (32 μM), a 1:1 $\text{NH}_4^+:\text{NO}_3^-$ nitrogen ratio, and aluminum treatments consisting of 0, 222, 444, 889, and 1332 μM Al supplied at equal ratios from $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$. The MINTEQA2 (version 3.11) chemical speciation model was used to predict activity of ions in solution. Shoot growth and root length were not affected by aluminum after 12 weeks in solution culture. Total plant nutrient uptake was monitored weekly. Preliminary results suggest that Helleri holly does not take up aluminum ions even though NH_4^+ is the preferred nitrogen source. Other studies have shown increased aluminum toxicity effects when NH_4^+ uptake exceeded NO_3^- uptake.

624 (PS 3)

SOIL AMENDMENT INCREASES GROWTH OF *SALVIA SPLENDENS* AND *CATHARANTHUS ROSEUS*

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Commonly used soil amendments for ornamental annual plantings were compared to determine their effect on growth of *Salvia splendens* 'Hotline Red' (*Salvia*) and *Catharanthus roseus* 'Peppermint Cooler' (*Vinca*). Transplants of the annuals were planted in amended outdoor beds on June 12, 1992. Treatments were 1) an unamended, rototilled control and 2) aged pine bark, 3) granite sand or 4) a regionally commercially available product Mr. Natural™. Mr. Natural™ consists of granite sand, expanded shale, and composted poultry litter. A 9-cm-deep surface layer of the amendments was rototilled into 20 cm of clay soil. One-half of the plants were fertilized with the recommended rate of 0.15 kg/m² of 14-14-14 Osmocote, and one-half were not fertilized. On July 14 and August 10, shoot dry weight was determined, and a growth index was calculated from the width and height of growth. Fertilizing increased the growth index and dry weight in all treatments. Plants in the aged pine bark and no fertilizer treatment were consistently the smallest plants and exhibited nitrogen deficiency symptoms. For both dates, the Mr. Natural™ *vinca* plants had the greatest dry weight and growth index. On the first date, Mr. Natural™ *salvia* plants did not differ from the sand treatment but had 50% greater dry weight than the unamended control. Mr. Natural™ *salvia* plants were the largest plants on the second harvest date.

625 (PS 3)

USE OF COMPOSTS AND ROCKWOOL IN PINE BARK POTTING SUBSTRATES

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Cotoneaster dammeri Schneid. 'Skogholm' liners were potted into combinations of pine bark (PB), horticultural rockwool (RW), composted municipal yardwaste (CYW), composted turkey broiler litter (TBL) and washed builders sand (S). The objective of the study was to characterize the physical and chemical properties, subsequent plant growth and foliar tissue levels of the resultant substrates. Of the physical parameters tested the three component substrates PB:RW:CYW and PB:RW:TBL (70:20:10 by v) appeared to have the most consistent favorable physical properties. Electrical conductivity (EC) was extremely high the first day after initiation in the substrates containing TBL but was not different for other sampling dates. Leachate pH initially ranged from 4.9 to 6.1 but through most of the study ranged from 5.2 to 5.8. As with EC all nutrient levels were very high one day after initiation. Leachate phosphate levels were maintained at recommended levels in the substrates containing TBL throughout most of the study while other substrates were generally deficient. The same was true for foliar tissue phosphate levels. The greatest top dry weight was yielded in the PB:RW:TBL (70:20:10, by v) substrate and least in the PB:S (80:20) substrate.

627 (PS 3)

Determination of the Photo-Perceptor Site for Overcoming the Early Stage of Endodormancy

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Buds, leaves, or stems of red-osier dogwood (*Cornus sericea* L.) plants were covered with aluminum foil and placed in a warm greenhouse under long day condition to determine the perceptor site for overcoming the early stage of endodormancy. The following categories with treatments were studied; Category A, foliated plants: A1) not covered, A2) buds covered, A3) stem covered, A4) stem and bud covered. Category B, foliated-covered plants: B1) leaves covered, B2) leaves and buds covered, B3) leaves and stem covered, B4) leaves, stem and buds covered. Category C, defoliated plants: C1) not covered, C2) buds covered, C3) stem covered, C4) stem and buds covered. These studies indicate the bud as the long day (LD) perceptor site for overcoming the early stage of endodormancy. However, the presence of leaves (Category A) stimulated terminal budbreak only in bud covered (A2) and stem and bud covered (A4) treatments. In addition, etiolation treatments (Stem covered, A3, A4 and C3) resulted in significant root initiation on stem internodes within 2 weeks. The rate of presence of roots in stem-covered treatment promoted shoot growth.

628 (PS 3)

CORNUS FLORIDA 'OZARK SPRING', A NEW, HARDY DOGWOOD
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The importance of regional adaptation of native plants is apparent in the selection of cultivars, especially in *Cornus florida* L., which grows in specific geographic areas of the United States where climate, soil and site characteristics are favorable. Selected from a seedling population in the western portion of the native range, 'Ozark Spring' was compared with cultivars from other regions. Growth and flowering were determined under moderately stressful conditions in south central Kansas. The selection was one of the few which flowered following -30°C (-23°F) during the winter of 1982. Hardiness was confirmed at -29 to -31°C by University of Minnesota freezing tests. Performance of 'Ozark Spring' has been superior to other cultivars in growth and bract development when planted in more exposed sites. The cultivar is expected to perform best in the Ozark region of Oklahoma, Arkansas, Missouri and eastern Kansas.

629 (PS 3)

ROOTING AND SUBSEQUENT GROWTH OF SUPERIOR LACEBARK ELM SELECTIONS BY HARDWOOD AND SOFTWOOD CUTTINGS
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Numerous cultivars of Lacebark elm (*Ulmus parvifolia* Jacq.) have recently been introduced which are produced vegetatively. Conventional propagation is usually by softwood cuttings under intermittent mist which is quite successful. Softwood cuttings taken off two mature specimen trees stuck in May and June rooted 73 and 93 percent respectively at 10,000 ppm IBA but required overwinter protection prior to lining out the following season. Hardwood cuttings of numerous selections, stuck in perlite: peat (70:30 v/v) over bottom heat at 21°C in a cool greenhouse rooted 60 to 100 percent at 10,000 and 20,000 ppm. Cuttings from winter prunings were taken on February 4, potted by March 12, lined out May 23 produced 69 to 97 cm of growth the same season. The latter procedure offers a low input method of propagation which has several advantages over using softwood cuttings.

630 (PS 3)

PRAIRIE PLANT SEED AND SEEDLING IDENTIFICATION

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Photographic resource materials to assist in identification of seeds and seedlings of grasses and forbs native to Minnesota prairies are being developed as part of a grant from the Legislative Commission on Minnesota Resources. Native plant materials are being used for habitat improvement,

roadside vegetation, and landscape plantings throughout Minnesota. Seeds were obtained from producers or collected from prairie remnants. Seed photographs were taken with a Wild Leitz stereomicroscope and camera unit using Kodak EPY 64T film. Magnification was 6.5X, 10X, or 16X based upon seed size. Seedlings were photographed with one or two true leaves using Kodak EPY 64T film with a Nikon FM2 camera with a 55 mm micro Nikkor macro lens and an extension tube when necessary. Nondestructive sampling using photographic techniques provides a relatively quick and inexpensive means of recording information on potentially rare species. The color pictures and slides developed in this project provide an effective tool for educators, producers and land managers to easily distinguish between seeds and seedlings of valuable native plants and those of weedy species undesirable in restoration projects or cropping systems.

631 (PS 3)

WOUND CLOSURE RESPONSE TO TRUNK INJECTION OF THE GROWTH RETARDANT FLURPRIMIDOL

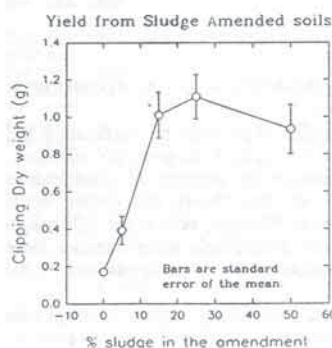
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Over \$1 billion per year is spent nationally for pruning trees to restrict growth. Plant growth regulators offer a means to extend pruning cycles, reducing future pruning costs. In this study, the influence of flurprimidol (flur) on wound closure rate was investigated. *Acer saccharinum*, *Celtis occidentalis*, *Fraxinus pennsylvanica*, *Gleditsia triacanthos*, and *Populus deltoides* were injected using an Arborchem pressure injection system, followed by trunk wounding. Injections of flurprimidol (at both 1X and 3X rates) and wounding were conducted in spring, early summer, and late summer. Without flur, wound closure rate was slowest in maple and most rapid in cottonwood. Flur suppressed wound closure rate most greatly in hackberry, and had the least influence on cottonwood. In addition, wound closure rates in the presence of flur were influenced by the timing of the treatments. These results will be of particular use to utility companies that prune trees under power lines.

632 (PS 3)

SEWAGE SLUDGE INCREASES YIELD AND COLOR OF PERENNIAL RYEGRASS WHEN ADDED TO YARDWASTE COMPOSTS. Jim Downer, Dennis Pittenger, Ben Faber, and Paul Rogers, U.Calif. Coop. Extn. 702 County Square Dr., Ventura, Ca 93003 and Kellogg Supply Inc., Carson CA.

Greenwaste from California landscapes has been disposed of in landfills for many years. Legislation now requires reduction of disposal by 25% in 1995 and 50% by 2000. Our goal was to find a sewage sludge (SS) rate which would most enhance yardwaste (YW) amendments. YW was co-composted with (SS) at ratios of: YW:SS 100:0, 95:5, 85:15, 75:25, and 50:50. Fresh YW and composts were blended (30% vol.) with clay, loam and sand soils, added to 3.7l containers and seeded with Manhattan II perennial ryegrass. Germination was greatest in unamended soil, but reduced 50% in soils with the 50% SS amendment, yet this treatment had the best turfgrass color ratings. Clipping yield was highest in sludge amended soils (Chart).



633 (PS 3)

CULTURALLY INFORMATIVE PLANT IDENTIFICATION LABELS TO ASSIST VISITORS AND STUDENTS IN PUBLIC GARDENS

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The Oklahoma Botanical Garden and Arboretum (OBGA) at Oklahoma State University was awarded an "America the Beautiful" grant from the Oklahoma Department of Agriculture and the Oklahoma Urban and Community Forestry Council to develop a plant database and plant identification labels.

Culturally informative plant identification labels are engraved by a computer-controlled Newing-Hall 300 Engraver. Labels include not only scientific, common, and family names, but also cultural icons, depicting light, water, and pH preferences. The cultural icons provide visual cues to students in OSU's plant identification courses, as well as to visitors in

OBGA's gardens and conservatories.

Additionally, the labels feature the common name engraved in Braille at the bottom of the label to facilitate education of visually impaired students and visitors. In anticipation of streamlining data collection on a plant's growth, flowering, fruiting, etc., each label also includes a barcode representation of the plant's accession number.

634 (PS 3)

CHOPPED NEWSPAPER FOR WEED CONTROL IN ORNAMENTALS
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Chopped newspaper was applied at 15 and 25 cm depths around field rows of *Gaillardia*, *Physostegia*, and *Daphne*. The paper was held in place by wetting, then rolling, or by a commercial binder used for hydroseeding. Both methods of holding paper in place were successful. All newspaper treatments controlled weeds very well. Fifteen cm paper depth resulted in higher weed count in September than 25 cm depth. Paper mulch held up well through the growing season and had little impact on crop growth.

635 (PS 3)

STIMULATING BUD BREAK AND IMPROVING OVERWINTER SURVIVAL IN ROOTED SOFTWOOD CUTTINGS.
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The objectives of this research were to stimulate and accelerate new growth in rooted cuttings of difficult-to-root woody plants, thereby increasing overwinter survival rates in the first propagation year. Etiolated and non-etiolated cuttings were taken from field grown stock plants of *Acer rubrum* 'October Glory', *Hamamelis vernalis*, *Hamamelis virginiana*, and *Stewartia pseudocamellia*. After a 4 week etiolation period, during which the bases of new shoots were covered in black velcro bands, cuttings were taken in June, 1992 and dipped in one of three levels of IBA: 500, 1,000, 5,000 ppm for *A. rubrum* 'October Glory', *A. rubrum* 'Red Sunset', and *H. vernalis*, and 1,000, 5,000 and 10,000 ppm for *H. virginiana* and *S. pseudocamellia*. After 3 to 8 weeks under mist, successfully rooted plants were given 16 hour days with 70/60 F. D/N greenhouse temperatures. Plants were treated with a foliar spray of thidiazuron 50 mg/L, thidiazuron followed by GA_{4/7} 250 mg/L ten days later, silver thiosulfate (STS) 1%, or STS followed by GA_{4/7} ten days later. Both STS and thidiazuron stimulated bud break in plants. After 4 weeks, plants were assessed for bud break, after which dormancy was induced under short days and cool temperatures (50/40 F. D/N). Once dormant, the plants' total growth and total nonstructural carbohydrates were measured, and plants were stored in 38 F. coolers for 3 months. At the end of 3 months, the plants were assessed for overwinter survival.

636 (PS 3)

ABSORPTION, TRANSLOCATION, AND METABOLISM OF DITHIOPYR IN SELECTED WEED SPECIES.

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Field studies have shown that dithiopyr has potential as a herbicide in ornamental nursery crops. Previous work has shown that certain weeds differed considerably in their tolerance to dithiopyr, both in the field and in the greenhouse. Four weeds were selected on the basis of these studies; large crabgrass and velvetleaf, both susceptible, and barnyardgrass and ivyleaf morningglory, tolerant species.

Root uptake was assessed in these four species by treating seedlings with 0.5 μ Ci of ¹⁴C-labelled dithiopyr dissolved in half strength Hoaglands solution. Whole plants were sampled at 12, 24, 48 and 96 hour after treatment (HAT) and plants were separated into roots and shoots. Dithiopyr levels and polar metabolites were measured after methanolic extraction of plant parts. Insoluble ¹⁴C levels were measured after biological oxidation. At 12 HAT, TLC and HPLC separation of extracts showed no detectable metabolites of dithiopyr. By 24 HAT, two polar metabolites were detected in both root and shoot extracts of all species. Total ¹⁴C and polar metabolites increased in roots and shoots over time. Species differences in total ¹⁴C uptake and metabolism were noted, potentially contributing to dithiopyr selectivity differences.

637 (PS 4)

SPORE VIABILITY AND GERMINATION OF THE ENDANGERED ALEUTIAN SHIELD-FERN, *POLYSTICHUM ALEUTICUM*

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Research was initiated in 1990 to study spore viability, spore germination *in vitro*, and methods of controlled environment culture for the endangered Aleutian shield-fern, *Polystichum aleuticum*. Examination of spores using scanning electron microscopy revealed from 24% to 78% deformed and possibly nonviable spores per plant. Normal spores germinated in 30-45 days on both Knop's solution and Hoagland's No 2 solution in aseptic culture. Germination was most rapid on cultures with less than 6 g/l agar. Cultures with no agar were susceptible to contamination by algae, and sporophyte losses during transfer to greenhouse media were high. Germination rate and subsequent appearance of the first leaf stage did not differ significantly within a medium pH range of 4.7 to 7.0. Spores exhibited a thermodormancy at 25°C, but germinated well at 18°C and required light for germination. Sporophyte transfer from aseptic culture was most successful after true fronds beyond the first leaf stage had developed. A commercial bedding plant mix composed of *Sphagnum* sp. peat and perlite provided an optimum rooting medium for the ferns.

638 (PS 4)

MICROPROPAGATION OF 'GERMAN RED' CARNATION (*DIANTHUS CARYOPHYLLUS*) FROM NODAL EXPLANTS

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Nodal explants were taken from both vegetative and flowering shoots of 'German Red' carnation and placed on MS medium supplemented with 2.0 mg/L benzylaminopurine (BAP) and 0.5 mg/L naphthaleneacetic acid. The explants taken from flowering shoots invariably produced flower buds *in vitro* and were of no value for micropropagation. With the vegetative explants, microshoots were observed after about 15 days. These were subcultured and the effect of cytokinins (kinetin, BAP, thidiazuron [TDZ]) on subsequent shoot production was evaluated. The cytokinins increased the number of shoots formed with TDZ and kinetin being the most and least effective, respectively. Shoots produced *in vitro* were rooted with 100% success *in vitro* or *ex vitro*. About 98% of the plants rooted *in vitro* or *ex vitro* survived transfer to the greenhouse and were successfully transplanted outdoors. In summary, starting from explants, well-branched flowering plants can be obtained in as little as 5-6 months. These results suggest that *in vitro* mass propagation of 'German Red' carnation is feasible.

639 (PS 4)

IMPROVING SEED GERMINATION OF *AQUILEGIA CHRYSANTHA* BY TEMPERATURE MANIPULATION

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Seeds of *Aquilegia chrysantha* Gray were germinated under a variety of temperature regimes. Germination was nearly 90% under a day/night temperature regime of 25/20°C but was reduced to 40% or less under constant 25°C or a 25/10°C day/night temperature regime. At day temperatures between 25 and 29°C (night temperature = 20°C), germination percentage dropped gradually to about 60% with increasing temperature. Above a day temperature of 29°C, germination declined dramatically such that no germination occurred at 31°C. Neither kinetin (1-10 mg/liter) nor ethephon (1-30 mg/liter) were able to reverse the inhibitory effects of a 33°C day temperature. Our results indicate that seed germination of *A. chrysantha* is quite sensitive to temperature and that germination percentages of 75% or greater can be obtained under a 25-27°C day/20°C night temperature regime.

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MAXIMIZING SHOOT REGENERATION FROM ANTHER CULTURES OF STRAWBERRY (*Fragaria x ananassa* Duch.)

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A sequential study was undertaken to optimize the cultural conditions for potential tetrahaploid plant regeneration from anthers of 'Chandler', 'Honeoye', and 'Redchief' strawberries. A comparison of auxins (IAA/NAA), cytokinins (BA/BPA/KIN) and carbohydrates (suc/glc/mal) showed highest regeneration with 2mg/l IAA, 1mg/l BA and 0.2M glc in agar-solidified MS medium after 60d (30d darkness + 30d 16h white light photoperiod). When comparing MS, Nitsch & Nitsch, and H1 (a new formulation based on the anther culture literature) media containing IAA/BA/glc solidified with Phytagar® or Gelrite®, highest regeneration occurred with H1 with Gelrite. Lastly, Fe-EDTA in H1 medium with IAA/BA/glc yielded more shoots than H1 medium containing Fe-Metalosate®, and anthers cultured in darkness for 30d followed by 30d in white light produced more shoots than those cultured in white or yellow light (16h photoperiod) for the initial 30d. Hence, the optimum regeneration medium and conditions for shoot regeneration from anthers of these 3 cultivars was H1 containing 2mg/l IAA, 1mg/l BA, 0.2M glc, Fe-EDTA, and Gelrite, followed by incubation in darkness for the first 30d. Although differences were noted for regeneration efficiency between cultivars, initial chromosome counts confirmed that tetrahaploids ($n=4x=28$) were obtained.

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CALLOGENESIS AND ORGANOGENESIS AS AFFECTED BY THE ORIENTATION AND SCARIFICATION OF LEAF DISC APPLE EXPLANTS ON THE MEDIUM. Gerson R. de L. Fortes* and Silvio L. Teixeira, P.O. Box 403, 96-100 9/0-Pelotas, RS, Brazil.

The aim of this work was to investigate the effect of orientation and scarification of apple leaf discs on the callogenesis and organogenesis. Leaf discs were collected from 4-months old apple seedlings cv. Hawaii along the midrib of the first pair of basal leaves. The treatments included the touching of the medium with the abaxial and adaxial surface and scarification and no-scarification of midribs. The leaf discs being placed in test tubes contained 10ml of a MS medium added to vitamin B₅ in mg/l: BAP(3.0); NAA(0.30); myo-inositol(100.0) glycine(2.0). Sucrose was added to the rate of 30.0g/l and agar 6.0g/l. After three weeks in dark condition and one month more in 16-hour photoperiod in a growth room at 23-25°C; 2000-2500 lux, the experiment was evaluated. Callus promptly formed at the cut surfaces and was both influenced by the surface touching the medium and scarification. Adaxial surface allows a better callus formation (100.0%) whereas abaxial ones promoted it partially (61.2%). Scarification did not improve the % of callus culture formed. No adventitious buds were observed and it was claimed to be due to the explants age. The better performance for the adaxial explants was due to the callus parenchyma which is the last tissue to cease growth and cellular division.

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PLANT REGENERATION OF TWO *ECHINACEA* SPECIES FROM LEAVES.

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The North American prairie genus *Echinacea* (Asteraceae) contains approximately nine species with medicinal, insecticidal and/or horticultural value or potential. *Echinacea tennesseensis*, a narrow-leaf prairie relic found only in middle Tennessee was among the first plants listed as endangered; *E. purpurea* is a common garden coneflower. This research focused on developing tissue culture techniques to generate propagules of both plants from leaves. Shoots were induced to form from 1 cm² leaf segments cultured for two weeks on MS medium supplemented with BA and IAA followed by three weeks of culture on MS medium without growth regulators. Nearly all (98%) of the shoots of *E. purpurea* rooted within three weeks in one-half strength MS salts medium without growth regulators and were easily acclimatized to greenhouse growing conditions. Shoots of *E. tennesseensis* rooted in MS medium amended with NAA or directly in soil with talc or alcohol-based NAA dips. However, only about 30% of the shoots could be rooted and acclimatized to field conditions.

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MICROPROPAGATION OF WHITE TARO (*COLOCASIA ESCULENTA* VAR. *ESCULENTA*) CV. PIKOEKA, POLOLU, NIHOPIU, AND HAUKEA).

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Axillary or apical meristem explants of white taro, a potential hypo-allergenic food, were used. 'Pikoeke', 'Pololu', 'Nihoپیو', and 'Haukea' varieties were taken directly from the field. Disinfection method was a 60-minute wash under running tap water, followed by a 40-minute soak in 10% commercial sodium hyperchlorite bleach, and then a double rinse in sterile water. Buds were excised and placed in sterile water. These were put in a 10% commercial sodium hyperchlorite bleach, and three sterile water rinses. Modified Murashige and Skoog media containing combinations of 5 and 10 uM indole-3-acetic acid (IAA) with 5 and 10 uM 6-benzylaminopurine (BAP) were used. A treatment of no growth regulators was included. Shoot development resulted in almost all explants in every treatment.

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HIGH-EFFICIENCY SHOOT REGENERATION FROM IN VITRO-GROWN LEAVES OF APPLE

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A high efficiency (up to 95%) shoot regeneration system for apple was developed. Explants consisted of young leaves harvested from in-vitro proliferating shoots cultures of several apple genotypes including 'Dayton', 'Gala', 'Golden Delicious', 'Malling 7a', 'McIntosh', and 'Royal Gala'. Leaf sections were cultured on a modified one-half strength Murashige and Skoog medium supplemented with thidiazuron (4.4 mg/L) and α -naphthaleneacetic acid (0.5 mg/L). Direct shoot organogenesis was often observed but sometimes was accompanied by callus formation. Regeneration frequency was genotype dependent. Other treatments investigated which enhanced regeneration included minced versus sectioned leaves and initial culture conditions of dark versus light. The possible applications of this regeneration system for *Agrobacterium*- and/or microprojectile-bombardment-mediated genetic transformation will be discussed.

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VITRIFIED VS. GLAUCOUS ONION (*ALLIUM CEPA* L.) LEAVES: A MICROSCOPIC COMPARISON

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The purpose of this study was to observe the difference in leaf surface characteristics between in vitro (vitrified and glaucous) and greenhouse grown onion leaves. Scanning electron microscopy (SEM) was used. Vitrified (hyperhydrated) leaves had little epicuticular wax and the leaf surface appeared twisted and wavy. Stomata were raised above the epidermis, appeared round instead of oblong and openings were malformed and clogged with waxy deposits. Both glaucous in vitro and greenhouse leaves had thick layers of epicuticular wax, the wax appearing to be heavier on greenhouse leaves. Stomata were oblong and sunken beneath layers of epicuticular wax. Vitrified plants died when transferred to the greenhouse under mist, while glaucous in vitro plants survived transplanting. Vitrified plantlets have a translucent, glass-like appearance and do not survive transfer to ex vitro conditions. Factors affecting vitrification will be addressed in later publications.

646 (PS 4)

PHOTOSYNTHETIC PHOTON FLUX AND UV-B EFFECTS ON ETHYLENE EVOLUTION, PROLIFERATION, AND LIPID CONTENT OF 'DOYENNE D'HIVER' PEAR IN VITRO

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In vitro-grown shoots of 'Doyenne d'Hiver' pear (*Pyrus communis* L.) were maintained for 7 days on 16-h photoperiods at photosynthetic photon fluxes (PPF) of 90, 180 or 270 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and irradiated 6 h per day midway through the photoperiod at 0.2 (-UV-B) or 12.0 (+UV-B) $\text{kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ of biologically effective ultraviolet-B (UV-B_{BE}) radiation. Increased ethylene production from UV-B_{BE}-treated shoots as compared to the control was recorded after 24 h in cultures grown under 90 and 180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and after 72 h in shoots grown under 270 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; PPF level had little effect on amount of ethylene evolved. Proliferation was highest at 270 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, but UV-B_{BE} decreased proliferation

and increased apical necrosis. UV-B_{BE} treatment increased lipid peroxidation after 24 h based on malondialdehyde (MDA) determination, but thereafter had no consistent effect on MDA. UV-B_{BE} exposure slightly decreased the monogalactosyldiacylglycerol/digalactosyldiacylglycerol ratio during the initial 24 h, showing an effect on chloroplast membranes.

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ADVENTITIOUS SHOOT REGENERATION FROM IN VITRO-CULTURED LEAVES OF *RUBUS* GENOTYPES

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Regeneration of adventitious shoots from in vitro-derived leaves of several *Rubus* genotypes was tested with various concentrations of cytokinins and auxins, different basal media, duration of dark incubation periods, temperatures and photosynthetic photon fluxes (PPF). Thidiazuron (TDZ) was significantly more effective than benzyladenine (BA), and indolebutyric acid (IBA) more effective than naphthaleneacetic acid (NAA). Leaves of 'Summit' and 'Sentry' raspberries and MD-ETCE-1 blackberry were most responsive (percentage of leaves responding, number of shoots formed) to 1 μ M or 10 μ M TDZ plus 0.5 μ M or 1 μ M IBA; MD-ETCE-1 leaves did not respond to BA or TDZ with either 2.7 or 5.4 μ M NAA. More organogenesis occurred on MS than on half-strength MS, Anderson modified, WPM or N6 media. For the five cultivars tested ('Autumn Bliss', 'Canby', 'Sentry', 'Summit', MD-ETCE-1), the most shoots formed when leaves were incubated at 20°C (vs. 25°C) for 1 week in the dark before they were exposed for 16 h per day to a PPF of 40 μ mol·m⁻²·s⁻¹. Significant differences in regeneration frequency were observed among four cultivars with the highest 71% for 'Autumn Bliss' and the lowest 29% for 'Summit'.

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PARTICLE BOMBARDMENT OF APPLE LEAF EXPLANTS INFLUENCES REGENERATION OF APPLE PLANTLETS

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Particle bombardment has been shown to be useful for genetic manipulation of many plants; however, a critical component for successful transformation is the ability of transformed cells to regenerate plants. This study describes factors that affect the regeneration efficiency of apple leaf explants following particle bombardment. Basal leaf segments of micropropagated 'Royal Gala' apple were treated with 1 μ m gold particles (0.5 μ g/10 μ l), accelerated at either 4.5, 6.2, 7.6, 9.3 or 13.8 MPa, and cultured on N6 salts + 10 μ M TDZ regeneration medium for 5, 10 or 20 days in the dark. Both microprojectile-treated and control explants exhibited 85-100% regeneration. However, only 30-60% of the explants bombarded at 7.6, 9.3 and 13.8 MPa had more than 10 regenerants and 6-10% had more than 20 regenerants, whereas for control explants and those bombarded at 4.5 and 6.2 MPa, 70-90% had more than 10 regenerants and 30-50% had more than 20.

649 (PS 4)

ISOLATION AND CULTURE OF DAYLILY MESOPHYLL PROTOPLASTS

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Mesophyll protoplasts were isolated from a diploid daylily (*Heimerocallis* cv. 'Red Magic') by enzyme digestion with a solution containing 0.5% Pectolyase Y-23, Cellulase R-10 1.0%, Driselase 0.1%, Sorbitol 0.6M and half-strength MS inorganic salts at 60 rpm for 4 h. The protoplasts underwent sustained division to produce multicellular colonies on a MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA. The optimal plating density for cell division was 5 X 10⁴ cells per ml. Cultures grown in agarose-bead media resulted in higher plating efficiencies than those in solidified or liquid media. Under the above conditions, formation of colonies occurred in 8 to

11% of the cultured protoplasts. Research is in progress for the production of callus from protoplast-derived colonies and for the generation of plantlets from callus.

650 (PS 4)

FIELD PERFORMANCE OF MUSCADINE GRAPE MICROPROPAGATED FROM SHOOT TIPS

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Phenotypic stability and yield of muscadine grape (*Vitis rotundifolia*) plants produced via micropropagation from shoot tips must be demonstrated to overcome reluctance to use micropropagated plants in vineyards. 'Golden Isles' plants produced from culture of fragmented shoot tips were planted in the vineyard, and yield data were collected three, four and five years after planting. Yield of four-year old vines was comparable to that collected in earlier years at this location from 'Golden Isle' plants from stem cuttings. Yield in the fifth year, however, declined significantly, perhaps due to late cold spring weather or heavy fruiting the previous year on these young vines. After five years in the vineyard, trunk diameter, shoot length, leaf area, berry size and soluble solids of the micropropagated plants were compared to those from stem cuttings. The only difference detected was for soluble solids, a trait highly influenced by environmental conditions. All micropropagated plants appeared similar to each other and to the 'Golden Isles' cultivar.

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REGENERATION OF HYPOCOTYL EXPLANTS OF GARDENEGGS (*SOLANUM GILLO*)

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The success of crop transformation is contingent upon the efficient regeneration of cocultivated explants. This study was initiated as part of a transformation program designed to solve some critical problems in gardenegg, an important solanaceous fruiting vegetable in many tropical African countries. A preliminary investigation involving the screening of cotyledons, hypocotyl, and leaf explants on zeatin-, kinetin-, or BAP-supplemented media produced regeneration in all explant types, but hypocotyl explants were consistently faster and produced more plantlets/explants. In this study, hypocotyl explants excised from 2-week-old seedlings cultured on media composed of MS salts supplemented with 2% sucrose, 0.9 mg/l thiamine-HCl, 2 mg/l glycine, 1-4 mg/l zeatin, zeatin riboside, or BAP, and 3.5% phytigel. Zeatin and zeatin riboside induced the fastest and most prolific shoot initiation. Rhizogenesis, however, was better on BAP-supplemented media. All plantlets rooted readily when transferred onto a hormone-free medium. As many as 28 plantlets/explants were obtained in some treatments. Rooted explants transferred into potted soil mix and acclimatized for 2 weeks in a growth chamber grew normally.

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LIQUID MEDIUM OVERLAYS ENHANCE GROWTH AND MULTIPLICATION OF IN VITRO APPLES AND GRAPES

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Previous studies have shown that liquid medium additions on established cultures enhance shoot growth and proliferation. In the present report, the growth and multiplication of apples and grapes were evaluated after the addition of liquid media to established cultures. Grapes and apples were micropropagated on agar solidified nutrient medium with 5 μ M BAP, and 4.4 μ M BAP, 1.4 μ M GA, 4.9 μ M IBA, respectively. A liquid overlay of similar medium was added after 4 to 6 weeks in both cultures. Improved growth, number of shoots, and a reduction in callus growth were observed in both species as compared to shoots transferred to fresh solid media. The number of micropropagated apple shoots and their height increased significantly by 35% and 69% respectively. Proliferation of grape shoots increased by 198% while callus growth decreased by 64% when compared to cultures transferred to fresh solid media.