12-1977

Laboratory Comparisons of Two Species of Liquidambar

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1977

LABORATORY COMPARISONS OF TWO SPECIES OF *LIQUIDAMBAR*

A Thesis

Presented to

the Faculty of the Department of Biology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Lynn H. Wellman

December, 1977

LABORATORY COMPARISONS OF

TWO SPECIES OF LIQUIDAMBAR

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Joe E. Winstead, Chairman of the Advisory Committee, for his help and encouragement throughout the extent of this study. Also, thanks are due to Advisory Committee members Drs. Kenneth A. Nicely and Gary E. Dillard for their helpful suggestions during the preparation of this manuscript.

The efforts of Professor Ching-Shia Chen, of Fu-Jen Catholic University, Hsinchuang, Taipei Hsien, Taiwan, Republic of China, in providing seed material of Liquidambar formosana are recognized with deepest appreciation.

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OF *LIQUIDAMBAR*

Lynn H. Wellman 43 pages

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Laboratory germinated seedlings of *Liquidambar styraciflua* L., sweet gum, from Barren County, Kentucky, subjected to a flooding or inundation test were shown to respond in significantly different ways than did the controls. Plants with root systems standing in water showed a much shorter period of non-dormancy than plants that were not subjected to submergence when both were grown under long day, warm temperature conditions in growth chambers. There was no evidence of transfer of a growth retardant or dormin-like compound in water transferred from plants grown under short day (long night) conditions to plants with roots submerged and grown under long day (short night) periods.

The root weights and shoot weights of plants grown under long day dry conditions were significantly higher (.001 level) than the root and shoot weights of seedlings subjected to root submergence although there was no significant difference between the root-shoot ratios.

Plants receiving the submerged treatment showed significantly higher (.001 level) values of wood specific gravity than control seedlings grown under dry or normal test conditions.

Root stocks of the submerged plants showed anatomical differences when compared with plants not submerged during the test. Submerged root stocks possessed structures with superficially resembled enlarged lenticels.

Laboratory germinated seedlings of Liquidambar formosana Hance obtained from Taiwan showed no response to photoperoid under the warm temperature cycle (32-24 C). Once these seedlings were placed under a cooler temperature cycle (24-10 C) they exhibited cessation of growth and formation of dormant buds.

Seedlings of Liquidambar formosana differed from L. styraciflua seedlings in having significantly fewer (.001 level) stomata per leaf area, a significantly lower (.001 level) leaf area, and a significantly lower (.001 level) seed weight.

INTRODUCTION

It has become evident in recent years that there will be an increase in the demand for production of renewable forest resources. While there have been numerous studies in the past on ecological relationships of important hardwood species in field trials, relatively little information is available that has been acquired under controlled laboratory conditions. One genus, Liquidambar, has received attention in recent years as its importance to the timber industry has grown.

Liquidambar styraciflua has been the subject of many studies concerning intraspecific variation because of its wide geographic range in North and Central America. Populations of L. styraciflua have been shown to vary in seed germination and stratification requirements (Wilcox, 1968; Winstead, 1971), in growth and photoperiod response (Farmer, 1968; Williams and McMillan, 1971; McMillan, 1974; Randel, 1975; McMillan and Winstead, 1976), in cell and wood characteristics (Winstead, 1972; Randel and Winstead, 1976a), in frost tolerance (Williams and McMillan, 1971b), in the Hill reaction (Williams, 1971a), and in the levels of soluble sugar and ATP (Williams, 1971b).

Liquidambar, in addition to the obvious economic benefits as a hardwood species, is also an organism that

has received brief mention in questions of the relationships between past floras. Asa Gray was one of the first botanists to notice the similarities between the flora of North America and that of Eastern Asia (Dupree, 1959). Liquidambar is one of approximately eighty genera with a discontinous distribution between North America and Asia. There is reason to believe that these genera may be the survivors of an ancient circumboreal flora which failed to survive in Europe and western Asia (Good, 1974).

Generally three species of Liquidambar are recognized. Harms (1930) split the genus Liquidambar into two sections. Euliquidambar contains the species L. styraciflua, found in the United States and Mexico, L. macrophylla Oerst., found in Central America (this species is included in L. styraciflua by most botanists), and L. orientalis Mil., which is distributed throughout southwestern Asia. The second section, Cathayambar, contains one species, L. formosana, which is found in Formosa and south China. All trees have a chromosome number of 2N=32 and it has been shown that crosses made between these trees produce viable seed (Santamour, 1972a, 1972b). L. formosana may provide certain genetic characteristics that could improve the commerical value of wood. If the products of these crosses show a hybrid vigor of better growth qualities, it might be profitable to further investigate L. formosana concerning the potential of hybrid trees.

There has been very little work done concerning Liquidambar formosana. This species differs from the North American species in having three-lobed leaves which are pubescent on both sides, a pubescent stem, and longer stipules. L. formosana is an economically important tree in China. The wood is used in the making of fine furniture (Wilcox, 1967). It has also been suggested that because of its brilliant spring and fall coloration, L. formosana might serve as an ornamental in the United States; it is used for this purpose in Japan.

The leaves of Liquidambar styraciflua are generally five-lobed but may also be three-lobed, particularily in specimens from Mexico and Central America (Winstead, personal communication). The leaves of L. styraciflua are glabrous and of a brighter green color as compared to the dull green of the leaves of L. formosana. It has also been noted that L. styraciflua is self-sterile requiring out-breeding by wind pollination (Schmitt and Perry, 1964) while it has yet to be proven for L. formosana (Santamour, 1972b).

Recent work in the laboratory at Western Kentucky University concerning Liquidambar styraciflua (Winstead, 1975; McMillan and Winstead, 1976; Randel and Winstead, 1976a, 1976b) has provided baseline data for further studies of this species. There has been little work concerning the effects of inundation on seedlings and the potential effects of flooding on wood quality. As part of this study it was decided to test the effects of inundation of the roots to determine the response of growth and wood development. Since

few comparative studies were known that involved both the North American and Southeastern Asian species, a limited investigation was undertaken in comparing the responses of plants to photoperiod when grown under controlled conditions. Due to the availability of seed material of L. formosana for testing under controlled conditions, morphological comparisons were also planned that could perhaps provide some clues for more detailed study in the future involving the relationships of these two species to past distributions of ancient floras.

METHODS AND MATERIALS

Laboratory germinated seedlings of both Liquidambar styraciflua and L. formosana were subjected to a variety of tests with limitations depending upon the total number of individual seedlings available. The North American species (L. styraciflua) was subjected to tests of flood tolerance, photoperiod, root and shoot weights and root-shoot ratios, and secondary tissues were analyzed for wood specific gravity. The Eastern Asiatic species (L. formosana), due to limited availability of seed, was tested only for photoperiod response. Both species were compared as to the number of leaf stomata per unit area, total leaf area, and seed weights.

Seed material for Liquidambar formosana was collected in the area of Taipai, Taiwan, the Republic of China (25° North Latitude). Seed material for L. styraciflua was collected from Barren County, Kentucky, (34° North Latitude). Seeds were kept in cold storage (4 C) until germination was attempted.

Seeds were germinated under controlled environmental conditions using Environator Corporation growth chambers (Model E3448). The seeds were placed in trays of sand, watered, and covered with plastic to prevent water loss. Upon germination, the plastic was removed. The environmental

chambers were programed for continuous light under a 12-hr temperature cycle (30-22 C). Light intensities averaged 4842 lux and humidity ranged from 30-100%. Trays contained both seeds from single seed trees (2 trays of L. formosana) and collections of seeds from mixed seed trees (1 tray of L. formosana and 1 tray of L. styraciflua). L. formosana seeds required additional cold treatments (one week at 4.5 C) in order to obtain sufficient germination. After two months, the seedlings were potted in 4-inch square pots, which were individually marked, in a 3:1 peat-perlite mixture. At this time the program was changed to a 14-hr day and 10-hr night period, keeping the same temperature cycle, with the higher temperature beginning with the light period. Plants were watered regularly with tap water and given full strength Hoagland's solution fortified with CIBA-GEIGY's Sequestrene when needed throughout the remainder of the experiment. Eighteen week old seedlings were divided into two groups and placed under different controlled environmental conditions in the growth chambers.

Both species were placed under a long day photoperiod (14-hr) with identical sets grown under a short day program (11-hr). Temperature programs of the growth chambers were kept identical with a 12-hr period set at 35 C corresponding with the light period and a 12-hr period of 24 C matching much of the dark period. Six seedlings of Liquidambar formosana were compared for response under each condition.

The Liquidambar styraciflua seedlings were divided into three groups (each group consisting of ten seedlings) within each chamber. The first group consisted of potted seedlings under regular (dry) conditions. The second group consisted of potted seedlings which were submerged in water 1/2 inch from the pot top. The water was held in plastic containers with five seedlings in each container. The third group was submerged in a similiar manner with the water from the containers transferred from the third group of the first (short day) chamber to the third group of seedlings in the second (long day) chamber every three to four days.

After three months, seedlings from the long day photoperiod were compared in specific gravity and shoot-root ratio. Five plants from the dry (group one) treatment and ten plants which were submerged (from both groups two and three) were used. Plants were taken from the pots, and the soil mixture was removed from the root stocks with the aid of a soap solution. The seedlings were cut at the point of attachment of the cotyledons. The shoots and roots were weighted on a triple beam balance (Ohaus Scale Corporation) to compute shoot-root ratios. A one cm section directly above the point of attachment was cut from the shoot and the bark was removed for specific gravity determination. The specific gravity determination was made using the maximum moisture content method developed by Smith (1954). The sections were saturated in water under a vacuum for 24 hours, then weighed on a Roller-Smith Precision balance (Federal Pacific Electric

Company). The sections were then placed in test tubes and oven-dried at approximately 100 C for two days after which they were weighed again. Stamm (1938) found the specific gravity of wood substances to be 1.53. The specific gravity was determined by substituting the values in the formula developed by Smith;

$$\frac{1}{\frac{\text{Weight saturated-weight ovendry}}{\text{weight ovendry}} + \frac{1}{1.53}}$$

The three month old seedlings from the short day photoperiod were transferred to a greenhouse. Greenhouse temperatures ranged from 18-41 C and the humidity ranged from 14-93%. The light intensities ranged from 21520-26900 lux. The plants from group one were repotted in 8-inch circular pots in Pro-Mix B, a commercial soil mix manufactured by Premier Brands, Inc. The remaining plants from groups two and three (those being submerged) from the long day treatment were transferred to similar submerged conditions in the greenhouse. The second group of seedlings from the short day treatment was transferred from the submerged treatment in the growth chamber to dry conditions in the greenhouse. The seedlings of group three from the short day condition were transferred to the greenhouse in similar conditions to those in the growth chamber. The remaining seedlings of group one from the long day treatment remained in the growth chamber. The seedlings of Liquidambar formosana remained in the growth chambers.

The number of stomata per unit area of leaves of Liquidambar styraciflua and L. formosana were compared. Leaves were taken from L. formosana seedlings from the short day treatment and long day treatment and leaves from L. styraciflua were taken from seedlings from the long day photoperiod and from the repotted seedlings in the greenhouse. Sections were cut from the leaf, avoiding major veins and margins. Epidermal peels were taken from the sections and the number of stomata were counted per unit area. A Whipple disc calibrated by a stage micrometer was used to determine the area. The leaves were pressed overnight and then traced and the outlines were measured by a planimeter to obtain the total leaf area.

The average seed weight of Liquidambar styraciflua and L. formosana was compared. Ten sets of fifty seeds for each species were weighed on the precision balance. The total weight of each 50-seed set was divided by 50 to obtain an average. The average for each group was then used to obtain the average seed weight for each species.

RESULTS

Analysis of the data indicates significant variances between seedlings of Liquidambar styraciflua which were subjected to different treatments involving varing photoperiods (long day verses short day) and different growing conditions (dry versus submerged). These differences involved photoperiod reaction (cessation of growth, formation of apical buds, and bud burst), growth (root weight, shoot weight, while maintaining a constant shoot-root ratio), wood quality (specific gravity), and structural differences in the root stock. Differences between the two species, L. formosana and L. styraciflua, were found in the leaf structure and in seed weights. The structural differences in leaves included the number of stomata present per unit area and total leaf area.

It was apparent that under the test conditions used in this study there was no evidence of transfer of a growth retardant or dormin-like substance in water from one plant to another. There was no difference in the reaction to photoperiod between the plants which were under the long day submerged treatment and receiving water from the short day submerged plants and the long day submerged control plants (those that were submerged but were not receiving any water transfer between the chambers).

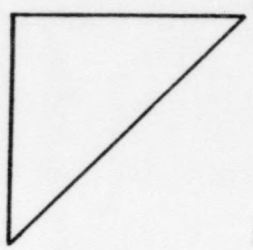
The trees under the four programs showed variation in the number of hours of darkness required for cessation of growth. The seedlings of the long day submerged program required significantly fewer (.05 level) hours of darkness for 50% cessation of growth (Table 1). There was no significant difference in this test for the other three treatments (short day dry, short day submerged, and long day dry). The plants of the long day submerged program also required significantly fewer (.05 level) hours of darkness for 100% cessation of growth (Table 2). There was also no significant difference between the other three treatments for this test.

The result of the test concerning the number of hours of darkness required for apical bud formation did not correspond with the results of the cessation of growth test. All treatments differed significantly in the requirement of hours of darkness required for 50% formation of apical buds (the long day submerged treatment still required the fewest hours of darkness, Table 3). The short day dry and short day submerged treatments did not differ significantly in the number of hours of darkness required for 100% apical formation. The other two treatments did differ significantly in this requirement (again the long day submerged treatment required the fewest hours of darkness, Table 4). It should be noted that the standard deviations for these results were extremely low.

The Liquidambar formosana seedlings showed no signs of reaction to photoperiod while under the 35-24 C temperature

Table 1. Mean number of hours of darkness required for cessation of growth of 50% of Liquidambar styraciflua seedlings

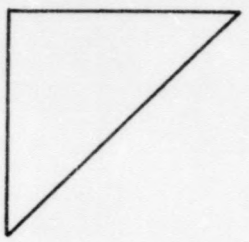
	Short Day Program	Long Day Program
Dry		
Mean	981	997.6
Standard Deviation	0	55.5
Submerged		
Mean	981	886.8
Standard Deviation	0	7.3



Lines connect means which are not significantly different at the .05 level.

Table 2. Mean number of hours of darkness required for cessation of growth for 100% of Liquidambar styraciflua seedlings

	Short Day Program	Long Day Program
Dry		
Mean	1053.8	1131.8
Standard Deviation	94.0	141.8
Submerged		
Mean	1053.8	949.4
Standard Deviation	112.8	80.6



Lines connect means which are not significantly different at the .05 level.

Table 3. Mean number of hours of darkness required for 50% apical bud formation in Liquidambar styraciflua seedlings

	Short Day Program	Long Day Program
Dry		
Mean	1163.0	1271.8
Standard Deviation	0	0
Submerged		
Mean	1186.4	1032.4
Standard Deviation	8.2	20.4

Lines connect means which are not significantly different at the .05 level.

Table 4. Mean number of hours of darkness required for 100% apical bud formation in Liquidambar styraciflua seedlings

	Short Day Program	Long Day Program
Dry		
Mean	1182.5	1285.8
Standard Deviation	28.2	9.3
Submerged		
Mean	1187.7	1076.8
Standard Deviation	5.8	84.5

Lines connect means which are not significantly different at the .05 level.

program. After receiving 3373 and 2325 hours of darkness, short and long day groups respectively, all plants were placed in a chamber programed for short days and cooler temperatures (8 hours of light with a day - night temperature cycle of 24-16 C). Evidence of cessation of growth was shown within three weeks as apical buds became evident in group one which previously received short day treatments. After four and one-half weeks, buds were noted in group two, those plants previously receiving long day treatments.

Plants of Liquidambar styraciflua transferred to the greenhouse also showed differences in growth responses based on their previous treatment in the growth chambers. Seedlings which received the short day submerged treatment in the chambers were transferred to submerged conditions in the greenhouse. These plants never underwent bud burst. Seedlings which received long day submerged treatments were also transferred to submerged conditions in the greenhouse and a few of these plants exhibited bud burst. Plants which received short day dry and short day submerged treatments in the chambers were transferred to dry conditions in the greenhouse. These plants also exhibited bud burst and their growth exceeded the minimal growth of the few submerged plants which underwent bud burst.

The root weights and shoot weights of the long day dry plants differed significantly (.001 level) from the root weights and shoot weights of the long day submerged plants. The weights of the dry plants were higher than those of the

seedlings receiving the submerged treatments. Although a difference in weights was observed there was no significant difference in the shoot-root ratio of both tests (Table 5).

Seedlings of the long day treatments also differed from the long day submerged seedlings in specific gravity. The plants receiving the submerged treatment have a higher value for specific gravity that was statistically significant at the .001 level (Table 6).

The root stocks of the submerged plants showed anatomical differences from the root stocks of the dry plants. Only the plants used for the shoot-root ratios and specific gravity tests were used for this observation; the other plants remained potted in the greenhouse or growth chambers. The submerged root stocks possessed structures which superficially resembled enlarged lenticels. Their internal anatomy as observed from free hand cross-sections also resembled that of a lenticel (Esau, 1962).

Leaves of Liquidambar formosana and L. styraciflua differed in certain leaf characteristics. The investigation of the number of stomata per unit area showed that the leaves of L. formosana had significantly more (.001 level) stomata per square centimeter than the leaves of L. styraciflua (Table 7). The three-lobed leaves of L. formosana also had a smaller individual leaf area than the five-lobed leaves of L. styraciflua (Table 8). It was also noted that the leaves of L. formosana were pubescent on both surfaces, had proportionally longer stipules, and their veins possessed a

Table 5. Comparison of root weight,, shoot weight, and shoot-root ratios of 23 week-old seedlings dry and submerged grown under controlled conditions

Dry

Plant Number	Root Weight(g)	Shoot Weight(g)	Shoot/Root Ratio
1	14.7	43.2	2.94
2	13.0	36.0	2.77
3	20.3	41.0	2.02
4	12.7	41.0	3.23
5	16.5	44.6	2.70
Mean	15.4*	41.2*	2.73
Range	12.5-20.3	36.0-44.6	2.02-3.23
Standard Deviation	3.1	3.3	.45

Submerged

Plant Number	Root Weight(g)	Shoot Weight(g)	Shoot/Root Ratio
1	8.6	28.6	3.33
2	11.7	27.0	2.31
3	6.6	21.9	3.32
4	8.4	19.3	2.30
5	5.2	16.7	3.21
6	4.8	14.6	3.04
7	9.2	18.4	2.00
8	5.0	14.2	2.84
9	4.5	11.4	2.53
10	6.3	14.7	2.33
Mean	7.0*	18.7*	2.44
Range	4.5-11.7	11.4-27.0	2.00-3.33
Standard Deviation	2.4	5.7	.49

* Means within column differ significantly at the .001 level.

Table 6. Comparison of wood specific gravity of 23 week-old seedlings dry and submerged grown under controlled conditions

Dry

Plant Number	Specific Gravity
1	.4765
2	.4461
3	.5221
4	.4884
5	.5138
Mean	.4894*
Range	.4461-.5221
Standard Deviation	.0316

Submerged

Plant Number	Specific Gravity
1	.5929
2	.5565
3	.5803
4	.5518
5	.5735
6	.5507
7	.5419
8	.5573
9	.5779
10	.5834
Mean	.5666*
Range	.5419-.5929
Standard Deviation	.0170

*Means within the column differ significantly at the .001 level

Table 7. Comparison of the number of stomata on the leaves of Liquidambar formosana and L. styraciflua. Numbers equal an average of ten counts per leaf.

L. formosana

Plant Number	Number of Stomata/cm^2
1	49568
2	39100
3	40191
4	54844
Mean	45926*
Range	39100-54844
Standard Deviation	7578

L. styraciflua

Plant Number	Number of Stomata/cm^2
1	32007
2	32958
3	24395
4	20848
Mean	27552*
Range	20848-32958
Standard Deviation	5887

*Means within column differ significantly at .001 level.

Table 8. Comparison of Leaf Area of Liquidambar formosana and L. styraciflua

L. formosana

Leaf Number	Leaf Area(cm^2)
1	43.43
2	46.54
3	31.65
4	58.03
Total Area	179.65
Mean	44.91*
Range	31.65-58.03
Standard Deviation	10.84

L. styraciflua

Leaf Number	Leaf Area(cm^2)
1	61.68
2	47.04
3	81.19
4	73.68
Total Area	263.59
Mean	65.90*
Range	47.04-81.19
Standard Deviation	14.92

*Means within column differs significantly at the .001 level

deeper red color, although at maturity some leaves of L. styraciflua also showed this deep red color.

A final difference between these two species was in the seed weights. The seed weights of Liquidambar formosana were significantly lower than the seed weights of the Kentucky population of L. styraciflua (Table 9).

Table 9. Comparison of Seed Weights of *Liquidambar formosana* and *L. styraciflua*

L. formosana

Seed Collection	Average Seed Weight(mg)
T-1	1.872
	1.656
T-2	2.964
	3.572
	3.556
	3.508
	3.224
T-5[1]	2.184
	1.836
	2.144
Mean	2.652*
Range	1.656-3.572
Standard Deviation	.7863

Table 9 (continued)

L. styraciflua

Seed Collection	Average Seed Weight (mg)
K-1	6.032
	6.316
	6.604
	5.948
	6.992
K-2	6.668
	6.036
	6.052
	6.676
	6.904
Mean	6.423*
Range	5.948-6.992
Standard Deviation	.3929

[1]Collection of seed from mixed seed trees

*Means within column differ significantly at the .001 level.

DISCUSSION

The data indicate the effects of submergence on Liquidambar styraciflua seedlings and morphological differences between the two species, L. styraciflua and L. formosana. It was shown that submergence was similar to photoperiod in being able to induce cessation of growth and formation of dormant buds. Submergence also resulted in a reduction of growth but not in altering biomass allocation. A final result of submergence was the production of lenticel-type structures on the root stocks of treated seedlings. The temperate species, L. styraciflua, was shown to differ from the sub-tropical species, L. formosana, in possessing a larger number of stomata over the same amount of leaf surface area, a greater leaf area, and a greater seed weight.

The transfer of water between submerged plants of the two different chambers was done to detect the possibility of a water soluble hormone which might be connected with dormancy. This test was attempted because of previous work in submerging populations of sweetgum from the United States and Mexico together under greenhouse conditions (Winstead, Personal Communication). In that particular test, Mexican plants went dormant when submerged but dry (unsubmerged) plants serving as a control did not. In that test there were no controls under different day-night cycles nor were any populations kept

separate under the submerged conditions. It was thought that the submerged plants under short day treatments might produce a water soluble hormone which would induce dormancy when introduced into the simulated pond conditions of the submerged plants receiving the long day treatment. The fact that the submerged control plants (those plants receiving the long day treatment but not receiving water transferred from the short day chamber) underwent cessation of growth and formation of apical buds at the same time as those submerged plants involved in the water transfer shows that placing the seedlings in water was enough to induce dormancy.

The presence of a growth regulating compound in the water of the submerged plants cannot be ruled out. Perhaps photoperiod of the stress of submergence is enough in itself to cause the production of a dormancy-stimulating compound (or a decrease in the production of a dormancy-inhibiting compound).

A reasonable explanation for the induction of dormancy is that the stress put upon the plant by the reduction of available oxygen to the root system is enough to stop growth and induce a dormant state. It should be noted that, but for a few exceptions, the plants never broke dormancy when they were submerged; those that did exhibit bud burst had minimal growth.

Liquidambar styraciflua is found in bottomlands and, therefore, seedlings are sometimes exposed to partial or complete flooding. It has been shown that seedlings of L. styraciflua can survive approximately two weeks (16 days) of

complete inundation. The recovery time is slower than that of other bottomland species such as willow and green ash (Hosner, 1958). It has been shown that mature trees of L. styraciflua can stand partial flooding for 3-6 months out of the year with the only noticable effect being that roots produced during that time were not associated with mycorrhizae and there was a reduction in the survival of mycorrhizae present during the test period (Filer, 1975). The results of the current study indicate that areas containing first year seedlings and becoming saturated or water-logged during the growing season would not provide a habitat for optimum growth. A practical application might be seen if some managed watershed area were subjected to periodic or continual saturation. If Liquidambar styraciflua was a significant species of that particular system the chances would be that the forest composition would change due to the probable decreased growth of this species.

The photochrome system provides a means by which plants may detect photoperiod. The system consists of a light-absorbing pigment, phytochrome, which exists in two forms, P_{660} or P_r, and P_{730} or P_{fr}. P_r has an absorption maximum at 660 mμ which changes P_r to P_{fr}. P_{fr} has an absorption maximum at 730 mμ which converts P_{fr} back to P_r. P_{fr} also converts back to P_r in the dark. It is theorized that daylight causes P_r to be converted to P_{fr}, with P_{fr} converting back to P_r during the dark (night) period (Hendricks and Borthwick, 1963). Longer daylight periods will result in a

longer period of time which the phytochrome is in the P_{fr} form. It appears that the length of the dark period is the important factor in photoperiod responses; a flash of red light in the middle of the dark period will convert P_r, obtained from the dark conversion of P_{fr}, back to P_{fr} negating the effect of the night periods (Hendricks and Borthwick, 1963).

An explanation of the biological activity of phytochrome would be its participation in some manner in the production of growth substances. It is thought that phytochrome itself might serve as an enzyme (Hendricks and Borthwick, 1963), a gene regulator, or in influencing membrane permeability (Quail, 1976). If this pigment (in the form of P_{fr}) governs catalytic activity in a reaction sequence involving the regulation or production of a sequence involving the regulation or production of a growth substance, then varying photoperiods would cause a change in the concentration of this growth hormone. Different photoperiods would regulate the duration of the P_{fr} form which in turn would regulate the amount of a growth hormone. An example of the above situation might be that long days cause the phytochrome to be in the P_{fr} form for a longer period of time. The longer time spent as P_{fr} results in greater amounts of a growth hormone and, therefore, more growth takes place during long days. In the reverse situation, short days reduce the amount of time that phytochrome exists as P_{fr} and, therefore, a reduction in the amount of a growth hormone present resulting in reduced growth. If the production of a dormancy compound is linked to the phytochrome system then an important

factor would be the total number of hours of darkness. The phytochrome will alternate daily between the two forms but the dormancy compound might be stable and would, therefore, accumulate over a period of time.

The seedlings of Liquidambar styraciflua were subjected to two factors, photoperiod and submergence. The seedlings had limits to these factors, the number of hours of darkness required to cause a reduction of growth and formation of an apical bud and the amount of time required for the effects of submergence to be shown. The chambers were programmed for different photoperiods; therefore, it would take the long day chamber more days to accumulate the same number of hours of darkness as the short day chamber. The submerged plants were placed in the trays at the same time so that at any given time the seedlings in both chambers had received identical treatments in terms of submergence.

Tables 1, 2, 3, and 4 list the mean number of hours of darkness required for cessation of growth of 50% of the Liquidambar styraciflua seedlings, the mean number of hours of darkness required for 100% of L. styraciflua seedlings, the mean number of hours of darkness required for apical bud formation in 50% of L. styraciflua seedlings, and the mean number of hours of darkness required for the formation of apical buds in 100% of L. styraciflua seedlings respectively. The standard deviations for Tables 1, 3, and 4 are extremely low. There are two possible explanations for this fact. First, the observations of the experimenter might have been

inaccurate and, second, the time of darkness was measured as total hours of darkness and not as applications of a constant dark period. The dark treatment was given in blocks of hours of darkness (8 and 12 hours). In analyzing the data in terms of hours of darkness, the values are restricted to those numbers which are multiples of the number of hours of the dark treatment. In future experiments, seedlings should be kept in continuous light until the start of the experiment. Once the seedlings are placed in chambers of different photoperiods the number of applications (nights) should be counted instead of the total hours of darkness. The total number of hours of darkness may be calculated after the data have been analyzed for significant differences. This would provide a more sensitive method of analyzing the experiment results. The fact that the standard deviations are so low might cause differences between means to appear to be significant when actually they fall within the range of expected variation.

The values summarized in Tables 1 and 2 were calculated from the graphs of seedling growth and, therefore, may be more representative than the values in Tables 3 and 4 which were obtained from physical observations. No significant differences were detected between the means of seedlings receiving long day dry treatments, short day dry treatments, and short day submerged treatments. The means of the short day dry and short day submerged were identical, but the standard deviations were zero (Table 1). In both cases, the number of hours of darkness for the long day submerged treatment was significantly less than the number required for the other treatments.

The explanation concerns the limits of the two different factors, photoperiod and submergence. These tables indicated that approximately 1000 hours of darkness are required for cessation of growth in terms of photoperiod response. There is also a limit to the amount of time the seedlings can remain submerged before showing the effects of inundation by the reduction of growth. The seedlings in the short day chamber received more hours of darkness during the same amount of time than the plants in the long day chamber. The short day submerged plants reached the limit of the hours of darkness before (or at the same time) the effects of submergence were shown. The long day submerged plants, however, received fewer hours of darkness in the same amount of time than did those plants in the short day chamber. The number of days required for the effects of submergence to be shown was reached before the total number of hours of darkness was received. In this case the effect of submergence was more important than the photoperiod effect.

Table 3 shows that all treatments required significantly different number of hours of darkness for 50% apical bud formation, and Table 4 shows that the only nonsignificant difference between the mean number of hours of darkness required for 100% apical bud formation was between the short day treatments, dry and submerged. Again it should be noted that the standard deviations are very low and, therefore, the differences test out significantly different even though they may not actually fall outside the range of normal variation.

The value for the long day submerged treatment in both tables was lower than the means of the other three treatments. This may continue the trend shown in Tables 1 and 2, or it may be that submergence results merely in the reduction of growth and that a certain number of hours of darkness is required for apical bud formation. The means of the hours of darkness required for apical bud formation fall within the range of previously reported data from similar treatments (Randel, 1975).

The plants which were moved to the greenhouse varied in their response to photoperiod according to previous and continued treatments. Plants which remained under dry conditions exhibited bud burst after apical bud formation (with one exception). Plants which were given continued submerged treatments rarely exhibited bud burst. The fact that the submerged plants did exhibit bud burst had previously received long day photoperiod, whereas no submerged plants which had previously received short day photoperiods initiated growth, may indicate a cummulative effect of the hours of darkness. The short day plants received more hours of darkness and, therefore, possibly accumulated more of a dormancy compound. The long day plants received fewer hours of darkness and, therefore, possibly had a lower amount of dormancy compound. The dormancy caused by the submergence was reinforced by the photoperiod response to a greater extend by the long nights. It is also possible that it was by mere chance that the only submerged plants to exhibit bud burst were those which had previously received the long

day treatment, only three seedlings out of forth submerged plants exhibited bud burst. The submerged seedlings which did exhibit bud burst showed only minimal growth. Submergence will reduce growth again, probably by the reduction of available oxygen. Plants which had previously been submerged and now receiving normal (dry) treatments in the greenhouse also exhibited bud burst. Once submerged conditions are removed seedlings may return to normal growth patterns. Plants surviving in saturated conditions must possess certain adaptions which enable them to survive such as decreased root respiration.

Submergence resulted in reduced growth but no difference in the shoot-root ratio. The primary effect of submergence is the reduction of available oxygen to the roots. The reduction of oxygen would produce the expected result of diminished growth. The weights of the root stock and shoots were significantly lower for the submerged seedlings than for the seedlings receiving the dry treatments (both groups were in the long day chamber) (Table 5). Although there was a reduction in growth there was no change in the shoot-root ratios. The shoot-root ratio is a measure of biomass allocation. There is some evidence that the shoot-root ratio is lower for some desert plants, more biomass is put into the production of roots since water is the limiting factor in the climate (Krause and Kummerow, 1977). It is apparent that submergence has no effect on the biomass allocation indicating that this characteristic is generally controlled and not subjected to

environmental conditions (if the trait were a plastic response the submergence would be expected to increase the shoot-root ratio).

Although specific gravity of the seedlings fell within the range of the specific gravity of the wood of mature trees reported in the literature, submergence has an effect on the quality of the wood of the seedlings. It has been previously shown that Liquidambar styraciflua seedlings from different latitudes vary in specific gravity, with plants of the lower latitudes having a higher specific gravity (Winstead, 1972). It has been speculated that the difference in specific gravity is related to different cell diameters, with wood having a higher specific gravity have smaller cell diameters (Randell and Winstead, 1976a). In this instance the environmental condition of submergence decreased specific gravity. A likely explanation is that submergence causes a decrease in growth, therefore, a decrease in cell diameter. It would seem that optimum growth conditions would result in a lower specific gravity. It would be expected that optimum growth conditions would be shown by larger growth rings. However, in field collected data there is no correlation between the width of growth rings and specific gravity (Taylor, 1977). It should be emphasized that this data was obtained from field collected material and not from trees grown under identical environmental controlled conditions.

A final effect of submergence on the seedlings was in the production of enlarged lenticels of the root stocks of the

submerged plants. Again this may be explained by the primary effect of the water on the seedlings, the reduction of available oxygen to the roots. Lenticels are structures which function in gaseous exchange. It might be that lenticels were produced in response to the stress of the lowered concentration of oxygen. This would seem to be an adaptive characteristic in reaction to an adverse environmental conditions.

It can be seen that submergence will have an effect on Liquidambar styraciflua in growth response and possibly in apical bud formation, in specific gravity, and lenticel formation.

The Liquidambar formosana seedlings showed a different response to photoperiod. The seedlings received 3373 and 2325.2 hours of darkness (short and long day chambers respectively) showing only a slight reduction in growth due either to photoperiod or shading (in future studies efforts should be made to minimize the effects of shading in the chambers if possible). When all L. formosana seedlings were moved into a short-day chamber with a day-night temperature cycle of 24-10 C, the seedlings showed cessation of growth and formation of apical buds. It is possible that temperature is more important factor than photoperiod in the dormancy response of L. formosana. It has been shown in this species that cambial activity increases with increasing temperature, is effected minimally by rainfall and relative humidity, and is correlated with the phenology of the tree (Lu and Chiang, 1975). An interaction of these factors is required

for the complete dormancy in L. styraciflua seedlings, apical buds are formed in response to photoperiod and lower temperatures are required for complete dormancy (the dry seedlings exhibited bud burst in the warmer temperatures, submergence was able to inhibit bud burst in the treated seedlings). It is possible that in a subtropical environment the temperature change is gradual with less fluctuation. In such a situation it might be more suitable for the plant to have a dormancy system which would react mainly to temperature. In the temperate zone, temperature change is also gradual but there might be more fluctuation (early and late frosts, etc.). A dormancy system geared to something other than temperature would be advantagous in preventing the killing of seedlings from a sudden temperature change. Apical buds might form in response to photoperiod, and dormancy might be reinforced by cooler temperatures. Therefore, it might be possible that each species has a different strategy for entering the dormant state, based on its natural environment conditions.

The fact that the first Liquidambar formosana seedlings to show cessation of growth and apical bud formation in the cool temperature cycle were those that had previously received the short day treatment might indicate the effect of an accumulation of a dormancy compound related to the total number of hours of darkness. This information was obtained from observations and was not subjected to statistical analysis; therefore, meaningful conclusions should not be drawn since these observations may be due strictly to chance.

This study was limited by the low number of Liquidambar formosana seedlings available for testing. More indepth research is needed to completely characterize the response of the populations used. Future studies, that test the interaction of photoperiod and temperature as well as compare different populations of this species, are needed to determine any ecotypic differentiation.

The number of stomata per area of the leaves of Liquidambar styraciflua was significantly lower (.001 level) than the number of stomata per area of the leaves of L. formosana. The number of stomata per cm^2 previously reported (22.443) from field collections falls within the range obtained in this experiment (Carpenter and Smith, 1975). A reasonable explanation of this fact may be related to the geographic location from which the seeds were obtained. The L. formosana seeds were collected on the island of Taiwan ($25^{o}N$) above the Tropic of Cancer, therefore, in a sub-tropical region. L. styraciflua seeds were collected in Barren County, Kentucky, ($37^{o}N$) which is a temperate region. It is possible that water is more available in the sub-tropical region than in the temperate zone (Lu and Chiang state that rainfall is abundant in Taiwan and water does not appear to be a limiting factor (1975)), therefore, the selection pressure against structures which are responsible for water loss (stomata) is much less severe than it would be in a situation in which water retention is important. If water is less abundant in the temperate region the selection pressure for the reduction in the number of stomata (and, therefore,

a reduction in water loss due to transpiration) would be much greater, resulting in a smaller number of stomates for the temperate species (L. styraciflua). It would be interesting to compare the number of stomates per area of leaves of Mexican populations of L. styraciflua with the Kentucky populations of L. styraciflua and L. formosana to determine whether the diminishing selection pressure for reduction of stomates holds for the sub-tropical population of L. styraciflua or whether the difference in stomata numbers is purely between species.

The individual leaves of Liquidambar formosana had a significantly smaller (.001 level) area that the individual leaves of L. styraciflua. This fact may be linked to the length of the growing seasons of their native geographical locations. Due to the shorter growing season of the temperate region, certain adaptations must be made by L. styraciflua in order to attain the same amount of growth as L. formosana.

It is possible that a larger leaf area is an adaptation of this type. The larger area of the individual leaves would result in a greater photosynthetic area. A greater photosynthetic area would result in a greater amount of photosynthetic activity and, therefore, a faster rate of growth. In this manner L. styraciflua might equal the growth per year of L. formosana even though the growing season of L. styraciflua is shorter than that of L. formosana.

A final difference between Liquidambar formosana and L. styraciflua was shown in seed weights. The seed weights of L. formosana were significantly lower (.001 level) than the

seed weights of L. styraciflua. It is probable that this difference in seed weight agrees with the fruit weight cline which has previously been shown with ash, ironwood, cherry, dogwood, and maple (Winstead, et al., 1977). It was suggested that these differences could reflect a reproductive strategy, heavier fruits are found in the more northern latitudes where the overwintering period is longer. This difference may also be part of a much wider concept of an overall production gradient correlated with latitude. Again it might be interesting to compare the fruit weights of Mexican populations of L. styraciflua to those of L. formosana and Kentucky populations of L. styraciflua to determine whether difference is related to latitude or to the difference in species. It also remains to be proven whether such responses are ecophenic (environmentally controlled) or ecotypic (genetically controlled).

It has been shown that in addition to the taxonomic differences reported in the literature the species Liquidambar formosana differs from L. styraciflua in photoperiod response, the number of stomata per leaf area, the area of individual leaves, and in seed weights. It is possible that these differences might be due largely to populations genetically adapted to different environmental conditions related to the latitude in which each population is found rather than to differences between species. An examination of the similarities and differences between L. formosana and Mexican populations of L. styraciflua would indicate whether these differences are due to the different species or are related more to the genetic adaptations of each population to its own environment.

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