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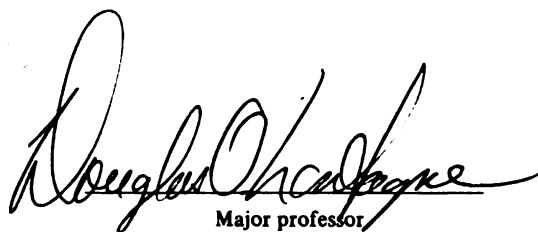
INDUCTION OF PRECOCIOUS FLOWERING
IN AMBURANA CEARENSIS

presented by

PAUL M. MUELLER

has been accepted towards fulfillment
of the requirements for

Masters degree in Forestry



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INDUCTION OF PRECOCIOUS FLOWERING
IN AMBURANA CEARENSIS

By

Paul M. Mueller

A THESIS

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in partial fulfillment of the requirements
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ABSTRACT

INDUCTION OF PRECOCIOUS FLOWERING IN AMBURANA CEARENSIS

By

Paul M. Mueller

A greenhouse experiment was conducted to induce precocious flowering in seedlings of *Amburana cearensis*, a high value tropical hardwood native to South America. Eighteen treatment combinations of chlormequat (CCC) (three levels), root pruning (two levels) and girdling (three levels) were applied to 10 month old trees. None of the treatment combinations induced flowering. Hypotheses are discussed to explain the lack of flowering: physiological age of the trees, environmental conditions and treatment levels are considered. Analysis of family means for ten month height growth data showed variation among families. No family differences in branch angle were detected in 12 month old trees.

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1 INTRODUCTION

For decades, Paraguay has used its forest resources indiscriminately and unsustainably. Because of this, Paraguay's most valuable timber species, *Amburana cearensis* (trébol), has been depleted to the point of commercial extinction. Degradation of the population is so severe that recovery of the species as an integral part of the forest industry is questionable. Trébol populations are also severely depleted in other countries of South America such as Bolivia (F. Bascopé, personal communication 1989) and Perú (A. Chung, personal communication 1989).

Once the most sought after species by loggers and consumers, *A. cearensis* has now disappeared from the Paraguayan market. In 1990, the Paraguayan Data Conservation Center classified *A. cearensis* as a species in danger of extinction. Although a new Paraguayan law (1991) prohibits cutting of the species, its population is likely to continue to decline for three reasons. First, although the intent of the law is sound, it is unlikely that the Paraguayan National Forest Service has the resources to enforce it; second, populations of young, unmerchantable trees will continue to be destroyed

through deforestation, and third, large trees left uncut after removal of the surrounding forest are threatened by fire and windthrow.

In 1986, the Trébol Recuperation Project (TRP) was started in an effort to restore the tree to commercial status, re-establish its position in the natural forest, and provide an opportunity for long term economic diversity for area farmers and ranchers. The TRP concentrated its initial efforts in collecting and developing information in the following areas; phenology, nursery production techniques, spacing for agroforestry and traditional plantation designs, plantation management, education and gene conservation. Long-term goals of the TRP included progeny and provenance testing, establishment of seed production areas, and breeding. Due to the highly degraded state of *A. cearensis* populations in Paraguay, breeding may become one of the more important of these long term goals if it is to be returned to a position of economic importance in Paraguay and elsewhere in its natural range.

Successful breeding of *A. cearensis* will be impacted in part by the number of individuals in the breeding population. In this light, maintaining a highly diverse genetic base of *A. cearensis* is of utmost importance. The frequency of phenotypically superior individuals in the population also

influences gain made through breeding. As the frequency of phenotypically superior individuals decreases, so does the frequency of alleles that favorably influence the traits of interest (assuming the trait is a heritable one). As a result, the probability of finding and capturing those alleles decreases. Not only is the present overall population of *A. cearensis* extremely low in Paraguay, but phenotypically good individuals are almost non-existent.

Another problem with a trébol breeding program is the long generation time of the species. Many tropical species, such as *Leucaena leucocephala* (NAS, 1977) and *Parapiptadenia rigida* (Mueller, personal observation) flower when less than four years old. The generation time for *A. cearensis* is not known, but is thought to be at least 15 years. If the time from seed to flower can be reduced in *A. cearensis*, the efficiency of a breeding program could be increased by reducing the time between successive generations. Other benefits of a reduced juvenile period include the ease of study of floral biology and natural pollination on small trees, and enhanced seed yields from seed production areas by inducing flowering in years that the tree does not flower naturally. Although the benefits of success are substantial, the induction of precocious flowering is difficult to achieve and has never been tried on *A. cearensis*. There is little published information in the

literature on this subject for hardwood forest tree species. Most work on precocious flowering has been done with the woody vine *Hedera helix* L., however Arshad (1980), Johnsson (1949) and others have conducted studies with *Betula* spp.

The objective of this study is to stimulate precocious flowering in *A. cearensis* using chemical and cultural treatments.

2 LITERATURE REVIEW

2.1 INTRODUCTION

Forest tree breeding for desired characteristics such as form, wood quality and growth rate is a time consuming process. Poor juvenile/mature correlation for important traits and the generally long time required for forest trees to flower are two key reasons. The period from germination to sexual maturity is termed the juvenile stage (Zimmerman, 1973). Some species, such as European beech (*Fagus sylvatica*) do not flower until age 30 to 40 years (Metzger, 1988). *Amburana cearensis* has been observed to require at least 15 years to flower in plantations.

Transition from the juvenile stage to the adult stage is influenced by a number of factors, although the exact mechanisms are not well understood (Zimmerman, Hackett, and Pharis, 1985; Zimmerman, 1976). Factors include chronological and physiological age, (Fortanier and Jonkers, 1976), nutritional condition of the plant (Hackett, 1976), environmental conditions (Wareing and Frydman, 1976), size (Zimmerman, 1972), genotype (Johnsson, 1949; Poethig, 1990) and hormone levels (Hackett, 1976). Experiments that deal with shortening the juvenile stage have produced varied results. Certain treatments may produce positive results in one species, but not in another (Zimmerman, 1972).

The greatest chance for success in working with trees comes not only from a thorough understanding of relevant literature, but also from an understanding of the species in question (Hanover, 1991 personal communication). Little is known about the ontogeny of *Amburana cearensis*.

Phenological studies have been initiated by the Trébol Recuperation Project in Paraguay, although no conclusions have been drawn from these observations. The rationale behind the treatments applied in this experiment comes mainly from the literature, but also from the experience of the author in his work with the species.

2.2 AMBURANA CEARENSIS

Amburana cearensis (LEGUMINOSAE), commonly known as trébol in Paraguay*, is a large sized, deciduous hardwood tree. It grows to 25 meters in height, and up to 110 cm in diameter at breast height. It has dark green foliage, with a rounded crown. Grown with competition, it can produce straight boles up to 12 meters long. Grown in the open, it produces a wide, spreading crown and makes an excellent shade tree.

In Paraguay the tree is found principally between the Aquidaban and Apa Rivers in the North of the *Región*

* Also known as yvyra pirirí guazu (Paraguay), tumi, serioco and roble (Bolivia), palo trébol, roble del país and roble criollo (Argentina), cerejeira, umbruna, imbruna do cheiro, cumaré and cumarú (Brazil) (Whitmore, et.al., 1990).

Oriental, and occasionally in the departments of Chaco, Presidente Hayes, and Alto Paraguay. It prefers sandy-clay soils, and is frequently found on calcareous soils (López, 1987).

Leaves of *A. cearensis* are pinnate and alternately when adult. Inflorescences are racemes, 2.0 to 7.0 cm long arising from the leaf axils. Flowers are off-white with one petal. Fruits range from 5.0 to 7.0 cm long, and contain one winged seed. Trébol flowers and seeds sporadically, although exceptions exist. One tree has been reported to flower yearly, but to varying degrees. Seed production by this tree ranges from zero to heavy, not necessarily corresponding to the intensity of flowering (Mueller, personal observation).

The wood of *A. cearensis* is moderately heavy, with a specific gravity of 0.550 Kg/dm³ (Chudnoff, 1984). It is easily dried and worked, and is prized for quality furniture production. The tree has no other principal uses, although it can be burned as fuelwood.

Trébol is the most valuable species in Paraguay, often bringing 200% of the cost of other commercially important species. It is also valued in other countries to which it is native, although specific dollar values are not

available. No figures are available on its worth on the international market.

2.3 ONTOLOGICAL DEVELOPMENT OF TREES

2.3.1 Maturation and ageing

As a tree grows it undergoes a series of developmental changes, or stages. Changes associated with the sexual development of the tree as well as certain morphological and physiological traits such as thorniness, leaf shape, phyllotaxy, disease resistance and ability to root are referred to as maturation (Wareing, 1959; Zimmerman, 1972; Poethig, 1990). Changes in morphological complexity (increased branching), vigor and growth rate are considered a result of ageing (Poethig, 1990; Zimmerman, et al., 1985).

Developmental changes due to maturation are generally not easily reversible. Nonetheless, since seedlings grown from the seed of mature trees are juvenile, reversion must take place during sexual regeneration (Brink, 1962). Work has been done to stimulate rejuvenation in a number of species (Muzik and Cruzado, 1958; Rogler and Dahmas, 1974; Greenwood, 1987), and has been observed in naturally grown trees (Brink, 1962).

In contrast, the effects of ageing are generally easily reversible. For example, a mature twig grafted onto

juvenile rootstock will exhibit increased vigor and a reduction in branching due to reversal of ageing, but will retain the characteristics of a mature tree, such as mature leaf form and the ability to flower.

The continued ability to flower signifies that a plant is in the adult stage (Hackett, 1985). However, a tree may not flower for a number of years after reaching maturity if all other requisite conditions such as photoperiod, temperature, and moisture level are not first met (Zimmerman, 1973).

Zimmerman (1973) terms the time from the end of the juvenile state to the first appearance of flowers (adult state) the transitional stage. Poethig (1990) uses the terms juvenile vegetative, adult vegetative (which would correspond to Zimmerman's transitional stage) and reproductive stage. Regardless of terminology used, the major identifying factor signaling the attainment of the adult (reproductive) stage is the ability to flower (Borchert, 1976).

It is possible, however, to induce plants to flower while still in the juvenile stage. Furr, et al. (1947) have shown that precocious flowering in three year old citrus is possible by ringing, and that anomalous flowering can occur at ages as young as three months. These results however, do not necessarily signify that the treatment has induced phase change; removal of the stimulus and subsequent growth of the

plant under normal conditions may not produce flowers for many years (Furr, et al., 1947).

The progression of a tree through stages of maturation and ageing are characterized by a number of traits. Marking the transition of the plant through these stages are morphological (Poethig, 1990), physiological (Fortanier and Jonkers, 1976) and genetic (Wareing and Frydman, 1976) changes in the plant.

2.3.2 Morphological characteristics of juvenile and mature trees

In some tree species distinct morphological changes take place as maturity is reached. These changes may include variation in leaf shape and size, thorniness and phyllotaxy. The changes that occur vary widely between species, with some species exhibiting more dramatic changes than others, while other species show no apparent change in morphology. Occasionally these changes can be used as indicators of the maturity of an individual.

The woody vine English Ivy (*Hedera helix* L.) has a number of morphological characteristics that can be used to distinguish mature from juvenile plants. These include phyllotaxy, stem pubescence and plagiotropic versus oligotrophic growth. Species of the tropical genus *Eucalyptus* typically exhibit marked morphological changes

between juvenile and adult plants. In most Eucalypts, juvenile trees exhibit decussate phyllotaxis, changing to spiral as they mature. In addition, four heterophyllous stages are recognized during the maturation of the genus: seedling, juvenile, intermediate and adult (Boland, et al., 1984). Phyllotaxis of *A. cearensis* changes from spiral to opposite during the seedling stage. How this change is related to the sexual development of the species is not yet known. *Gleditsia triacanthos* (Chase, 1947) as with many species of the genus *Citrus*, are characterized in the juvenile stage by the production of thorns. As the trees mature, fewer thorns are produced (Furr, et al., 1947).

2.3.3 Physiological characteristics of juvenile and mature trees

Physiological changes in a tree, such as shifts in hormone production as it passes from juvenile to adult, are difficult to quantify. To date, little work has been done in this area (Zeevart, personal communication, 1991). Nonetheless, phenomenon that reflect at least some of these physiological changes are rooting ability, leaf retention and disease resistance.

Western red cedar's (*Thuja plicata*) resistance to the leaf blight fungus *Didymascella thujina* (Dur.) Maire, has been shown by Soegaard (1956), to be related to maturity.

Juvenile Western red cedar (< four years old) are more susceptible than older, more mature trees.

In many species of woody plants, cuttings from juvenile plants, or juvenile parts of mature plants will root easier than cuttings from mature tissue (Greenwood, 1987). Nolasco and Schwyzer (1985) showed that basal portions of young trees of *A. cearensis* rooted better than terminal portions. In addition, juvenile tissues in beech (*Fagus spp.*) and oak (*Quercus spp.*) retain leaves during the dormant season while leaves abscise from mature tissue (Wareing, 1959).

Leaf retention, the ability of cuttings to root, and disease resistance could possibly be explained by changes in hormonal balances/concentrations in the plant. However, research on linking particular hormones to phase change has been inconclusive (Metzger, 1987) due principally to the complex nature of the problem. In addition, whether changes in hormone levels are a result of or the cause for phase change is unclear. The five known hormones: auxin, gibberellins, ethylene, cytokinins and abscisic acid, each have specific and at the same time overlapping roles in the growth and development of a tree. The role of hormones in the growth and development of plants has been thoroughly reviewed in the literature by Davies (1987), Wareing (1982) and others and therefore will not be covered here.

2.3.4 Transition to the mature stage

The majority of the literature focuses on two requisites for a plant to mature: growth through a predetermined number of cycles, or attainment of a minimal critical size. The latter appears to be the currently favored hypothesis (Hackett, 1985), although no definitive proof has been presented to account for it. Other factors, such as distance of the meristem from the roots, the number of cell divisions gone through by the meristem (Robinson and Wareing, 1969), hormone balance (Srinivasan and Mullins, 1981) and nutritional status (Sachs, 1977) may also play a role in triggering phase change.

Relative distance of the meristem from the roots may factor into the timing of transition. Roots supply hormones such as cytokinins (Davies, 1987) and possibly gibberellins (Davies, 1987; Arshad, 1980)) to the upper parts of the plant. These may inhibit initiation of flower primordia. As the meristem's distance from the roots increases, a diffusing factor could play a role in minimizing their effect as a flower inhibitor.

Conversely, the shoots may direct translocation of certain hormones to the roots such as gibberellins and abscisic acid. These substances may act in the root to inhibit flower formation. Their effectiveness in the root may

decrease as the meristem's distance from the root increases. Arshad (1980) showed that IAA, which is synthesized in the shoot, may have at least partial control over the basipital movement of GA through the stem.

2.4 GENETICS OF PHASE CHANGE

Ultimately, all a plant's functions are controlled either directly or indirectly, wholly or partly by the genetic makeup of the individual. It is not unlikely that the prerequisites for the initiation of floral primordia, be it photoperiod, age or other conditions, trigger transcription and/or translation of certain genes (Metzger, 1988). The product of these genes may then be what initiates phase change, not the prerequisite conditions as we see them.

Studies have been conducted to identify genes that exhibit control over various aspects of flowering (Reid, 1979; Johnsson, 1949). Research on the heritability of the juvenile period has also been conducted (Greene, 1991; Couranjou, et al., 1988), which can be important in breeding programs.

The current study deals with mechanical and chemical treatments to disrupt the physiological processes in *Amburana cearensis* to stimulate flowering. Nonetheless, information in the following section on genetic control and

heritability is useful as background in understanding the problem.

2.4.1 Genetic regulation and differential gene activation

Genetic control of the length of the juvenile stage appears to be complex and varies between species. In peas the *Sn*, *Hr* and *E* alleles interact in control of photosensitivity, age and size of the plant as regards flowering (Reid, 1979; Kelly and Davies, 1986). The number of days from germination to flowering in dry bean (*Phaseolus vulgaris* L.) is controlled by few genes but is also under control of photoperiod and temperature (Wallace, et al. 1991) which may implicate additional genes. Conversely, Johnsson (1949) found segregation ratios for early flowering in *Betula verrucosa* and *B. pubescens* that suggested control by single, dominant genes. Teich and Holst (1969) also found Mendelian ratios for precocious flowering in scotch pine (*Pinus sylvestris*).

Genes connected to phase change in corn mutants have been investigated by Poethig (1988). He succeeded in identifying a mutant gene that may control the synthesis or distribution of a diffusible factor that maintains the juvenile phase. It may be hypothesized then, that the product of the wild-type form of this gene triggers transition to the mature, or reproductive phase.

Meeks-Wagner, et al. (1989) showed differential transcription in tobacco roots between mature and juvenile plants, indicating that activation of specific genes in the root may trigger phase change.

What is not clear in any of the examples above is what regulates the activity of the genes in question. The most likely answer, however is that a combination of factors is responsible.

It is known that at least some hormones can have a regulatory effect over particular genes. Gibberellin (GA_3) regulates mRNA synthesis of the α -amylase gene (Jacobsen, et al., 1982), and auxin has been shown to regulate transcription of a number of soybean genes (Hagen, 1987). It is possible therefore, that hormone balance and/or distribution may ultimately be the catalyst for phase change through gene regulation.

2.4.2 Inheritance of the juvenile period

Although studies such as the present one may result in temporary induction of flowering, permanent shifts in reproductive status are desirable. Selecting and breeding for shorter juvenile periods can result in earlier flowering progeny.

Variation in the length of the juvenile period exists in a number of temperate tree species. Where sufficient variation coupled with adequate heritabilities exist, breeding for the trait can be worthwhile. Variability has been shown in birch (*Betula spp.*) (Johnsson, 1949; Huhtinen, 1976), apple (Visser, 1976), pear (Bell and Zimmerman, 1990; Visser, 1976; Zielinski, 1963), longleaf pine (*Pinus palustris*) (Boyer and Evans, 1967), scotch pine (Teich and Holst, 1969) and southern magnolia (*Magnolia grandiflora*) (Greene, 1991).

Heritability, the ratio of additive genetic variance to total phenotypic variance (Falconer, 1989), is used in calculating gain in breeding programs. High heritability estimates with the same selection intensity will give greater gain than low estimates. Breeding experiments have shown heritability of the juvenile period to be generally high, but variable among species. Greene (1991) estimated family heritability of early flowering in 36 families of magnolia to be greater than 0.80. Couranjou, et al. (1988) found heritability to be from 0.8 to 0.9 in 7 apricot varieties using parent-offspring regression. In working with pear and apple, both Visser (1976) and Bell and Zimmerman (1990) found control of the juvenile period to be principally through additive gene action as indicated by high general combining ability and low specific combining

ability. Maternal (cytoplasmic) effects have been shown in cherry (*Prunus sp.*) (Schmidt, 1976), scotch pine (Teich and Holst, 1969). In these species, it may therefore be important to designate individual plants as male or female parent.

In summary, research in a number of species indicates that genetic variability exists for length of the juvenile period, and that improvement is possible. High heritability estimates for several species, in addition to indications that principal control is through additive gene action, suggest that efficient breeding programs can be developed to shorten the time to flowering.

2.5 RESPONSE TO DIFFERENT TREATMENTS

Three treatments in eighteen different combinations were applied to *A. cearensis* seedlings. The following section discusses these treatments and their major known effects.

2.5.1 Chlormequat

Chlormequat, also known as CCC, Cycocel or Chlorocholine chloride, ([2-Chloroethyl]trimethylammonium chloride) is a growth retardant used in agricultural crops to reduce lodging (Sponsel, 1988; Nickell, 1979) by stunting vegetative growth. CCC acts in the plant by inhibiting activity of ent-kaurene synthetase A, an enzyme in the

biosynthetic pathway of gibberellic acid (GA) (Sponsel, 1988). *Ent*-kaurene synthetase A catalyzes the conversion of geranylgeranyl pyrophosphate (GGPP) to copalyl pyrophosphate (CPP), an intermediary in the process. Treatment with CCC should therefore result in a reduction of endogenous gibberellins. Hedden (1988) however, states that the exact site of action of CCC is yet unclear, and that it may also act on other pathways not related to GA but that inhibit growth. Arshad (1980) found higher levels of gibberellin-like compounds in the bark of CCC treated birch, although no mention was made of growth. Reid and Crozier (1970) found that small concentrations of CCC (1 ppm) increased levels of GA in pea plants (*Pisium sativum* L. cv. Alaska), whereas higher concentrations (1000 ppm) reduced levels.

In addition to growth retardation, CCC has also been shown to affect flowering in a number of species. In hollyhock (*Althaea rosea* cv. Summer Carnival), CCC was shown to increase total flowers per plant, and inhibit flower abortion (Tezuka, et al., 1989). Arshad (1980) found that application of CCC in combination with stem girdling increased flowering in seedlings of *Betula pendula*. Treatment of *Mattiola incana* and *Althaea rosea* with CCC induced flowering and increased levels of nitrogen, phosphorus and potassium in the shoots (Hamza and Helaly, 1983).

Although CCC has been shown to induce early flowering in several species, the exact pathway by which this is accomplished is not known. Hormone balance and nutritional status, two factors thought to play a role in phase change (see section 2.1), are affected by CCC. How CCC affects these and/or other physiological aspects in trébol is not known.

2.5.2 Girdling

Girdling, (ringing, scoring) a tree is the act of removing the phloem in a continuous band around the bole (or branch). When girdled around the bole, transport of carbohydrates to the roots is stopped, killing the tree. Girdling upsets the balance of both carbohydrates and growth regulators (Noel, 1970), and can have a promotory effect as regards flowering.

Lahav, et al. (1986) showed that girdling increased the number of flowering trees in 9 avocado (*Persea americana*) crosses. They concluded that girdling shortened the juvenile stage, depending on time of girdle. The validity of this conclusion is doubtful since 47.3% of the control plants also flowered. Dennis (1967) showed that scoring increased flowering on those apple progenies that had already commenced to flower, but had no effect on pear seedlings. He concluded that scoring did not affect juvenility per se, but may induce trees in the transition

period to flower slightly sooner than normal. Arshad (1980) found that girdling induced flower initiation in young birch (*Betula pendula*) seedlings. He inferred that floral induction was due to either an accumulation of metabolites above the girdle, or to interruption of the supply of photosynthates to the root. Starvation of the root may have caused a reduction in a flower inhibitor synthesized there and translocated to the shoots (Arshad, 1980).

2.5.3 Root pruning

Root pruning results in two major changes in a plant. First, there is a reduction in transport of hormones from the root to the shoots, principally cytokinins and gibberellins. Second, due to subsequent increased root growth there is an increase in the translocation of photosynthates to the roots (Richards, 1986). It has been shown that root pruning apple trees can increase flowering (Geisler and Ferree, 1984). Most of these studies have dealt with already mature trees and the effect therefore is not necessarily related to phase change.

Hendry, et al. (1982) have shown that cytokinin levels in buds of juvenile citrus plants (*Citrus sinensis* (L.) Osbeck) were higher than levels in mature plants. Since the experiment was conducted with grafted material on same age rootstock, the site of control of either cytokinin

production or translocation appears to reside in the shoot. Regardless, root pruning used in combination with other treatments that are known to affect juvenile-mature transition may stimulate the expansion of flower initials.

After a time lag, pruned roots will produce a flush of new growth. This increase in root growth creates a demand for photosynthates from the crown. It has been shown by Kramer and Kozlowski (1979) that an increase in demand for photosynthates by the root will result in an increase in photosynthetic rate. Floral initiation is dependent in part on high levels of photosynthates (Kozlowski, 1971). By creating a demand in the root, increased photosynthesis in the shoots may produce an excess of photosynthates which could be used to set floral initials. Nonetheless, research to support this hypothesis was not found.

3 MATERIALS AND METHODS

3.1 FACILITIES DESCRIPTION

The study was conducted at Michigan State University in East Lansing, Michigan at the Department of Forestry's Tree Research Center. It was initiated in mid-June 1991 and terminated in mid-June 1992. The entire study was conducted in a greenhouse because *A. cearensis* is a tropical to sub-tropical species native to South America.

The greenhouse was of domed fiberglass construction. Heat was provided by two gas burners at the east end of the house from September through June. Cooling during summer months was provided by variable speed exhaust fans and evaporative cooling pads. Supplemental lighting was used to maintain a day length of approximately 13 hours. Lights were on during the entire 13 hour photoperiod. Regular fluorescent lights of 110 watts were used over the first seven months. These lights proved to provide insufficient light during the winter months. Six additional fluorescent fixtures (12 bulbs) were installed on 14 January 1992. All bulbs in all fixtures were changed to 215 watts for the remainder of the experiment.

3.2 EXPERIMENTAL PROCEDURES

Stem girdling, root pruning and chemical treatment in a 3x2x3 factorial were applied to 10 month old container grown *A. cearensis* seedlings to induce precocious flowering.

3.2.1 Soil and container preparation

Seedling containers measured 10x10x58 cm, and were made of heavy gauge waxed paper. Nine containers were placed in each of 54 30x30x58 cm plastic cases. The 58 cm plastic cases were constructed by removing the bottom of one 29 cm case, and stacking it on a second case. This was done to accommodate the typically long tap root of the species. The containers were filled with a prepared potting mix of 75% peat and sphagnum moss and 25% perlite. To reduce soil settling after seeding, the soil was compacted by dropping the cases several times during filling. Cases were then placed on pallets approximately 10 cm high to facilitate drainage and air pruning of the roots. Cases were staggered on the pallets by a space equivalent to one case to allow for crown development.

3.2.2 Seeding and seedling care

Seed of *A. cearensis* was obtained from the School of Forestry at the National University of Asunción and the Trébol Recuperation Project in Paraguay. Nine open pollinated half-sib families were used for the study (Table

1). One seed was planted in each container on 10 June 1991, and covered with approximately 0.5 cm of soil. Each family was represented once per case. Trébol has a permeable seed coat so seed scarification was unnecessary.

Table 1. Trébol Recuperation Project accession numbers and geographic origin of seed of *Amburana cearensis*.

Paraguayan Accession number	Location
021.001	Estancia Errante Tranquerita, Concepción, Paraguay
021.002	Peguajó-San Fransisco, Concepción, Paraguay
021.003	Invernadero La Novia, Arroyito, Concepción, Paraguay
021.004	Escuela Agropecuaria de Concepción, Concepción, Paraguay
021.006	Colonia José Félix López (Peuntesiño), Concepción, Paraguay
021.007	Naranjaty, Concepción, Paraguay
021.008	Yvy ja'y, Amambay, Paraguay
021.009	Alfonso cué, Concepción, Paraguay
021.010	Calle 16 - Norte, Arroyito, Concepción, Paraguay

3.2.2.1 Watering

Containers were watered after seeding to a depth of several centimeters. Containers were then watered as necessary to maintain a suitable soil moisture level for germination. On 26, 27, and 28 July 1991 after germination had occurred, all plants were again watered to ensure that the soil mixture throughout the container was moist. All water added to this point was regular tap water. All watering subsequent to this point was with a nutrient solution (Table 2).

Table 2. Composition of nutrient solution.

Nutrient	Concentration (ppm)
Nitrogen	200
Potassium	100
Phosphorus	107
Magnesium	1.1
Iron	.75
Manganese	.42
Boron	.15
Zinc	.12
Copper	.07
Molybdenum	.07

Frequency of watering after these dates was approximately once per week from 28 July 1991 through 12 August 1991. In early August it was determined that tap roots were not developing as expected, and overwatering was the suspected cause. In an effort to stimulate tap root development no watering occurred from 12 August to 22 October 1991. After 22 October 1991 through to the end of the experiment the plants were watered every three weeks.

3.2.2.2 Staking

In late July trees were staked with bamboo poles to maintain the plants in an upright position. Low light levels and lack of wind stimulation were suspected causes of spindly tree growth. Trees continued to be staked as necessary over the study period.

3.2.2.3 Environment

Air temperatures were monitored daily during the initial stages of germination and growth, and then weekly to monitor maximum and minimum temperatures. Mean air temperature during the germination period was approximately 34.2 degrees celsius (C). Overall summer temperatures during 1991 ranged from 27.2°C to 38.9°C. Winter greenhouse temperatures ranged from 7.8°C to 30.0°C. Target temperatures were approximately 32.2°C. The heaters were unable to adequately heat the greenhouse during the winter months.

3.2.2.4 Pesticide and fertilizer application

Fungicides (captan and metalaxyl) to control damping off and other soil borne fungi were applied approximately two weeks after seeding. Nicotine gas bombs were used three times to control insects, and two miticides, dienochlor and fluvalinate were used twice to control mites. Osmocote brand slow release 18-6-12 fertilizer (15 grams/tree) was also applied two weeks after seeding, and again on 30 January 1992 at 6 grams per tree.

3.2.3 Treatment application

Treatments were applied on 14 and 15 April 1992. The trees were approximately 10 months old and averaged 88.5 cm tall.

3.2.3.1 Root pruning

Two levels of root pruning were applied; hand pruned at the case interface (approximately 50% of the root length), and air pruned out the bottom of the containers. Hand pruned roots were cut with a sharp machete, slicing through the containers and roots at the case interface. Cases were lifted apart to assure that all roots had been cut.

3.2.3.2 Girdling

Three levels of stem girdling were applied with grafting knives: 0, 50 and 90 percent of the circumference. Girdles were one centimeter wide, starting at a height of 10 cm from the soil surface. Any branches that originated below 10 cm on the stem were also girdled at the same level (0, 50, 90%) as the main stem. Percentages of circumference were ocularly estimated. In most cases the bark slipped off easily. In cases where the bark was not easily removed, the girdled area was scraped until no inner bark remained. Callus tissue was prevented from bridging the girdle during the experiment. Sprouts originating below the girdle were removed.

3.2.3.3 Chlormequat

Three levels of chlormequat (CCC) were applied: 0, 750 and 1500 ppm. CCC was dissolved in tap water and applied as a soil drench in two 0.5 liter applications one week apart.

Two applications were used to assure that the solution did not penetrate past the case interface. Solution penetrating past the case interface in root pruned treatments would not have been available for uptake.

CCC solutions were prepared separately for each replication and applied to each tree individually. Tap water was added to those treatments that received 0 ppm CCC. Before treatment initiation trees were not watered for six weeks to increase water stress and encourage uptake of the solution.

3.3 EXPERIMENTAL DESIGN

The experiment was established as a randomized complete block factorial (3x2x3) design with four replications. A total of 18 treatments were tested. Each treatment contained nine trees. Treatments were randomly assigned to each case of nine trees. Families were randomly assigned to containers within each case. Initially, each treatment contained one representative of each family. Due to poor germination, only enough plants for three replications were available. Seedlings from the fourth replication were used to replant empty pots in replications one, two and three. Because germination was not equal across all families, final representation of families was not equal across replications. Inequality of families across replications did not affect tests of significance for flowering, branch

angle or height since these analyses were conducted as though bulked seed had been used. Family identity was maintained throughout the experiment to facilitate the identification of individuals showing better than average or unusual growth.

3.4 DATA COLLECTION

3.4.1 Flowering

At the conclusion of the experiment, each tree was individually checked for flowers, or flower buds arising from leaf axils. Treatment values were the sum of the racemes on the individual plants within each treatment.

3.4.2 Height

Total height of each plant, from soil level to the terminal meristem, was measured monthly. Total height was recorded to the nearest tenth of a centimeter.

3.4.3 Branch angle

Main stem branch angle was measured on four randomly selected trees of each family in each replication. Inside branch angle was measured in degrees from the main stem using clear plastic protractors. The first three branches were measured on each of the trees selected.

3.5 DATA ANALYSIS

3.5.1 Flowering

Flowering data was to be analyzed as a completely randomized block design with three replications of 18 treatments.

3.5.2 Height

Statistical analysis of family means for ten month height growth was not possible because staking may have influenced total heights among families. Family differences in height growth detected by statistical analysis would not necessarily reflect true differences. Simple means were used as an indicator of possible differences in family height growth.

3.5.3 Branch angle

The angle at which a branch arises from the main stem can influence the quality of the lumber produced. Branch angles on 12 month old seedlings were measured to provide an estimate of juvenile variation among and within families.

Branch angle data were analyzed as a completely randomized block design with sub-samples (Table 3). There were three replications of eight treatments (families)(Table 1).

Family 021-008 was not used in the analysis because approximately 33% of the trees measured had no branches.

Data were checked for normality using the Kolmogorov-Smirnov

Table 3. Source, df and EMS for main stem branch angle.

Source	df	EMS
Total	95	
Rep.	2	$\sigma_w^2 + T\sigma_{rf}^2 + TF\sigma_f^2$
Family	7	$\sigma_w^2 + T\sigma_{rf}^2 + TR\sigma_f^2$
Family x rep.	14	$\sigma_w^2 + T\sigma_{rf}^2$
Trees w/in plot	72	σ_w^2

two sample test (Steel and Torrie, 1980). Variance components and small sense individual tree and family heritabilities (h^2) were also estimated. Individual tree h^2 was estimated using the following formula from Zobel and Talbert (1984)

$$h^2 = \frac{\sigma_a^2}{\sigma_{ph}^2}$$

Family heritability was calculated by

$$h_f^2 = \frac{\sigma_f^2}{\frac{\sigma_w^2}{tR} + \frac{\sigma_{rf}^2}{t} + \sigma_f^2}$$

(Zobel and Talbert, 1984).

where: $\sigma_a^2 = 4\sigma_f^2$ σ_{rf}^2 = family x replication variance
 $\sigma_{ph}^2 = \sigma_w^2 + \sigma_{rf}^2 + \sigma_f^2$
 σ_f^2 = family variance σ_w^2 = within plot variance
t = number of trees per plot r = number of replications

4 RESULTS

4.1 FLORAL INITIATION

No flowers or flower buds were observed during the experiment. Possible reasons for the failure of the plants to respond are varied, and will be discussed in section 5. Information not related to flowering was also gathered on the species, and is presented below.

4.2 BRANCH ANGLE

Analysis of variance for family differences in main stem branch angle showed significant differences at the five percent level of confidence for family x replication interactions (Table 4).

Table 4. Analysis of variance for main stem branch angle.

Source	df	sum of squares	mean square	F value	EMS
Total	92				
Rep.	2	94.41			$\sigma_w^2 + T\sigma_{rf}^2 + TF\sigma_r^2$
Family	7	550.43	78.63	0.74	$\sigma_w^2 + T\sigma_{rf}^2 + TR\sigma_f^2$
Family x rep.	14	1479.64	105.69	2.19*	$\sigma_w^2 + T\sigma_{rf}^2$
Trees w/in plot	69	3333.32	48.31		σ_w^2

* Significant at $\alpha = 0.05$

Table 5 contains ranked family means for branch angle across replications.

Table 5. Ranked family means for branch angle.

<u>Family</u>	<u>Branch angle (degrees)</u>
021-002	62.7 $\pm$ 5.9
021-007	59.1 $\pm$ 6.5
021-006	58.4 $\pm$ 7.2
021-003	57.5 $\pm$ 8.2
021-001	57.3 $\pm$ 6.6
021-009	56.7 $\pm$ 7.1
021-004	55.0 $\pm$ 8.2
021-010	54.4 $\pm$ 10.1

Individual tree heritability estimate was -0.15. Family heritability estimate was -0.41. Negative heritabilities are a result of the failure of statistics to accurately estimate the amount of variation present as the variability between families approaches zero. Since negative heritabilities are theoretically impossible, both these estimates can be assumed to be zero and will not be discussed further.

4.3 TREE HEIGHT

A comparison of family means at 10 months of age showed that genetic variation in early height growth may be present. Family 021-008 was 146% of the population mean, while the second ranked family, 021-007, was 104% of the population mean. Family means ranged from 77% to 146% of the population total (Table 6).

Table 6. Ranked Family means for ten month total height growth.

<u>Family</u>	<u>Number of individuals</u>	<u>Mean height (cm)</u>	<u>% of pop. mean</u>
021.008	58	164.9 $\pm$ 43.9	146
021.007	54	117.6 $\pm$ 36.2	104
021.001	77	113.1 $\pm$ 37.0	100
021.002	26	105.5 $\pm$ 33.2	94
021.009	56	103.7 $\pm$ 29.0	92
021.006	74	102.2 $\pm$ 26.8	91
021.004	59	100.2 $\pm$ 32.6	89
021.003	39	93.4 $\pm$ 26.5	83
021.010	20	86.4 $\pm$ 23.5	77

5 DISCUSSION

5.1 FLORAL INITIATION

It would be difficult, if not impossible, to determine why the trees did not respond as expected to the treatments. Several hypotheses, however, can be drawn to explain the results based on different aspects of the experiment. It should be noted that each of these aspects are discussed individually. Nonetheless, they are all related and the observed results are likely a consequence of many interactions between them.

5.1.1 Stage of maturation

The appearance of flowers is typically used as an indicator of maturity. No observable indicators, however, have yet been identified to distinguish the end of the juvenile stage or the end of the transitional stage. Supposedly, transitional trees are "ready" to flower, but only lack the necessary external stimuli to do so. Since it is currently impossible to distinguish between the two phases the problem becomes apparent: studies that have induced "juvenile" trees to flower may have actually induced trees in the transitional stage to flower. Successful treatments in one species may fail to produce results in another of the same chronological age due to the developmental differences between the species.

Although the treatments used in this study induced flowering in *Betula pendula* seedlings (Arshad, 1980), they did not in similarly aged seedlings of *A. cearensis*. It may be hypothesized that *B. pendula* seedlings were actually transitional as regards physiological development, and *A. cearensis* was juvenile, thereby rendering the treatments ineffective.

5.1.2 Treatment levels

Physiological differences between species give rise to variation in developmental processes. Artificial stimuli are therefore likely to produce different reactions in different species. The treatment levels applied in this study were based on previous studies and a review of related literature. Since work had never been carried out with *A. cearensis*, knowledge of appropriate treatment levels were based on work with other species.

One assumption made was that *A. cearensis* was similar enough to these other species that positive results would be produced by application of similar treatments. It may be concluded that this assumption did not hold, as evidenced by the lack of flowering. It should not be assumed however, that the treatments *per se* were not appropriate.

Higher resistance of *A. cearensis* to the action of CCC may have necessitated increased levels of the chemical to stimulate flowering. Similarly, more intense root pruning or stem girdling may also have been required.

5.1.3 Environmental conditions

Although attempts were made to simulate environmental conditions similar to those of *A. cearensis*' native habitat, actual conditions fell short of optimum. Large changes in temperature from day to night were common over the colder months, often dropping to temperatures (7.0°C) that occur infrequently in its natural range. Also, light intensities within the greenhouse, even after the replacement of the original bulbs with higher intensity bulbs, was significantly less than direct sunlight due to the opaque plexiglass roof. These conditions may have sufficiently stressed the trees to upset normal physiological processes which thereby inhibited floral initiation.

Photoperiod may play a role in flowering in *A. cearensis*. Photoperiod was maintained at approximately 13 hours during the experiment. It is not known at what point in *A. cearensis*' phenological cycle floral initials are formed. It is possible, however, that the photoperiod at that time in Paraguay is shorter than that in central Michigan when the treatments were applied. If *A. cearensis* is photoperiod

sensitive as regards flowering, too long (or short) a photoperiod at the time of treatment could be enough to inhibit flower formation.

5.2 BRANCH ANGLE

Analysis of variance for branch angle showed no statistically significant differences among families, and a significant family by replication interaction at the 5% level. Although the family x replication interaction was statistically significant, it is doubtful that there is any practical importance in it. Although family rankings change substantially over replications, no environmental differences between replications could be identified to account for these changes. Since among family variance can be assumed to be near zero, it follows that a small change in value for a family mean could result in a significant change in rank relative to other families. This change in rank would be reflected in a significant interaction. The change in value over replications could have been a result of microenvironment differences, sampling errors, measurement errors or any combination of these factors.

Two points should be mentioned about this analysis that are of interest. First, it is possible that the environment in which the trees were grown affected branch angles. A test of the same families in a native environment may produce

quite different results. Second, correlation of traits between juvenile and mature trees is often very poor. In other words, what is true in a juvenile tree will not necessarily be the case in a mature tree.

Regardless of these two drawbacks, the analysis is also important for two reasons. First, the angle at which branches arise from the main stem is of principal importance only during the initial years when the harvestable stem is being formed. Plantation grown *A. cearensis* must be pruned to produce quality boles. Branches that arise at a near 90° angle are much easier to prune, leave smaller scars, and smaller knots in the final product. If the branch angle changes after the merchantable portion of the stem is formed, it is of little practical importance. Second, this study could shed some light on the effect of environment on branch angle in *A. cearensis* if similar studies are carried out in its natural range.

5.3 TREE HEIGHT

As mentioned in a previous section, statistical analysis was not possible with ten month height data. Comparison of means, however, showed that variance among families may be present for early height growth. Although this was an extremely small trial in terms of number of families, one family, 021-008, performed noticeably better than the rest.

Rapid early growth can prove to be a survival advantage. Weeds are a common problem in forest plantations. Individuals that out-compete weeds for canopy space early on may have a better chance for survival. In addition, this initial advantage could take years off the rotation age by minimizing the establishment time required for the plantation.

5.4 FASCIATION

During the course of the experiment three trees exhibited fasciated stems. Similar fasciations on *A. cearensis* had been previously observed by the author in Paraguay. The fasciation appeared as a widening and flattening of the terminal meristem. Appearance of the fasciation temporarily stopped growth of the affected meristem. After an undetermined length of time, height growth resumed and the abnormality in the stem was covered over by diameter growth.

Transmission electron micrographs of an affected meristem taken by the Michigan State University Plant & Pest Diagnostic Center showed what could possibly be mycoplasma-like-organisms in some cells. Positive identification of the cellular inclusions could not be made due to lack of sufficient affected material.

6 SUMMARY

Induction of precocious flowering is a complex matter, and it is doubtful if continuing research in this area will be fruitful until more basic knowledge is gathered on the species. Detailed phenological studies combined with environmental data will aid in defining conditions necessary for flowering, as well as natural flowering and fruiting cycles.

Early flowering trees, whether naturally or through artificial stimulation, would be useful in a breeding program of *A. cearensis* for reasons already discussed. It is this author's opinion that a more productive use of TRP resources at this time is germplasm collection. Conserving a wide array of genotypes from the Paraguayan population will be critical if future breeding efforts with the species are to be successful.

Data collected on early height growth indicated that genetic variation likely exists among half-sib families in *A. cearensis*. Properly conducted progeny and/or provenance trials in Paraguay or elsewhere in its natural range would aid in determining the extent of variability for this trait. Increased early growth can decrease both the time needed for establishment and the time to harvest. Practically

speaking, this will reduce establishment costs by requiring less maintenance in the early life of the plantation, and increase the rate of return on investment for the plantation (all other things being equal).

No genetic variation was found among the eight families tested for branch angle. This is not to say however that variation does not exist for the trait within *A. cearensis*. The present study looked at only eight families. To accurately assess the amount of variability, or lack of variability present in the species, many more families should be tested. To determine if a breeding program to increase branch angle is appropriate, additional information is needed. More plantation data is required to determine the relative importance of branch angle in high quality timber production of *A. cerensis*.

The fasciation noted in three of the trees is interesting, and could be of major importance in future work with the species. Past experience of the author in Paraguay has shown that the fasciation can affect stem quality, although in most cases it does not result in serious damage. The abnormality is typically covered over with new wood as the tree's diameter increases. In all cases however, regardless of the extent to which the stem is deformed, growth is temporarily stopped. This pause in growth may seriously

retard a plantations progress.

Observations in Paraguay indicate that the appearance of the fasciation may be cyclic in nature (Zarza, personal communication 1992.). If this is true, then it would have impact on a planting program principally during the high points of the cycle, and would be of minor consequence at other times. The importance of identifying the cause and possible ways to deal with it depends in part then, on the frequency of the cycles. The more frequent the cycle, the more important it is to initiate research on the subject.

The results of this study are important in that they serve as a starting point for further research on *A. cearensis*. Identifying superior performing families for growth rate, branch angle or other traits will be a step towards ultimately providing superior planting stock to those interested in planting *A. cearensis*.

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