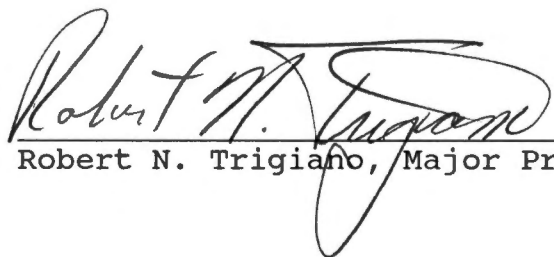
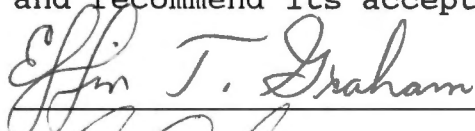
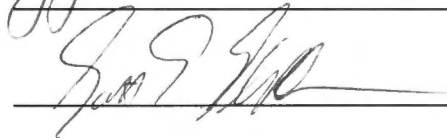


To the Graduate Council:

I am submitting herewith a thesis written by Lori Anne Weaver entitled "Axillary Bud Proliferation And Somatic Embryogenesis In American Yellowwood [*Cladrastis lutea* (Michx.) K. Koch]". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ornamental Horticulture and Landscape Design.


Robert N. Trigiano, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Provost and
Dean of the Graduate School

ABSTRACT

The potential for *in vitro* propagation of *Cladrastis lutea* (Michx) K. Koch [American yellowwood] by axillary bud proliferation and somatic embryogenesis was investigated. Axillary buds were removed from elongating branchlets during the first week in April 1989 and aseptically cultured on modified Murashige and Skoog (MS) medium amended with either 0.5, 1.0, 3.0 or 5.0 mg·liter⁻¹ 6-benzyladenine (BA). The numbers of shoots and buds were recorded at four week intervals for 24 weeks. The average length of harvested shoots decreased with increasing BA concentrations. Conversely, the average number of buds per explant increased with higher BA levels. Axillary buds cultured on medium supplemented with 1.0 mg·liter⁻¹ BA produced the most consistent number of elongated shoots and buds over the six month culture period. Elongated shoots produced on any of the BA treatments were excised and transferred to rooting medium. Excised shoots could be rooted only when cultured in the dark on half-strength MS medium supplemented with 1.0 mg·liter⁻¹ indole-3-butyric acid (IBA).

The somatic embryogenic potential of yellowwood was investigated by aseptically culturing 4-10 week post-anthesis ovules at weekly intervals. Zygotic embryos were

excised and placed on Shenck and Hildebrandt (SH) medium supplemented with either 1.0, 2.0, 3.0 or 5.0 mg·liter⁻¹ 2,4-D. Explants were transferred after 2 and 3 weeks to SH medium amended with 5.0 mg·liter⁻¹ 1-naphthaleneacetic acid (NAA). Somatic embryos were initiated on all treatments and usually formed as pairs at the proximal end of the cotyledonary tissue. The greatest embryogenic response occurred from 6-8 weeks post-anthesis. Somatic embryos typically had fasciated cotyledons but were capable of producing a radicle when transferred to half-strength SH medium lacking hormones. Whole plants were regenerated from 3 embryos and established in soil.

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INTRODUCTION

Cladrastis lutea (Michx.) K. Koch [American yellowwood] is a 40-60 foot ornamental tree and a member of the Fabaceae (Leguminosae). The tree was first described as growing along the banks of the Cumberland River in Tennessee in 1796 by the French taxonomist Andre' Michaux and classified as *Virgilia lutea* Michx. (cf. Robertson, 1977). The German taxonomist, Karl Koch, removed yellowwood from the genus *Virgilia* in 1869 and erected the genus *Cladrastis* to accommodate the tree. The genus name is derived from the Greek words *klados* (branch) and *thraustos* (fragile) that refer to the brittleness of the branchlets (Everett, 1981). The common name can be traced to the early American pioneers who used the bright yellow heartwood to dye homespun cloths (Crockett, 1972). The natural range of American yellowwood is limited to areas in Kentucky, Tennessee, North Carolina, Missouri and Indiana (Fig. 1). The three other species of *Cladrastis* include Chinese (*C. sinensis* Hemsl.), Japanese (*C. platycarpa* Mak.) and Oriental yellowwood (*C. wilsonii* Takeda), all of which are native to southeastern Asia and are not commonly planted in the United States.

American yellowwood is admired for its flowering habit, attractive yellow fall color and landscape value as a single specimen or avenue tree. The wood was used

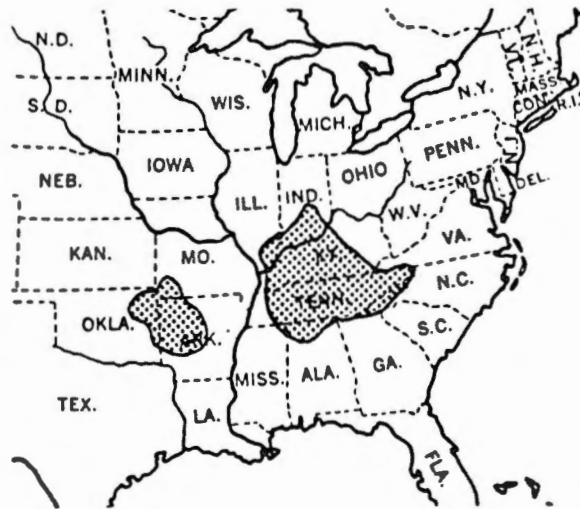


Figure 1. Natural range of *Cladrastis lutea* in the United States (Source: Harrar, 1971)

occasionally as a substitute for walnut in gunstocks and for handmade novelty gifts (Harrar, 1971). The tree bears hanging panicles of white or occasionally pink pea-shaped flowers in late April followed by light brown pods in the summer. Blooms are abundant in alternate years or every third year (Dirr, 1975).

The only methods of commercial propagation of yellowwood are seeds and root cuttings taken from young seedlings in late December (Dirr and Heuser, 1987). Germination of some tree and woody ornamental seeds require scarification of the seed coat. As with many other leguminous species, yellowwoods have impermeable seed coats capable of inhibiting germination if seeds are planted immediately after maturation (Frett and Dirr, 1979). Scarification of the seed coat with concentrated sulfuric acid is usually necessary to facilitate water imbibition and to provide uniform germination (Olson and Barnes, 1974). An important factor for commercial producers is that the seed supply may be limited from year to year due to sporadic blooming cycles.

Tissue culture is an alternative method of clonal propagation that potentially offers rapid mass multiplication without seasonal constraints. Micropropagation is the *in vitro* production of plant material in which proliferation may occur by the following three

processes: 1) axillary bud proliferation, 2) organogenesis and 3) somatic embryogenesis. Axillary bud proliferation is the most widely used commercial method of plant regeneration (Lutz *et al.*, 1985) and involves the elongation and development of shoots from preformed meristems of shoot tips or axillary bud explants (Pierik, 1987). Organogenesis is the monopolar formation of either shoots or roots with vascular connections to the parent tissue (Ammirato, 1985). Somatic embryogenesis is a process that occurs without fusion of gametes or sexual recombination and resembles zygotic embryo ontogeny in true seeds (Williams and Maheswareen, 1986). Somatic embryos have closed vascular systems without connections to the explanted material and are bipolar with root and shoot apices capable of regenerating entire plantlets identical to the parent (Haccius, 1978; Ammirato, 1985).

American yellowwood has ornamental qualities and the potential for wider geographic availability. However, its value in nursery and landscape production can be limited by lack of an efficient method of propagation. Seed supplies are not reliable from year to year and vegetative propagation is difficult from mature trees. As a result, the availability of named clones or cultivars has been limited. Micropropagation offers a means to enhance the efficiency of propagation and the horticultural value of

American yellowwood in the United States.

This thesis investigation was undertaken to assess the potential of *C. lutea* to regenerate *in vitro*. The first objective of the study was to induce axillary bud proliferation and whole plant regeneration. The second objective was to determine if yellowwood was capable of somatic embryo formation from immature zygotic embryo explants and, then to achieve germination and whole plant development.

The following thesis includes an Introduction and is divided into three sections. Section I is the Literature Review and Sections II and III are presented as separate journal articles which were prepared in a format suitable for submission to The Journal of The American Society for Horticultural Science.

I. LITERATURE REVIEW

The Leguminosae (Fabaceae) is the third largest angiosperm family and includes 600 genera and 12,000 species of annuals, perennials, vines, shrubs and trees (cf. Hammatt *et al.*, 1986) (Fig. I.1). More than 35 legumes, including forage, grain and tree species, have been regenerated using various tissue culture techniques (Styer and Chin, 1983; Flick *et al.*, 1984; Hammatt *et al.*, 1986).

Tissue Culture Propagation

In vitro propagation has been utilized in the production of ornamental plants since the early 1960's. Tissue culture techniques were recognized as a means for mass clonal propagation of ornamental crops while attempting to obtain virus-free orchids (Morel, 1964). Currently, over 1,000 ornamental and agronomic species have been regenerated through tissue culture techniques (Brown and Thorpe, 1986; Murashige, 1988). Tissue culture as a plant propagation tool offers several advantages over conventional production, including rapid clonal multiplication, reduced stock plant area and year-round production of high quality plants (Pennell, 1983).

Distinctions have been made between the three primary

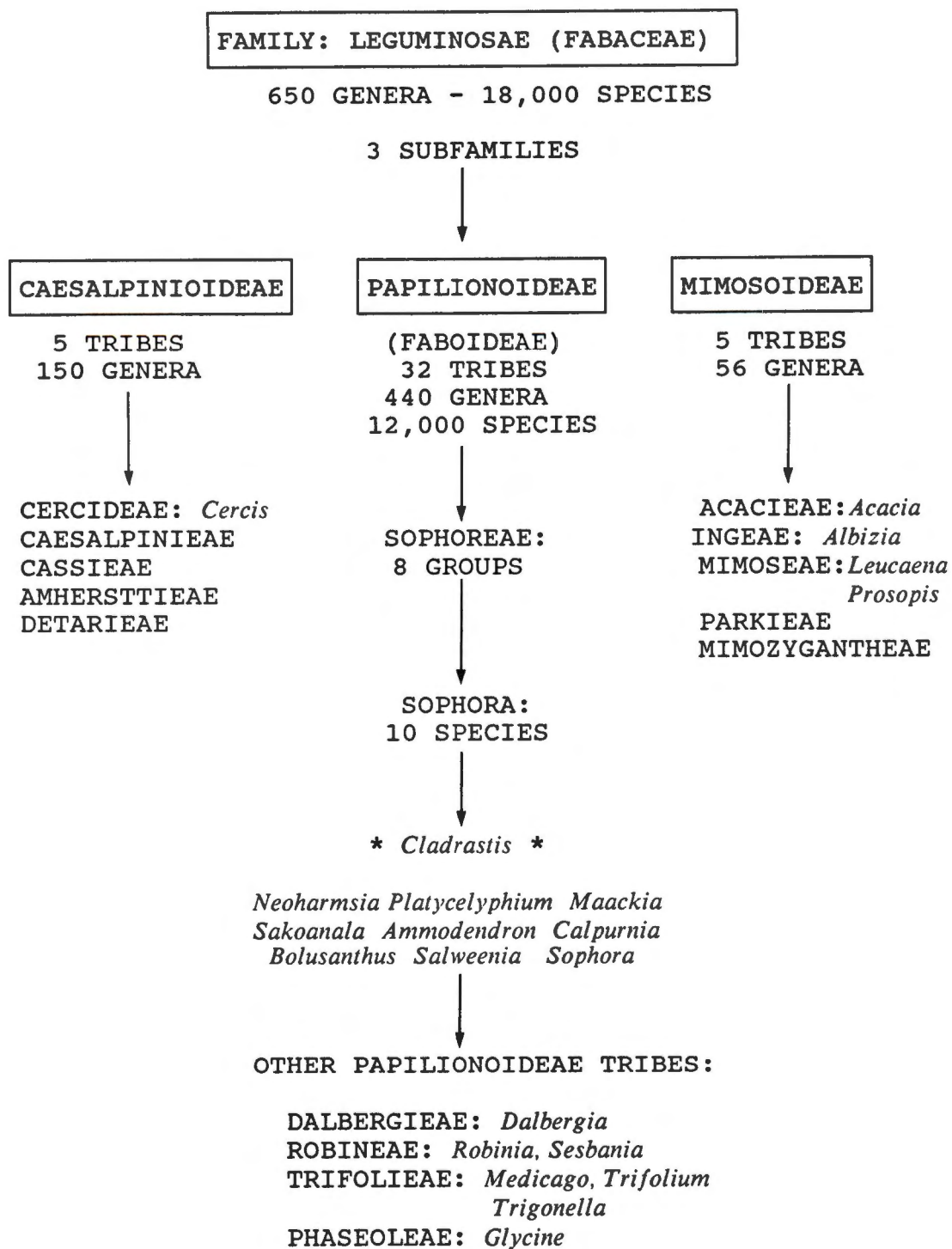


Figure I.1. Taxonomic placement of *Cladastris* in Leguminosae.
(Source: Pohill and Raven, 1981)

modes of tissue culture regeneration: axillary bud proliferation, organogenesis and somatic embryogenesis (Hu and Wang, 1983). Axillary bud proliferation from shoot tips or axillary bud cultures differ from the other modes since organized meristems are differentiated *in vivo* with elongation and root initiation necessary in forming complete plantlets. In organogenic and somatic embryogenic systems, however, *de novo* organization and developmental changes occur before either shoots, roots or other organs will be formed.

Stages of Micropropagation

The *in vitro* production of plants is accomplished through a three step sequence as outlined by Murashige (1974) as Stages I, II and III. The methodology has been extended to include two additional stages, Stage 0 (Debergh and Maene, 1981) and Stage IV (George and Sherrington, 1984). Stage 0 emphasizes the selection of the appropriate explant material, considering age, genotype, health and time of year the material is obtained. Stage I is designated as the establishment of sterile explants in culture.

The objective of Stage II is rapid production of organs which would give rise to complete plants. The development of these organs has been demonstrated to be

directly related to the auxin-cytokinin ratio of the culture medium (Skoog and Miller, 1957). Cytokinins are used extensively to enhance axillary bud cultures by lessening the effects of apical dominance and enhancing the branching of lateral buds (cf. Tisserat, 1985). Somatic embryogenic systems most often utilize auxins (Wann, 1988).

Stage III in axillary bud systems involves root formation on excised shoots and preparation for transfer to soil. High auxin to cytokinin ratios usually favor root production (Skoog and Miller, 1957). Germination or 'conversion' of somatic embryos into plantlets on medium with little or no hormones was the objective of Stage III in somatic embryogenic systems.

Greenhouse acclimation of plantlets is considered the goal of Stage IV. Following transfer to soil, maintenance of the plantlets in high humidity and under low light intensity is essential for changing plants from an autotrophic state in culture to a heterotrophic state in the greenhouse.

Somatic Embryogenesis

The process by which somatic cells develop into entire plants in a manner similar to zygotic embryo ontogeny has been characterized as somatic embryogenesis (Wann, 1988). Somatic embryogenesis was first

by cultural treatment to a permissive state. Directive induction does not entail structural changes in the cells, but only physiological alterations. After cells have been redirected, somatic embryogenesis occurs through permissive cultural conditions. Difference in 2,4-D levels and source of nitrogen between the somatic embryo proliferation medium and expression medium were the critical directive elements involved in somatic embryo development in soybean (Christianson, 1981).

Liquid suspension cultures must be developed to fully realize the potential of somatic embryogenesis in the production of ornamental plants. Somatic embryos in numbers sufficient for mass propagation may be grown and harvested from large, continuous batch, fermentation-like cultures (Styer, 1985). The advantages of such a system are many, including mass clonal propagation of embryos from few cultures and the development of synthetic seeds. Reports have documented that 60,000 carrot embryos may be produced in one liter of liquid medium (Ammirato, 1987).

The majority of reports of somatic embryogenesis for legume species have been made for agronomic crops. Somatic embryos have formed indirectly from leaf tissue callus of *Medicago sativa* L. [alfalfa] (Saunders and Bingham, 1972) and *Trifolium pratense* L. [red clover] (Phillips and Collins, 1981). Somatic embryos have also been produced from immature

cotyledonary tissue of *Glycine max* (L.)Merr. [soybean] (Beversdorf and Bingham, 1977; Lazzeri *et al.*, 1985; Ranch *et al.*, 1985).

Tissue Culture Of Woody Ornamentals

Many woody ornamentals are well suited for tissue culture propagation because they are either slow or difficult to propagate by conventional cuttings or seed are difficult to germinate (Ma and Wang, 1977). Introduction of new varieties of azalea and rhododendron may have taken over ten years using conventional vegetative methods. Tissue culture reduced the time required to develop sufficient stock of new clones for market introduction (Anderson, 1975; Ma and Wang, 1977). Early work with other woody ornamental species included *in vitro* propagation of roses via shoot tips and lateral buds (Hasegawa, 1979; Skirvin and Chu, 1979).

The number of tree species which have been successfully propagated through tissue culture have been increasing rapidly during recent years (Mott, 1981; Sommer and Wetzstein, 1984; Bajaj, 1986; Dunstan and Thorpe, 1986). The first tree micropropagated *in vitro* was derived from shoots produced from subcultured callus of *Populus tremuloides* Michx. [aspen] (Winton, 1970). *Betula platyphylla* [white birch] was the first ornamental tree species

commercially propagated from shoot tip cultures (McCown and Amos, 1979). An average of twenty shoots were produced per culture in six to eight weeks. The investigators estimated that only 125 ft² of culture area was needed to produce half a million plants per year.

Tissue Culture Of Leguminous Trees

Organogenesis And Axillary Bud Proliferation

Propagation of leguminous trees has been successful through organogenic and axillary bud systems (Table I.1). Mass clonal production of a leguminous tree, *Robinia pseudoacacia* L. [black locust] has been reported recently (Barghchi, 1987). Axillary bud proliferation was accomplished on Murashige and Skoog medium (1962) amended with 0.25-1.0 mg·liter⁻¹ 6-benzyladenine (BA). Rooting of microshoots was successful in half-strength MS supplemented with 0.5-1.0 mg·liter⁻¹ indole-3-butyric acid (IBA).

Somatic Embryogenesis

Reports of somatic embryogenesis in hardwood tree species are not as prevalent as documentation on herbaceous species (Table I.2). The number of reports of somatic embryogenesis in leguminous trees are relatively few and include five species (Table I.3). Various

Table I.1. Examples of micropropagation of leguminous trees by organogenesis or axillary bud proliferation.

TREE	AUTHORS (explant)
<i>Albizia lebbek</i> (L).Benth [<i>Albizia</i>]	Gharyal and Maheshwari, 1983 Rao and De, 1987 Tomar and Gupta, 1988a (seedling hypocotyl)
<i>Acacia koa</i> Grey [<i>Acacia</i>]	Skolmen and Mapes, 1976 (shoot tips)
<i>Acacia auriculiformis</i> A. Cunn. ex. Benth [<i>Acacia</i>]	Mittel <i>et al.</i> , 1989 (axillary buds)
<i>Cercis canadensis</i> L. var. <i>mexicana</i> [<i>Mexican redbud</i>]	Bennett, 1987 (axillary buds)
<i>Dalbergia latifolia</i> Roxb. [<i>East Indian rosewood</i>]	Lakshimi Sita <i>et al.</i> , 1986 Rao, 1986 (shoot tips)
<i>Dalbergia sissoo</i> Roxb. [<i>Dalbergia</i>]	Suwal <i>et al.</i> , 1988 (seedling cotyledon)
<i>Leucaena leucocephala</i> (Lam) de Wit. [<i>Leucaena</i>]	Goyal <i>et al.</i> , 1985 (axillary buds)
<i>Prosopis cineraria</i> [<i>Prosopis</i>]	Goyal and Arya, 1984 (axillary buds)
<i>Robinia pseudoacacia</i> L. [<i>black locust</i>]	Davis and Keathley, 1987 Barghchi, 1987 (axillary buds)

Table I.2. Examples of somatic embryogenesis reported in hardwood trees species.

TREE	AUTHORS
<i>Aesculus hippocastanum</i> L. [horsechestnut]	Radojevic, 1988
<i>Carya illinoensis</i> (Wangenh.) C. Koch [pecan]	Merkle <i>et al.</i> , 1987
<i>Castanea sativa</i> Mill. [Spanish chestnut]	Gonzalez <i>et al.</i> , 1985
<i>Cercis canadensis</i> L. [eastern redbud]	Trigiano <i>et al.</i> , 1988
<i>Cornus florida</i> L. [dogwood]	Trigiano <i>et al.</i> , 1989
<i>Corylus avellana</i> L. [filbert]	Radojevic <i>et al.</i> , 1975
<i>Eucalyptus</i> sp. [eucalyptus]	Quyang <i>et al.</i> , 1981
<i>Fraxinus americana</i> L. [white ash]	Preece <i>et al.</i> , 1989
<i>Juglans regia</i> L. [walnut]	Tulecke and McGranahan, 1985
<i>Ilex aquifolium</i> L. [English holly]	Hu and Sussex, 1971
<i>Liquidambar styraciflua</i> L. [sweetgum]	Sommer and Brown, 1980
<i>Liriodendron tulipifera</i> L. [yellow poplar]	Merkle and Sommer, 1986
<i>Paulownia tomentosa</i> Steud. [empress tree]	Radojevic, 1979
<i>Populus nigra</i> [lombardy poplar]	Park and Son, 1988
<i>Quercus rubra</i> L. [red oak]	Gingas and Lineberger, 1989
<i>Quercus alba</i> L. [white oak]	
<i>Robinia pseudoacacia</i> L. [black locust]	Merkle and Weicko, 1989
<i>Santalum album</i> L. [sandalwood]	Rao, 1965

Table I.3. Reports of somatic embryogenesis from seedling hypocotyl or immature zygotic embryo explants of leguminous trees.

TREE	AUTHORS (explant)
<i>Acacia koa</i> Grey [Acacia]	Skolmen, 1986 (seedling hypocotyl)
<i>Albizia lebbek</i> (L.) Benth [Albizia]	Gharyal and Maheshwari, 1981 (seedling hypocotyl)
<i>Albizzia richardiana</i> (L.) Benth [Albizzia]	Tomar and Gupta, 1988a (seedling hypocotyl)
<i>Cercis canadensis</i> L. [Eastern redbud]	Trigiano et. al, 1988 (immature zygotic embryo)
<i>Robinia pseudoacacia</i> L. [Black locust]	Merkle and Wiecko, 1989 (immature zygotic embryo)

explants of *Acacia koa* Grey [acacia] were cultured on solid and liquid MS or Schenk and Hildebrandt (SH) medium. Fifty-two combinations of basal medium and growth regulators were used and embryo-like structures were induced on only SH liquid medium amended with 5.0 mg·liter⁻¹ p-chlorophenoxyacetic acid, 5.0 mg·liter⁻¹ kinetin and 0.2 mg·liter⁻¹ 2,4-D (Skolmen, 1986).

Somatic embryos were formed on root, hypocotyl and cotyledon explants of *Albizia lebbek* Hemsl. [East Indian walnut] (Gharyal and Maheswaren, 1981). B5 medium (Gamborg *et al.*, 1968) without the usual auxin supplement supported somatic embryo development. The average number of embryos reported per hypocotyl was between 20-25. Somatic embryogenesis in an additional *Albizia* species, *A. richardiana* Hemsl. was reported from callus derived from hypocotyl explants (Tomar and Gupta, 1988a). Callus was induced on B5 medium amended with BA and subsequently transferred every five weeks to MS also supplemented with BA. Two-to-twenty somatic embryos were observed per culture following the seventh subculture.

Cercis canadensis L. [eastern redbud] was the first temperate leguminous tree reported to be capable of somatic embryogenesis (Trigiano *et al.*, 1988). Immature zygotic embryos were cultured on SH medium amended with ammonium sulfate and various concentrations of 2,4-D.

Somatic embryos were observed on 96-110 day post-anthesis zygotic embryo cultures. Media containing either 2.0 or 3.0 mg·liter⁻¹ 2,4-D produced more embryos than media that contained either 1.0 or 5.0 mg·liter⁻¹ 2,4-D.

More recently, somatic embryogenesis has been reported for *R. pseudoacacia* L. [black locust] (Merkle and Weicko, 1989). Immature zygotic embryo explants formed somatic embryos after 7 weeks following a one week exposure to 4.0 mg·liter⁻¹ 2,4-D and subsequent transfer to hormone free medium.

Tissue culture of American yellowwood

No tissue culture work to date has been reported for *Cladrastis lutea*. Although axillary bud proliferation and somatic embryogenesis have been accomplished in the Leguminosae, requirements for *in vitro* propagation of legumes is species dependent. Research with both tissue culture systems show that although related, protocols may differ for the members of the same family.

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II. AXILLARY BUD PROLIFERATION IN AMERICAN YELLOWWOOD

Abstract

Axillary buds of *Cladrastis lutea* (Michx.) K. Koch [American yellowwood] were removed from elongating branchlets in early April 1989, surface sterilized and placed on Murashige and Skoog medium (MS) amended with either 0.5, 1.0, 3.0 or 5.0 mg·liter⁻¹ 6-benzyladenine (BA). Elongated shoots were excised and the original buds and any callus subcultured to fresh MS medium every four weeks for 24 weeks. Elongated shoots (0.5 cm or greater) were excised and transferred to a rooting medium. The average length of shoots harvested decreased with increasing BA concentrations. Conversely, the average number of buds per explant increased with higher BA concentrations. The most consistent bud formation and number of elongated shoots formed over the six month period was observed from explants cultured on 1.0 mg·liter⁻¹ BA. Adventitious roots were only formed when shoots were transferred to half-strength MS medium supplemented with 1.0 mg·liter⁻¹ indole-3-butyric acid (IBA) and cultured in the dark.

Introduction

American yellowwood, *Cladrastis lutea* (Michx.) K. Koch, is a 40-to-60 foot ornamental tree of the Fabaceae (Leguminosae). The attractive, white or pink flowering tree is native to southeastern U.S. and is used as a specimen or avenue tree. Yellowwood is commercially propagated by seed and to a lesser extent by root cuttings. Seed supply, however, can be limited due to heavy flowering in alternate years or every third year (Dirr, 1975).

Tissue culture production of woody plants is becoming a viable alternative to conventional propagation methods. The emphasis of tissue culture programs in horticulture lies in the rapid propagation of selected genotypes (Haissig *et al.*, 1987). Mass propagation via tissue culture techniques would prove beneficial for increasing stock of *Cladrastis lutea* 'Rosea', a scarce pink flowering yellowwood. Axillary bud proliferation is widely used for ornamental crops because it insures a high degree of clonal fidelity (Hu and Wang, 1983). Many legumes are capable of axillary bud, organogenic or embryogenic regeneration (Styler and Chin, 1983; Flick *et al.*, 1984; Hammatt *et al.*, 1986). Regeneration of leguminous trees by *in vitro* techniques has been reported for the following leguminous species: *Albizia lebbek* (L.) Benth (Gharyal and Maheshwari, 1983; Rao and De,

1987; Tomar and Gupta, 1988), *Acacia koa* Grey (Skolmen and Mapes, 1976), *Cercis canadensis* L. (Bennett, 1987), *Dalbergia latifolia* Roxb. (Lakshimi Sita *et al.*, 1986; Rao, 1986) and *Robinia pseudoacacia* L. (Davis and Keathley, 1987; Barghchi, 1987).

The commercial availability of *C. lutea* is dependent on seed supply. Inconsistent seed production from year to year is a limiting factor in yellowwood propagation. Establishment and proliferation of axillary buds *in vitro* could yield large numbers of plantlets on a regular basis, alleviating the need for seed. The objective of this study was to establish American yellowwood axillary bud cultures. This is the first report of *in vitro* propagation of *C. lutea* via axillary bud proliferation.

Materials And Methods

Elongating yellowwood branches were collected in early April of 1989 from trees located on the University of Tennessee's Agricultural Campus, Knoxville, Tennessee. After the lamina and most of the petiole were removed, nodal segments were cut 1 cm above and below the enlarged clasping petiole bases enclosing the axillary buds. Stem segments were first dipped in 95% ethanol and flamed for 10 seconds, then surface sterilized in 20% v/v commercial bleach (1.05% sodium hypochlorite) with 0.1% Triton X-100

and rinsed three times in sterile water. Axillary buds were excised and placed in 20mm X 150mm culture tubes containing Murashige and Skoog (MS) basal salts (1962) amended with $100 \text{ mg} \cdot \text{liter}^{-1}$ thiamine, $1.0 \text{ mg} \cdot \text{liter}^{-1}$ myo-inositol, 3.0% sucrose and 0.7% agar. Non-contaminated buds were transferred after one week to similar MS medium amended with either 0.5, 1.0, 3.0 or $5.0 \text{ mg} \cdot \text{liter}^{-1}$ 6-benzyladenine (BA) to induce multiplication. The pH of all media was adjusted to 5.7 before steam sterilization at $1.06 \text{ kg} \cdot \text{cm}^{-2}$ for 20 minutes. Cultures were incubated at 25C growth chamber with $70 \mu\text{E sec}^{-1} \text{ m}^{-2}$ light provided by cool white flourescent tubes for 16 hr. Treatments were arranged in randomized complete blocks with twelve replications.

Each culture was examined at four week intervals (one cycle), and data recorded on axillary buds of each explant and number and length of harvested shoots. Excessive callus was trimmed and explanted buds were subcultured to fresh multiplication medium at this time. Data were analyzed by analysis of variance (ANOVA) and means separated using Duncan's New Multiple Range Test ($\alpha = 0.05$). Analyses were conducted within the individual cycles; comparisions between cycles over the course of the 24 week experiment were not made.

Elongated shoots were harvested and transferred to half-strength MS or to MS medium supplemented with either

indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA) or activated charcoal. Treatments were incubated in either 16 h light or in the dark. Rooting treatments consisted of the following: (1) control (2) $0.1 \text{ mg} \cdot \text{liter}^{-1}$ IBA in the light (3) $1.0 \text{ mg} \cdot \text{liter}^{-1}$ IBA in the light (4) $0.5 \text{ mg} \cdot \text{liter}^{-1}$ NAA in the light (5) $1.0 \text{ mg} \cdot \text{liter}^{-1}$ (NAA) in the light (6) $1.0 \text{ mg} \cdot \text{liter}^{-1}$ IBA in the dark for 7 days then into light (7) $1.0 \text{ mg} \cdot \text{liter}^{-1}$ NAA in the dark for 7 days then into light (8) $1.0 \text{ mg} \cdot \text{liter}^{-1}$ IBA + 0.25% activated charcoal in the light (9) $1.0 \text{ mg} \cdot \text{liter}^{-1}$ NAA + 0.25% activated charcoal in the light (10) 1.0 mg/L IBA in the dark.

Results and Discussion

The initial axillary bud explants formed three shoots during the first culturing cycle. Examination under the dissecting microscope revealed the presence of three winter buds at the base of each yellowwood petiole, indicating that the three shoots were of *in vivo* origin. Analyses of data on shoot length and number of buds after six cycles showed that the BA levels had a significant effect within some of the individual cycles. There were no significant differences in shoot length after two cycles among any of the treatments. However, significant differences were evident in the subsequent cycles between

the 0.5 and 1.0 mg·liter⁻¹ BA concentrations and the 3.0 and 5.0 mg·liter⁻¹ treatments (Table II.1). After six cycles, significantly more shoots were produced on the 1.0 mg·liter⁻¹ treatment than the other treatments. No significant difference was detected in the number of axillary buds produced during the first three cycles or in the last cycle. Statistical differences were determined between the lower and higher BA concentrations in both cycle 4 and 5 (Table II.2). Significant differences in number of axillary buds proliferated in cycle 4 occurred between the 5.0 mg·liter⁻¹ treatment which had an average of 19 buds per explant and 0.5 mg·liter⁻¹ level which had 13 buds per explant.

The average length of shoots from axillary buds decreased with increasing BA concentrations. Shoots from the 0.5 mg·liter⁻¹ treatment exhibited rapid shoot elongation during the first three cycles. Shoot elongation at 5.0 mg·liter⁻¹ level was slow and suppressed as compared to the other treatments. Conversely, the average number of axillary buds per explant increased with increasing BA concentrations (Fig. II.1). Shoots were harvested from all BA treatments. The 3.0 and 5.0 mg·liter⁻¹ treatments formed the greatest number of buds, but elongation of shoots to a harvestable length (over 0.5 cm) did not occur as readily as in the other two treatments (Plate I. Figs. A-D). Axillary buds explanted

Table II.1. Mean number of shoots (S) and range of shoots (R) produced per *Cladrastis lutea* axillary bud explant for each of the six culturing cycles.

BA (mg/L)	Cycle											
	1			2			3			4		
	S	R	S	S	R	S	S	R	S	S	R	S
0.5	0.5 a ^z	0-2	0.8 a	0-2	1.7 a	0-4	1.1 ab	0-2	0.7 ab	0-2	0.4 b	0-2
1.0	0.5 a	0,1	1.0 a	0-4	1.5 a	0-3	1.4 a	0-3	1.5 a	0-6	1.2 a	0-5
3.0	0.5 a	0-2	1.3 a	0-4	0.3 b	0-2	0.5 b	0-4	0.4 b	0-4	0.4 b	0-2
5.0	0.5 a	0,1	0.6 a	0-2	0.3 b	0,1	0.3 b	0	0.0 b	0	0.0 b	0

^z Mean separation in columns by Duncan's Multiple Range Test, alpha = 0.05.

Table II.2. Mean number of buds (B) and range of buds (R) produced per Cladrastis lutea axillary bud explant for each of the six culturing cycles.

BA (mg/L)	Cycle											
	1			2			3			4		
	R	B		R	B		R	B		R	B	
0.5	2.5 a ²	1-5	5.0 a	1-8	11.0 a	1-18	12.8 b	1-25	13.6 b	1-25	10.8 a	1-20
1.0	2.7 a	1-5	5.3 a	0-15	11.3 a	1-25	12.1 b	0-25	16.0 ab	1-40	14.6 a	0-40
3.0	3.2 a	1-6	6.0 a	1-18	15.1 a	0-22	16.9 ab	0-35	24.0 ab	1-50	18.1 a	0-40
5.0	2.4 a	1-4	5.1 a	1-16	13.9 a	1-20	19.1 a	1-35	18.0 a	1-35	14.8 a	0-38

² Mean separation in columns by Duncan's Multiple Range Test, alpha = 0.05.

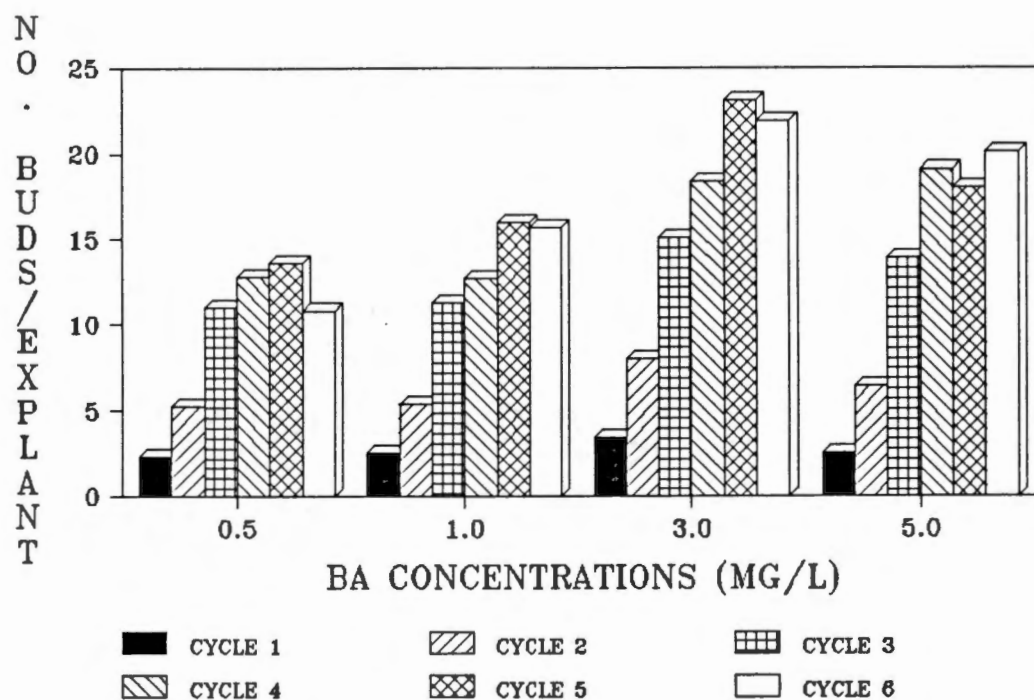
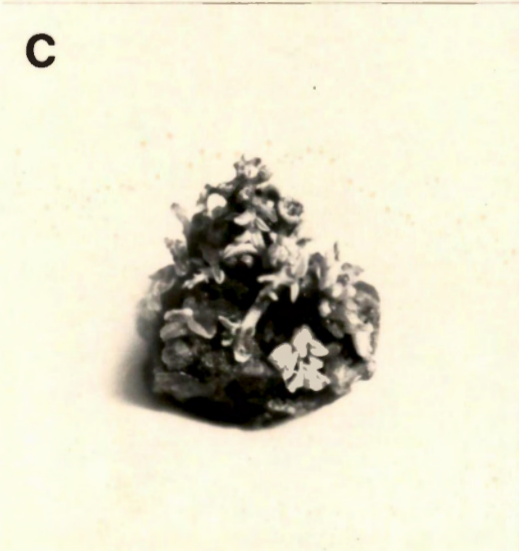


Figure II.1. Effect of BA concentrations on mean number of *Cladrastis lutea* buds per explant after six cycles.

Plate I. Axillary bud proliferation and shoot elongation in *Cladrastis lutea* after three culturing cycles on MS medium supplemented with 6-benzyladenine (BA).

- A. Elongated shoots on the $0.5 \text{ mg} \cdot \text{liter}^{-1}$ BA treatment.
- B. Increased shoot elongation on the $1.0 \text{ mg} \cdot \text{liter}^{-1}$ BA treatment.
- C. Buds and limited shoot elongation on the $3.0 \text{ mg} \cdot \text{liter}^{-1}$ BA treatment.
- D. Masses of buds (arrows) at the base with suppressed shoot elongation on the $5.0 \text{ mg} \cdot \text{liter}^{-1}$ treatment.



LORI WEAVER
PLATE I
P. 37

to $0.5 \text{ mg}\cdot\text{liter}^{-1}$ treatments medium initially produced the most harvestable shoots, but gradually decreased over the 24 week culture period (Fig. II.2). The most consistent bud formation, elongation and number of harvested shoots over the six month culturing period occurred on medium containing $1.0 \text{ mg}\cdot\text{liter}^{-1}$ BA.

Optimum cultural concentrations of cytokinins for axillary bud multiplication may be different depending on the leguminous tree species. *Dalbergia spp.* are perhaps the closest related arborescent species to yellowwood in the subfamily Papilionoideae. An average of 10-to-15 shoots were harvested per cotyledonary node explant of *D. sissoo* on medium with $1.0 \text{ mg}\cdot\text{liter}^{-1}$ BA and $0.1 \text{ mg}\cdot\text{liter}^{-1}$ NAA every four weeks (Suwal *et al.*, 1988). Cultures were maintained for two years without loss of multiplication potential. Shoots were also induced from callus of *D. latifolia* on medium supplemented with $3.0 \text{ mg}\cdot\text{liter}^{-1}$ BA and $0.5 \text{ mg}\cdot\text{liter}^{-1}$ naphthaleneacetic acid (NAA) (Lakshmi Sita *et al.*, 1986).

Cercis canadensis var. *mexicana* [Mexican redbud] produced the greatest number of shoots; elongation was the greatest on medium containing $5 \text{ mg}\cdot\text{liter}^{-1}$ BA (Bennett, 1987). These cultures were also successfully maintained for one and one-half years. After a year, multiplication rates decreased and BA levels were reduced to 2-to-3 $\text{mg}\cdot\text{liter}^{-1}$. Medium supplemented with $0.25 \text{ mg}\cdot\text{liter}^{-1}$ indoleacetic acid

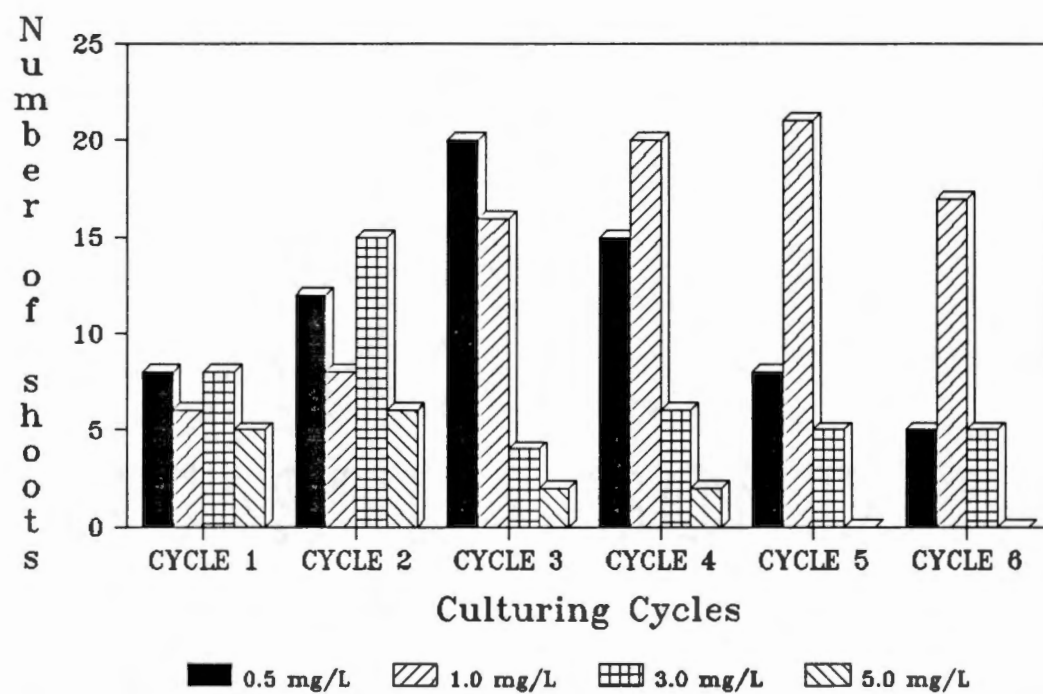


Figure II.2. Effect of BA concentration on number of *Cladrastis lutea* shoots harvested per cycle after six cycles.

and $0.5 \text{ mg}\cdot\text{liter}^{-1}$ gibberellic acid maintained shoot production. Unlike *Cercis* and *Dalbergia*, production of yellowwood shoots declined rapidly after six months and could not be maintained.

Vitrification or 'glassiness' of tissue is a physiological condition reported in *in vitro* systems and is associated with high cytokinin levels (Pierik, 1987). Vitrification of yellowwood tissue was observed only in the $5.0 \text{ mg}\cdot\text{liter}^{-1}$ treatments after five cycles. Similar observations have been reported in cultures of another leguminous tree, *R. pseudoacacia*. Optimum concentrations of BA for axillary bud proliferation were reported between 0.25 and $1.0 \text{ mg}\cdot\text{liter}^{-1}$ (Barghchi, 1987). However, $4.0 \text{ mg}\cdot\text{liter}^{-1}$ BA limited shoot elongation and cultures became vitrified and necrotic. Other studies with black locust axillary buds also report relatively high BA levels induced tissue vitrification (Davis and Keathley, 1987).

One of the principle problems encountered in propagation of many leguminous plants via tissue culture is the difficulty in rooting of excised shoots (Davis and Kealthey, 1987). Protocols used to produce roots on leguminous woody shoots have been shown to be species specific, although most required an auxin (Pierik, 1987). Forty-seven percent of the yellowwood shoots rooted on medium containing $1.0 \text{ mg}\cdot\text{liter}^{-1}$ IBA when incubated in the

dark for two weeks. Shoots transferred to the nine other rooting treatments elongated, but produced only callus from the cut surface. Difficulty in rooting shoots produced *in vitro* of other leguminous trees has been reported (Rao, 1986; Rao and De, 1987).

This report indicates that yellowwood is capable of regeneration by axillary bud proliferation. The 1.0 mg·liter⁻¹ BA treatment produced the most consistent response during the course of the experiment. Further research is necessary to improve bud proliferation, shoot elongation and rooting percentages before a rapid and efficient *in vitro* method of yellowwood propagation is commercially feasible.

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III. SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION IN AMERICAN YELLOWWOOD

Abstract

Somatic embryos developed on 4-9 week post-anthesis immature zygotic embryos from five *Cladrastis lutea* (Michx.) K. Koch [American yellowwood] trees. Seed pods were collected at weekly intervals, ovules removed and zygotic embryos explanted on modified Shenck and Hildebrandt medium (SH) amended with either 1.0, 2.0, 3.0 or 5.0 mg·liter⁻¹ 2,4- dichlorophenoxyacetic acid (2,4-D). Approximately one-third of the explants were transferred to SH medium supplemented with 5.0 mg·liter⁻¹ 1-naphthaleneacetic acid (NAA) after 2 and 3 weeks respectively. The remaining explants were incubated on the initial 2,4-D containing media for 6 weeks. All treatments supported somatic embryogenesis. Pairs of somatic embryos formed at the proximal end of the cotyledons; only a few formed as single embryos. The greatest number were formed from zygotic embryos explanted from 6-8 weeks post-anthesis. Six of 23 somatic embryos germinated on half-strength SH medium without hormones and three plantlets were transferred to soil. In experiments completed during the second year, somatic embryos were

produced from 6-9 week post-anthesis zygotic embryo explants.

Introduction

Cladrastis lutea (Michx.) K. Koch or American yellowwood is an arborescent species of the Fabaceae (Leguminosae) native to southeastern United States. The tree is used as an ornamental and commercially propagated by seed. Trees bloom heavily only in alternate years or every third year (Dirr, 1987), a limiting factor in the production and availability of yellowwood trees.

Somatic embryogenesis has been reported for many agronomic crops, ornamentals and a number of woody species (Ammirato, 1984; Williams and Maheswaren, 1986; Wann, 1988). Although mass propagation of any tree species via somatic embryogenesis has yet to be commercially feasible, many researchers have been investigating its potential for rapid and clonal production of difficult-to-propagate tree species. Prerequisite to the development of this type of system is the demonstration of the ability of a plant to produce somatic embryos.

Reports of somatic embryogenesis among leguminous tree species has been limited and includes *Acacia koa* Grey (Skolmen et al., 1976), *Albizia lebbek* (L.) Benth (Gharyal and Maheshwari, 1981), *Albizzia richardiana* (L.) Benth

(Tomar and Gupta, 1988), *Cercis canadensis* L. (Trigiano et al., 1988) and *Robinia pseudoacacia* L. (Merkle and Weicko, 1989).

The commercial availability of American yellowwood is limited because of unreliable seed production from year to year. Mass, clonal propagation through an *in vitro* somatic embryogenic system could alleviate the need for seed, provide uniform plants and increase the availability of *C. lutea*. The objective of this research was to investigate the potential for somatic embryo development in cultures of American yellowwood. This is the first report of *in vitro* propagation of *C. lutea* by somatic embryogenesis.

Materials And Methods

Sampling And Tissue Culture Techniques

Seed pods were collected from four yellowwood trees located on the University of Tennessee's Agricultural campus in Knoxville, TN. Anthesis or the time at which the trees were at full bloom, was the last week of April. Immature pods from each tree were randomly sampled once a week beginning four weeks post-anthesis (May 30, 1988) and ending ten weeks post-anthesis (July 15, 1988). Samples were not collected after ten weeks because the pods had senesced and the seed coats had hardened; excision of the zygotic embryo was not possible. Fruits were surface

sterilized for five minutes in 50% v/v commercial bleach (2.62% sodium hypochlorite) and 0.1% w/v Triton X-100 followed by three rinses in sterile distilled water.

Immature zygotic embryos were excised from the ovules and cotyledons separated from the hypocotyl. The individual embryo parts were placed on Schenk and Hildebrandt (SH) basal medium (1972) supplemented with 330 mg·liter⁻¹ ammonium sulfate, 100 mg·liter⁻¹ myo-inositol, 1.0 mg·liter⁻¹ thiamine, 30 g·liter⁻¹ sucrose and 8 g·liter⁻¹ agar in 60 mm petri plates. Initial treatments consisted of either 1.0, 2.0, 3.0 or 5.0 mg·liter⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D). The pH of all media was adjusted to 5.7 before sterilization at 1.06 kg·cm⁻² for 20 minutes. Each seed pod contained from one-to-eight ovules, many of which had naturally aborted, leaving only one to three useable ovules per pod. All the zygotic embryos from one pod were cultured in the same plate and considered a replication. The four treatments were arranged in a randomized complete block with 9 replications or plates cultured/tree/week for a total of 7 weeks. The culture dishes incubated at 25C with light provided by cool white fluorescent tubes (approx. 70μE sec⁻¹ m⁻²) for 16 hour·day⁻¹. Each 2,4-D treatment was also further divided into a control (no transfer), and 2 or 3 week transfer treatments of 12 plates each. Transfers were made from the initial SH medium containing 2,4-D to

SH basal medium amended with $5.0 \text{ mg} \cdot \text{liter}^{-1}$ 1-napthalene-acetic acid (NAA). The cultures were monitored weekly for somatic embryo development.

The experiments were repeated the next year with some modifications. The number of pods available from the same four trees was limited due to poor seed production. Sampling was restricted to six weeks post-anthesis (June 23, 1989) to ten weeks (July 21, 1989). Additionally, Tree #3 did not set fruit and was replaced with another yellowwood designated as Tree #5. Initial treatments consisted of either 2.0 , 3.0 or $5.0 \text{ mg} \cdot \text{liter}^{-1}$ 2,4-D. Half of the explants from each treatment were incubated six weeks on the 2,4-D containing media; whereas, the remaining cultures were transferred after three weeks to SH basal medium supplemented with $1.0 \text{ mg} \cdot \text{liter}^{-1}$ abscisic acid (ABA). Similar plating and culturing techniques described previously were followed.

Cotyledonary-stage somatic embryos were removed from the explant or resulting calli and transferred to half-strength SH basal medium with $6.0 \text{ g} \cdot \text{liter}^{-1}$ agar contained in 60 mm petri plates. Germinated embryos were transferred to the same medium in Magenta^R boxes after the plantlets had at least one true leaf and then established in soil-less potting medium (Fafard's Pro-Mix^R).

Histological Studies

Somatic embryos were removed and fixed in CRAF III (Sass, 1958) (30% chromium trioxide, 20% acetic acid, 10% formalin and 40% distilled water) for histological examination. Embryos were aspirated, dehydrated in a standard ethanol: t-butanol graded series and embedded in paraffin blocks. Ten μ m-thick sections were cut on a rotary microtome, mounted on slides, rehydrated and stained in a modified Flemming's triple stain using safranin, crystal violet and fast green (Johansen, 1940).

Results And Discussion

Somatic embryogenesis was limited to 5-9 or 6-9 weeks post-anthesis explants for the first and second year respectively. Similar results were reported for *C. canadensis* [redbud], where 13-15 week post-anthesis zygotic embryos supported somatic embryo development (Trigiano *et al.*, 1988). Very few somatic embryos formed from the earliest 4 weeks post-anthesis zygotic embryos; only one explant produced 5 embryos. All other explants produced little callus and developed necrotic margins. The 10 week post-anthesis zygotic embryo cultures did not form somatic embryos; the immature cotyledons expanded and formed copious amounts of callus and occasionally formed roots.

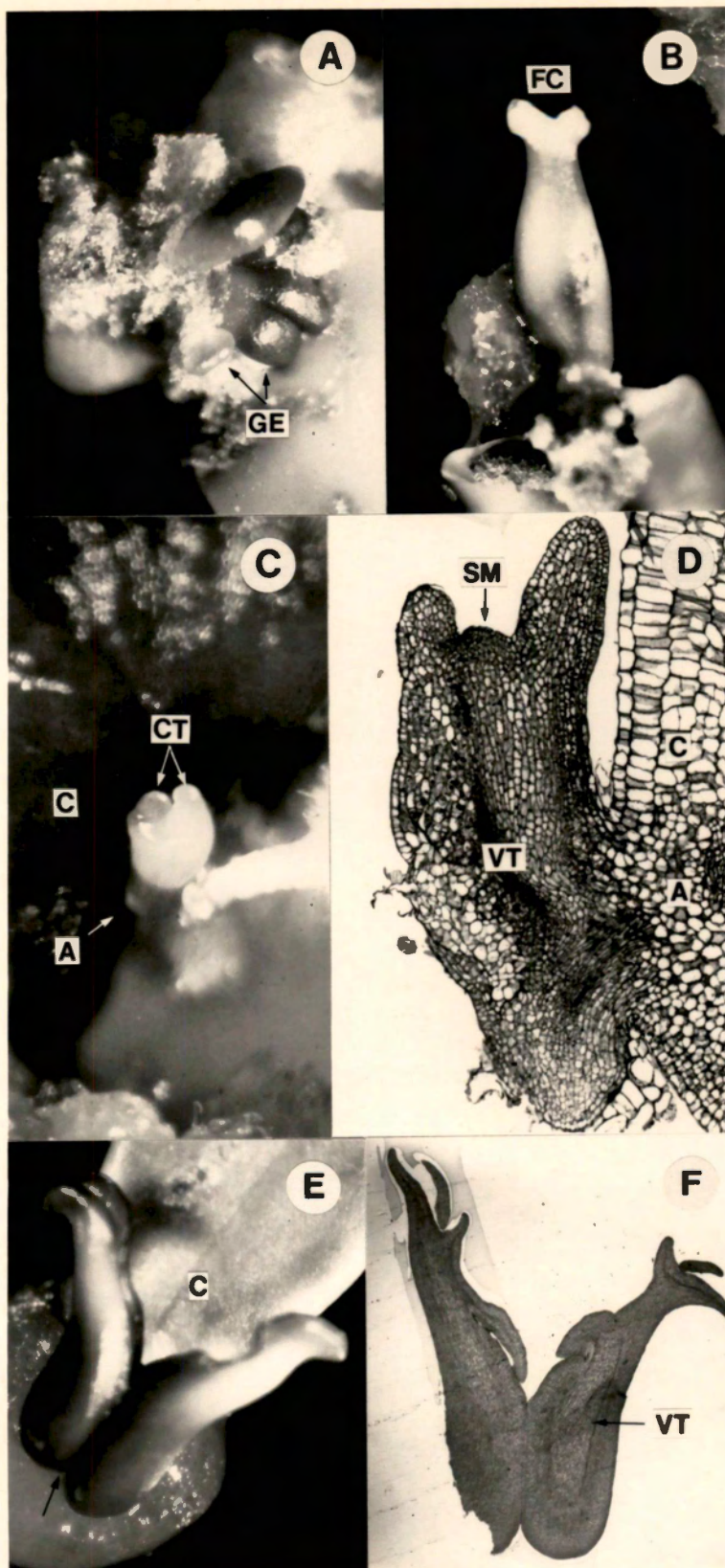
The ability of the explants to form somatic embryos is

a difficult to predict using weeks post-anthesis. During the second culturing season, anthesis was determined to be a week later than the previous year, yet the pods matured a week earlier. Depending on the environmental factors, anthesis may be difficult to use to predict or accurately anticipate the limited window of response in the American yellowwood.

Somatic embryos were observed from direct and indirect origins and in various stages of development including globular, cordate and cotyledonary (Plate II. Fig. A-D) In most cases, two or more fused somatic embryos developed from the base or proximal end of the cotyledons (Plate II. Fig. E,F); only a few formed as single embryos (Plate III. Fig. A,B). Fasciated cotyledons were observed in a majority of the abnormal embryos. The histological studies were conducted to examine the structure and origin of the somatic embryos. Ammirato (1987) detailed the morphological, anatomical and chemical evidence necessary to identify somatic embryos. The developmental sequence must be similar to their zygotic counterparts. Anatomically, the somatic embryos must be bipolar and develop procambium or vascular tissue between the root and shoot apices (Plate II. Fig. D,F and Plate III. Fig. B) Additionally, embryo-specific storage products should be, and were, evident in the histological sections. A majority of the embryos were morphologically abnormal,

Plate II. Somatic embryo development in *Cladrastis lutea*.

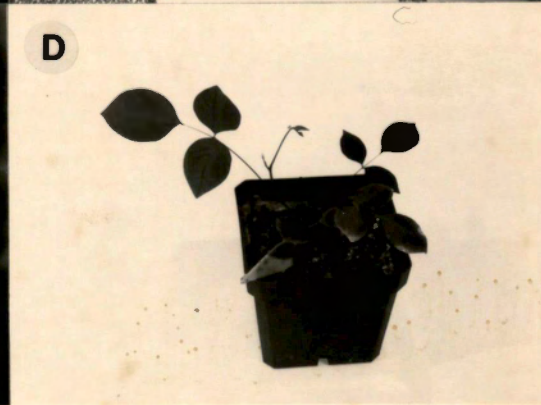
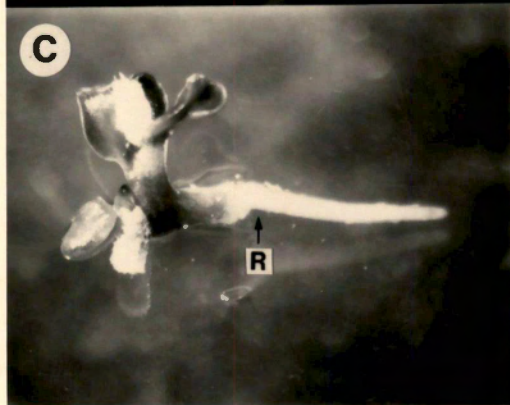
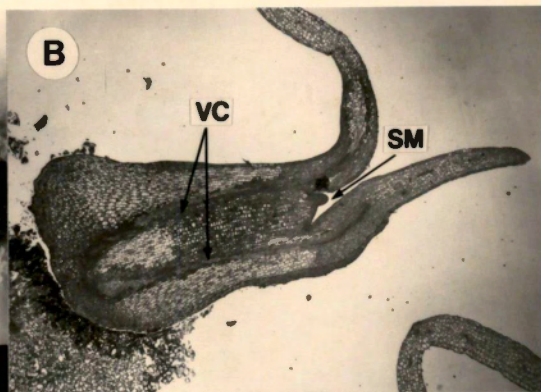
- A. Green, globular somatic embryos (GE) originating from the proximal end (base) of the cotyledonary explant. X 16
- B. Somatic embryo with fasciated cotyledons (FC). X 18
- C. Early cotyledonary stage somatic embryo with expanded cotyledons (CT) and attached directly to the zygotic cotyledon tissue (C). X 37.5
- D. Longitudinal section of embryo shown in Fig. C. with a broad attachment (A) to zygotic cotyledon (C). VT = vascular tissue and SM = shoot meristem. X 28
- E. Two somatic embryos fused along the lower half of the hypocotyl (arrow) that originated from the proximal end of the zygotic cotyledon. X 27
- F. Longitudinal section of fused embryo shown in Fig. E. VT = vascular tissue. X 15



LORI WEAVER
PLATE II
P. 52

Plate III. Somatic embryogenesis and plantlet re-generation in *Cladrastis lutea*.

- A. Large somatic embryo with fully expanded cotyledons arising from base of zygotic cotyledonary explant (C). X 16
- B. Longitudinal section of the somatic embryo shown in Fig. A. Note the shoot meristem (SM) and well-developed vascular cylinder (VC). X 18
- C. Somatic embryo germination. R = radicle. X 12
- D. *Cladrastis lutea* plantlet from the somatic embryo germination shown in Fig. C after three months growth.



LORI WEAVER
PLATE III
P. 54

forming as clusters and often lacking one of the apices or vascular tissue.

All treatments used in all experiments induced somatic embryo development. The greatest number of somatic embryos for all four trees occurred on either the 7 or 8 weeks post-anthesis zygotic embryos. Approximately one-half of the zygotic embryos initially cultured on 2 mg·liter⁻¹ 2,4-D from 8 weeks post-anthesis explants transferred to 5 mg·liter⁻¹ NAA produced somatic embryos (Table III.1). Cultures which remained on the 2,4-D induction medium for the entire six weeks also produced somatic embryos, but at a lower frequency and number.

Although the rate and frequency of embryogenesis was greater than control cultures, transfer of the explants to NAA after 2 and 3 weeks had little effect on embryo ontogeny. In an attempt to normalize ontogeny, zygotic embryos explanted during 1989 were transferred to SH medium containing 1.0 mg·liter⁻¹ ABA after initial culture on 2,4-D medium. ABA did not effect ontogeny; somatic embryos were induced by all hormone treatments (Table III.2) but had fasciated cotyledons and formed in clusters at the base of cotyledons. Most embryos were formed on 6-8 weeks post- anthesis explants.

The ability to produce somatic embryos in leguminous species has been reported to be genotype specific (Flick

Table III.1. Effect of weeks post-anthesis, time on induction media and 2,4-D concentrations on the number of somatic embryos formed from immature zygotic embryos of *Cladrastis lutea*.

Weeks Post Anthesis	Weeks On Induction Media ^z	2,4-D mg/l			
		1	2	3	5
4	2	4 (5.0) ^y	0 (0)	0 (0)	0 (0)
	3	0 (0)	0 (0)	0 (0)	0 (0)
	6	0 (0)	0 (0)	0 (0)	0 (0)
5	2	3 (1.0)	6 (2.0)	0 (0)	3 (3.0)
	3	0 (0)	3 (1.0)	11 (2.5)	3 (3.0)
	6	4 (1.0)	3 (2.0)	0 (0)	3 (2.0)
6	2	11 (2.5)	17 (2.0)	0 (0)	7 (3.0)
	3	6 (1.5)	4 (1.0)	3 (4.0)	0 (0)
	6	6 (1.0)	0 (0)	3 (1.0)	5 (2.0)
7	2	0 (0)	21 (1.0)	4 (2.0)	17 (1.0)
	3	20 (1.5)	9 (1.0)	24 (2.4)	25 (1.4)
	6	8 (2.0)	16 (2.2)	9 (3.5)	28 (2.4)
8	2	7 (1.0)	13 (1.5)	22 (1.8)	24 (1.2)
	3	29 (2.0)	46 (2.6)	13 (1.0)	35 (2.2)
	6	0 (0)	37 (2.2)	17 (2.0)	32 (3.3)
9	2	0 (0)	7 (1.0)	20 (2.0)	6 (1.0)
	3	16 (2.5)	9 (2.0)	0 (0)	6 (1.0)
	6	20 (4.0)	13 (4.5)	0 (0)	0 (0)
10	2	0 (0)	0 (0)	0 (0)	0 (0)
	3	0 (0)	0 (0)	0 (0)	0 (0)
	6	0 (0)	0 (0)	0 (0)	0 (0)

^z transfers for 2 and 3 week treatments made to 5 NAA media, 6 weeks = no transfer

^y percent of explants which responded (average number of embryos per explant which responded)

Table III.2. Effect of weeks post-anthesis and 2,4-D concentrations with or without ABA transfers on number of somatic embryos formed from immature zygotic embryos of *Cladrastis lutea*.

WPA ^z	2,4-D concentrations (mg/L)					
	2		3		5	
	-ABA	+ABA ^y	-ABA	+ABA	-ABA	+ABA
6	10(1.0) ^x	9(7.0)	11(3.0)	8(1.0)	11(3.5)	20(1.5)
7	8(1.0)	10(2.0)	5(4.0)	14(3.0)	13(3.3)	10(2.0)
8	9(2.0)	0(0.0)	12(2.6)	0(0.0)	15(3.6)	8(2.0)
9	5(1.0)	0(0.0)	4(1.0)	0(0.0)	4(1.0)	0(0.0)
10	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(2.0)	0(0.0)

^z weeks post-anthesis (June 23 - July 21, 1989)

^y transfers were made after three weeks to SH + 1 mg/l ABA.

^x percent of explants which responded (average number of embryos per explant)

et al., 1984). This phenomenon has been observed in pea (Malmberg, 1979), red clover (Phillips and Collins, 1979) and alfalfa (Bingham *et al.*, 1975). Immature zygotic embryos from all five yellowwood trees supported somatic embryo development, although at different rates (Fig. III.1). The origin of these trees is unknown, but assumptions based on commercial production methods indicate that they are of heterozygous seed origin. Therefore, the ability to produce somatic embryos does not appear to be genotype dependent.

Yellowwood somatic embryo formation was limited to excised immature cotyledons. Hypocotyl explants only formed callus. However, either complete developing zygotic embryos or seedling hypocotyl explants of other leguminous trees have been demonstrated to be capable of producing somatic embryos. *A. lebbek* (Gharyal and Maheshwari, 1981), formed somatic embryos directly from hypocotyl explants on hormone free B5 medium (Gamborg, *et al.*, 1968). Unlike the previous protocols, *A. richardiana* formed somatic embryos on explants cultured on medium containing cytokinins instead of auxin (Tomar and Gupta, 1988). Immature zygotic embryos of black locust formed somatic embryos after a one week exposure to $4 \text{ mg}\cdot\text{liter}^{-1}$ 2,4-D and $0.25 \text{ mg}\cdot\text{liter}^{-1}$ BA (Merkle and Wiecko, 1989). Somatic embryos which differentiated on the 2,4-D medium for a more than a few

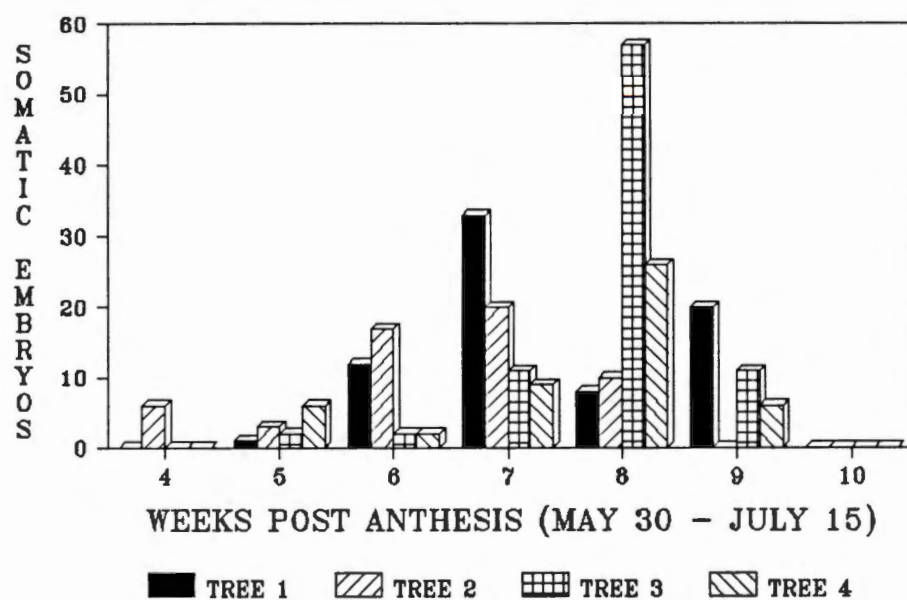


Figure III.1. Somatic embryo formation from immature zygotic embryos of four *Cladrastis lutea* trees.

weeks were difficult to convert into plantlets; whereas, short pulse treatments of 2,4-D produced embryos capable of conversion. Additional studies will be needed to examine the effects of auxins and cytokinins on frequency and rate of somatic embryogenesis as well as normalization of somatic embryo development in yellowwood cultures.

Somatic embryogenesis in the five leguminous trees reported have different requirements for basal medium and hormone treatments. Yellowwood appears to be similar to redbud and acacia in their ability to develop somatic embryos on 2,4-D medium without transfer to reduced auxin or hormone free medium. The existence of single pre-embryogenic determined cells (PEDC's) that are already competent at the time of culturing in the immature zygotic embryo (Sharp *et al.*, 1982), would account for the ability to form primarily direct somatic embryos. Yellowwood somatic embryos may be formed through a permissive induction system; cells require only growth regulators and the appropriate cultural conditions to express somatic embryo development (Christianson, 1985). Fewer indirect somatic embryos were observed that would have formed through a directive induction system; whereas, the indirect embryogenic determined cells (IEDC'S) are redirected through cultural conditions to a permissive state.

Twenty-one embryos were removed from explants for germination and radicles emerged from six within two weeks. Three embryos exhibited both shoot and root development (Plate III. Fig. C) and were transferred to a potting medium three weeks later. Only one plantlet however, was successfully established in potting medium (Plate III. Fig. D) Lack of conversion of somatic embryos to plantlets may be due to anatomical abnormalities. Many of the abnormalities in somatic embryogenic systems may be the result of deviations in the normal patterns of embryo development (Ammirato, 1987). Extra cell divisions or premature cell enlargement in early proembryo stages may lead to multiple growing points producing fused embryo clusters, fasciated cotyledons and incompetent meristems, especially at the shoot apex.

This research indicates that American yellowwood is capable of plantlet regeneration by somatic embryogenesis. Somatic embryos were formed directly and indirectly from tissue at the base of the immature cotyledon. Further research is needed to increase the frequency, normality and germination of yellowwood somatic embryos before implementation of such a system would be commercially feasible.

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VITA

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