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Interpopulational & Species Comparisons of the Genus Liquidambar

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Duckworth,

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1980

INTERPOPULATIONAL AND SPECIES COMPARISONS OF THE GENUS LIQUIDAMBAR

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Diana L. Duckworth

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INTERPOPULATIONAL AND SPECIES COMPARISONS

OF THE GENUS LIQUIDAMBAR

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OF THE GENUS LIQUIDAMBAR

Diana L. Duckworth 42 pages

Directed by: J. E. Winstead, M. R. Houston, and K. A. Nicely

Department of Biology Western Kentucky University

Growth patterns under controlled conditions and chemotaxonomic techniques involving protein and flavonoid analysis were used to investigate both interpopulational and species comparisons in the genus Liquidambar. Striking differences in budbursting patterns after a dormant period were seen when comparing L. styraciflua and L. formosana. The latter species showed earlier initiation of growth. Dormancy patterns of L. styraciflua grown under growth chamber conditions showed a latitudinal cline. Cessation of growth occurred later in a Georgia population under both long and short day experimental periods than populations from Missouri and Kentucky. When grown under controlled conditions, flavonoid patterns from L. styraciflua and L. formosana showed few differences. Differences in flavonoid patterns were suggested when comparing the North American L. styraciflua and a single leaf sample of L. orientalis from Turkey. Extensive trials attempting to examine protein banding patterns by electrophoretic methods were unsuccessful even though the techniques have been used successfully in examinations of other plants.

INTRODUCTION

The American sweetgum, Liquidambar styraciflua L., has been the subject of research studies of populational differences at Western Kentucky University (Randel and Winstead, 1975; Wellman and Winstead, 1979; Winstead, 1971, 1972, 1977) and elsewhere (Williams and McMillan, 1971) over the past few years. However, this genus has a wide geographic distribution and an extensively documented geologic history. Extant species occur in Turkey (L. orientalis Mil.), in China and Taiwan (L. formosana) and in the southeastern United States and Central America (L. styraciflua). The published literature has concentrated on populations from the United States and Mexico and there has been only a cursory examination of the Asiatic species in comparison with the North American species (Wellman, 1977). As demands for renewable resources such as wood fiber increase world-wide, increased efforts are likely in the utilization of species that have to the present been little studied. The lack of laboratory work on species of the genus Liquidambar prompted this preliminary investigation centering on biochemical techniques to study taxonomic relationships.

Current taxonomy based on Harms' (1930) treatment of the genus outlines four species (Figure 1). Harms also

Figure 1. Taxonomy of Liquidambar Harms (1930)

Order--Ranales

Family--Hamamelidaceae

Subfamily--Liquidambaroideae

Genus--Liquidambar

Section I--Euliquidambar

Species--styraciflua
orientalis
macrophylla

Section II--Cathayambar

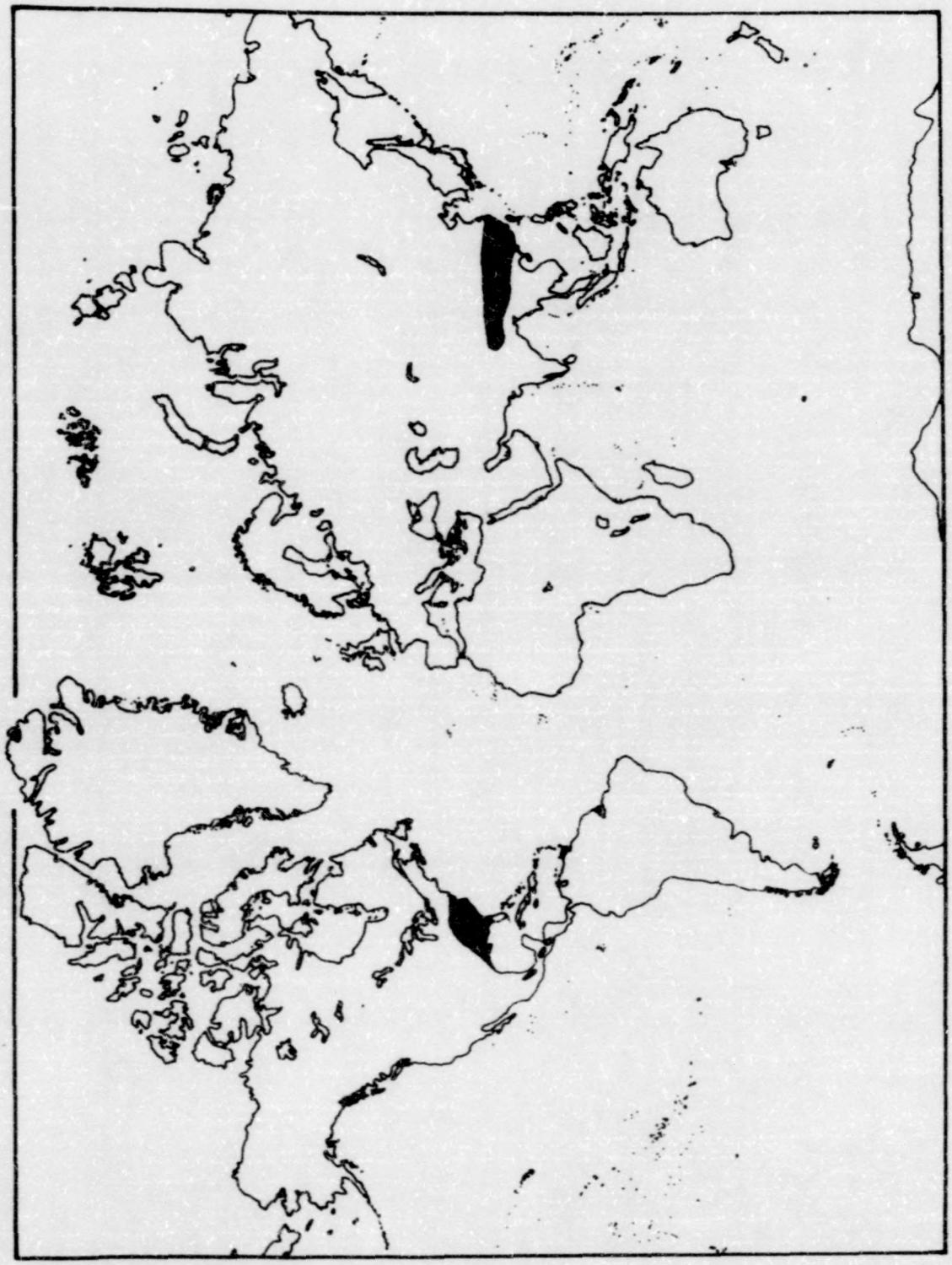
Species--formosana

divided the genus into two sections. Euliquidambar contains the species L. styraciflua found in the United States and Mexico, L. macrophylla Oerst. found in Central America and L. orientalis found in Turkey. The second section, Cathayambar, contains the single species L. formosana which is found in China and Taiwan. The ability of three of the species to hybridize (Santamour, 1972) may question the taxonomic outline, and there has been a general trend to treat the species L. macrophylla as part of L. styraciflua by various workers (McMillan and Winstead, 1976; Winstead, 1972; Williams and McMillan, 1971).

The geographic distribution (Figure 2) of the genus may give evidence of the age and former distribution of the genus and its family. The family, Hamamelidaceae, contains 19 genera, nine of which are monotypic (Berry, 1920). Monotypic genera indicate either young, recently evolved genera or geologically old genera representing the remaining descendants of long now extinct lines. Liquidambar's distribution indicates that it has a long geologic history with a distribution which included areas linking its now isolated locations. Another alternative, that of spontaneous origin at more than one site, is highly unlikely.

The fossil records show 20 extinct species of Liquidambar and a cosmopolitan distribution (Berry, 1920). The oldest authenticated form was from the upper Eocene in Greenland, Alaska, and Oregon. Following the Eocene was a period known as the Oligocene. The only known Oligocene species was from

Figure 2. Modern geographic distribution of the genus Liquidambar



Italy. This period was a time of unsettled climate and typographic changes; thus, fossil records from this period are sketchy. Fortunately, records from the following period, the Miocene, are extensive. Liquidambar is recorded from Japan to southwestern Asiatic Russia. Its distribution was cosmopolitan in Europe and America. Pliocene records show Liquidambar covered an extensive area. However, this period was followed by the Pleistocene or Glacial Period. During this time, many of the forest trees of Europe were destroyed by glacial movement. Why this same fate did not extend to the trees in Asia and North America can be seen by comparing the geography of the three areas. In Europe, the Alps run east and west blocking migration southward to a milder climate as was possible in the United States and Asia. After the glacial retreat, Liquidambar reestablished much of its former distribution in the United States and is presently found from eastern Massachusetts southward to the Florida peninsula and from the east coast to Texas.

There is an increasing interest in the use of biochemical analyses as a taxonomic tool. Proponents of biochemical systematics or chemotaxonomy think of it as a complement to the classical taxonomy based on morphological features. The validity of this tool has been verified numerous times (Alston and Turner, 1963 and Clarkson, Huang and Cech, 1975). Proteins, flavonoids, alkaloids, and terpenoids are among the compounds utilized for taxonomic purposes.

Lack of biochemical data on Liquidambar prompted this study. Because of documented successes with flavonoids (Alston and Turner, 1962 and Bate-Smith, 1973) and proteins (Clarkson, Huang and Cech, 1975; Liu, Sharitz and Smith, 1978; Payne and Fairbrothers, 1973) these compounds were employed in an attempt to clarify the taxonomy of the genus. Also, evidence of certain relationships might elucidate the origin and manner of distribution of the species. For example, if L. formosana could be shown to be closely related to L. styraciflua from a Georgia population (which was an isolated population during the Glacial Period) this would be evidence supporting the theory that forests in Asia and the United States were connected before this period.

Seedlings and two-year-old trees from Taiwan and various areas of the United States became readily available from previous studies at Western Kentucky University. Since the protein studies were to be the bulk of the work, much of the material was reserved for that portion. A single leaf sample from Turkey allowed comparison of flavonoid samples from all three species. Due to the necessity of daily inspection of the plant material, budbursting patterns were collected as well as apical bud formation data.

MATERIALS AND METHODS

Plant material for analysis was provided by field collections and tree seedlings grown in the laboratory. Plants grown under controlled laboratory conditions were placed in Environator Corporation growth chambers (Model E3448). Humidity inside the growth chambers ranged from 30-100% and the light intensity ranged from 5597-9903 lux. Plants were watered daily and given nutrient solutions at regular intervals.

One growth chamber was programmed for a 16-hr day under a 12-hr temperature cycle (39-22 C) and held sixteen two-year old trees left from a study by Wellman (1977). Eight of the plants were L. formosana from a single parent tree from near Taipai, Taiwan, and eight represented L. styraciflua being progeny of trees in Barren Co., Kentucky. These trees, having been maintained in the greenhouse since the end of Wellman's study, were in a dormant condition when placed in the growth chamber. The plants were potted in 8-in plastic pots in Promix growth medium. These trees were used in budbursting and flavonoid studies. The flavonoid studies also included dired mature leaf material representing a single leaf sample from Turkey, and leaf material from progeny of trees from Texas and Mexico which were grown in a garden in Wichita

Falls, Texas. Fresh collected mature leaves from the growth chamber were oven-dried for 24 hrs. The dried material was crushed and allowed to sit in 5 ml of 1% HCl (v/v) in methanol for 24 hrs. The sample was obtained by allowing the leaf material to settle out and the supernatant was used to spot the chromatograph sheets. At a point one inch from the corner of the chromatograph sheets, 100 drops of supernatant was applied. Two dimensional paper chromatography was used and the chromatographs developed within Precision Scientific Chromatocabs. The long dimension was run for 24 hrs in a solution of tertiary butanol/glacial acetic acid/ water (3/1/1/ v/v). The short dimension was run for 4 hrs in a solution of glacial acetic acid/water (85/15 v/v). The chromatographs were read under visible light in a saturated atmosphere of ammonia, UV light (wavelength 254 nm), and UV light in a saturated atmosphere of ammonia. Composite chromatographs were made for comparison purposes. The data from the flavonoid study were used to make three principal comparisons: (1) Texas L. styraciflua a representative of a Mexico population when both had been grown in a garden in Wichita Falls, Texas for 11 years, (2) comparing a population of Kentucky L. styraciflua with Taiwan L. formosana when both were grown as seedlings under growth chamber conditions, (3) a single sample from L. orientalis was run to determine any differences between it and the other two species.

After the preceding study, it was learned that phenols could interfere with flavonoid patterns (T. F. Stussey,

personal communication). An ether extraction procedure was added to remove the phenols from the samples. The sample obtained by the previous extraction method was placed in a separatory funnel and ether was added. The methanol layer was discarded and chloroform added to the ether layer. Upon standing, a white precipitate formed. The precipitate was discarded, and the remaining liquid was used as the sample to be chromatographed. This procedure was the only change in the original extraction procedure. Leaf material was from plants held in the greenhouse from a Georgia population of L. styraciflua and L. formosana. These plants were left over from protein studies outlined subsequently in this thesis. Samples were prepared by extracting with and without ether. Unfortunately, flavonoid controls were not available.

The protein studies were to be used to make two main comparisons: (1) L. formosana with several populations of L. styraciflua to determine species and population differences, the degree of relationship between L. formosana and the various populations of L. styraciflua was also of interest; (2) the effect of photoperiod on protein banding patterns was of interest. Comparisons were to be made only between populations of L. styraciflua due to lack of sufficient numbers of seedlings of L. formosana.

Two growth chambers were used in the protein studies. Plants for these growth chambers represented seedlings from single parent trees from Taipai, Taiwan; Alvaton, Kentucky;

Atlanta, Georgia; and Farmington, Missouri. For germination, all seeds were placed in autoclaved sand, watered, and placed at 4 C where they remained at this temperature for 36 days. At this time, they were removed from the cold and placed in the growth chambers under continuous light and a 12-hr temperature cycle (32-21 C). After germination, the seedlings were planted in 4-in plastic pots in peat/perlite (3/1, w/w). They were divided into two groups and placed in separate growth chambers. Until the experiment started, chambers were set for a 16-hr day, 12-hr temperature cycle (30-22 C).

During the experiment, one group remained on the original program, the other was placed on a 12-hr day cycle. The temperature program remained the same. Samples were taken from each chamber at two-week intervals. Sampling was continued until all plants in the 16-hr growth chamber showed apical bud formation. Dormant bud formation data was recorded for both chambers.

Polyacrylamide gel electrophoresis was used throughout the study. Acrylamide concentrations were varied from 7.5-20%. Acrylamide concentrations of 7.5-8% gave the best combination of running time and protein separation as determined by a protein control.

Initial runs employed the slab gel apparatus (Figure 3). Electrophoretic times for these experiments were 3-4 hrs. Reagents and buffers were prepared as described by Laemmli (1970) (Tables 1 and 2). Samples were prepared by grinding

Figure 3. Slab gel electrophoretic apparatus. Reservoir and gel molds are shown.

DARK DOCUMENTS "May Not Film Well."

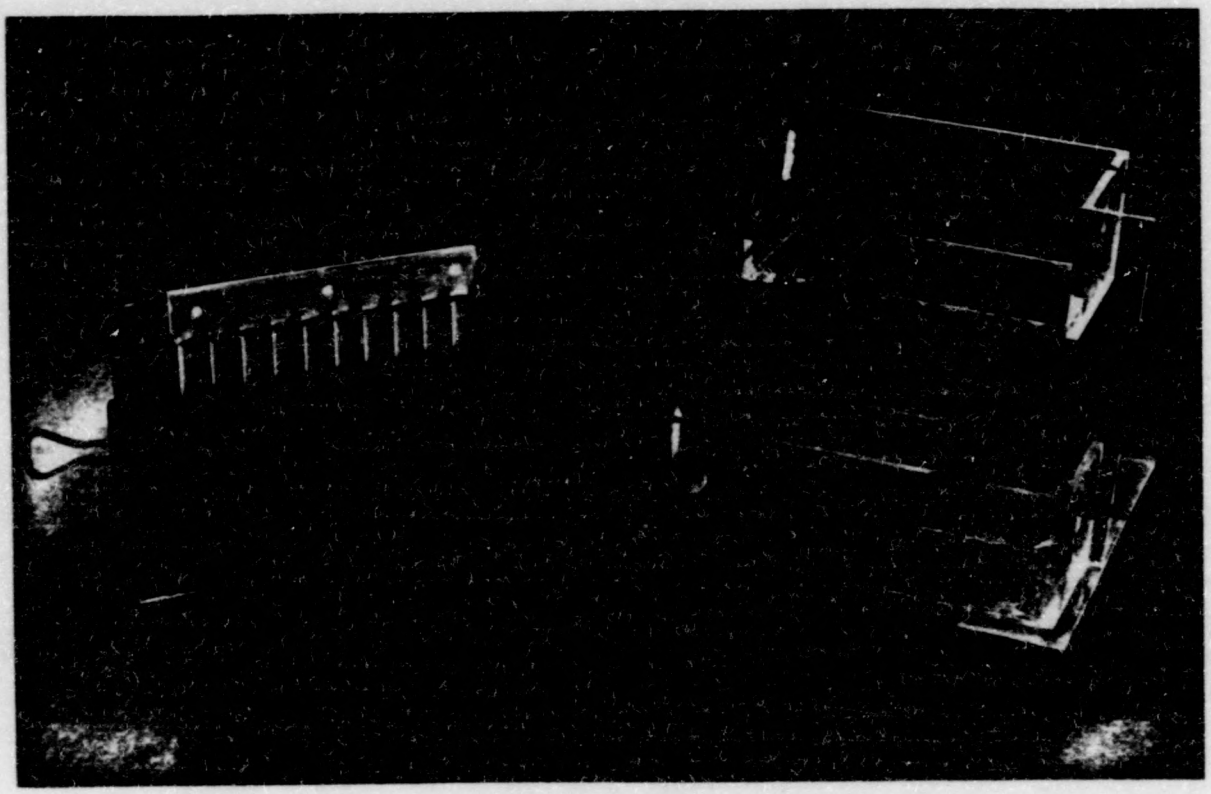


Table 1. Stock solutions for Laemmli acrylamide gels and buffers

10% SDS (w/v) Stock		
Sodium dodecylsulfate		10 g
Water		to 100 ml
A: 4X-concentrated Stacking Buffer		
Tris		6.06 g
10% SDS Stock (0.4%, final 0.1%)		4.00 ml
Conc. HCl to pH 6.8	approx.	3.60 ml
Water		to 100.00 ml
B: 4X-concentrated Separating Buffer		
Tris (1.5 M, final 0.375 M)		18.17 g
10% SDS Stock (0.4%, final 0.1%)		4.00 ml
Conc. HCl to pH 8.8	approx.	11.77 ml
Water		to 100.00 ml
C: 30% Acrylamide/0.8% Bis Stock		
Acrylamide		30.00 g
Methylene Bisacrylamide		0.80 g
Water		to 100.00 ml
10% Persulfate Solution		
Ammonium persulfate		50.00 g
Water		0.50 ml
TEMED		
Tetramethylethylenediamine		Full strength
or		
Tetramethylethylenediamine		10.00 ml
Water		to 100.00 ml
10X-concentrated Reservoir Buffer (pH 8.3)		
Tris (final 0.025 M)	60.60 g	121.20 g
Glycine (final 0.182 M)	288.20 g	576.40 g
SDS (final 0.1%)	20.00 g	40.00 g
Water	to 2 l	to 4 l

Table 1 (cont.)

0.1% Bromphenol Blue Stock		
Bromphenol blue		0.10 g
Water	to	100.00 ml
2X-concentrated Sample Buffer, no 2-ME		
Buffer A (0.125 M Tris-HCl, pH 6.8)		5.00 ml
10% SDS Stock (total 4%)		7.80 ml
Glycerol (15% v/v, note Laemmli used 10%)		4.00 ml
0.1% Bromphenol Blue Stock (0.002%)		0.50 ml
Water	to	25.00 ml
2X-concentrated Sample Buffer, with 2-ME		
Same as above plus:		
2-Mercaptoethanol (5%)		1.25 ml

(Note: diluted 2-ME tends to oxidize over a month or so. Refrigeration to slow oxidation will solidify SDS, but it will remelt easily at room temperature.)

0.05% Commassie Blue		
25% Isopropyl Alcohol		125.00 ml
10% Acetic acid		50.00 ml
0.05% Commassie blue		0.25 g
Water	to	500.00 ml

Keep slab or disc in overnight

7% Acetic Acid (Destainer)		
7% Acetic acid		70.00 ml
Water	to	1 L

Table 2. Laemmli acrylamide gels

Separating (Lower) Gel (pH 8.8) 20.04 ml					
Desired Acrylamide Concentration:	7.5%	8%	10%	12%	20%
Water:	9.88 ml	9.61 ml	8.27 ml	6.94 ml	1.53 ml
C:	5.01	5.33	6.67	8.00	13.36
B:	5.00	5.00	5.00	5.00	5.00
10% TEMED:	0.05	0.05	0.05	0.05	0.05
10% Persulfate:	0.10	0.10	0.10	0.10	0.10

Stacking (Upper) Gel (pH 6.8) 10.04 ml	
Water:	6.50 ml
C:	1.00
A:	2.50
10% TEMED:	0.10
10% Persulfate:	0.10

fresh leaf material in de-ionized water. The resulting mixtures were centrifuged in a Sorvall (RC-2B) refrigerated centrifuge for 20 min at 18,800 x g. The resulting supernatant was centrifuged in an IEC International Preparative Ultracentrifuge (Model B-35) for one hour at 90,000 x g. The supernatant was used for electrophoresis.

After the preceding electrophoretic procedure proved futile, a different extraction procedure (Ester Iglich, personal communication) and gel and reservoir buffers (M. R. Houston, personal communication) were used (Table 3). Both slab-gel and disc-gel electrophoretic methods were used in this portion of the experiment. Running times were 5.5 and 1.5 hours, respectively. Samples were prepared from newly germinated seedlings and from mature leaves collected 1-3 weeks after leaf expansion representing L. formosana and a Kentucky population of L. styraciflua. Samples were prepared by grinding fresh leaf material in 0.10 M KH_2PO_4. The resulting mixture was then centrifuged in a Sorvall (RC-2B) refrigerated centrifuge at 17,300 x g for 20 min. The supernatant was used in the electrophoresis.

The procedure was altered during two experiments. In one, the sample was dialysed against the reservoir buffer, and in the other, a 3% aqueous methanol solution (v/v) was added to the extraction buffer in order to inhibit phenol interference (F. R. Toman, personal communication).

Table 3. Extraction, acrylamide gel and reservoir buffers used in the last phase of the electrophoretic runs

Extraction Buffer

KH_2PO_4	0.136 g
Cysteine	0.36 g
EDTA	0.336 g
Sucrose	13.6 g
Tris-base	2.4 g
Citrate	0.38 g
Water	to 100 ml

Adjust pH to 7-7.5 with concentrated phosphoric acid. Keep refrigerated and do not store for longer than two weeks.

Stock Gel Buffer

Histidine-HCl	1.045 g
TEMED	0.336 ml
Glycerol	200 ml
1M NaOH to pH 8.5	
water to	1000 ml

0.03M Borate/NaOH Reservoir Buffer

Boric Acid	3.72 g	5.58 g	7.44 g
1M NaOH to pH 8.5			
Water to	2l	3l	4l

Figure 4. Disc gel electrophoretic apparatus.
Above: Reservoir and assembled gel mold.
Below: Apparatus assembled for an electrophoretic run.

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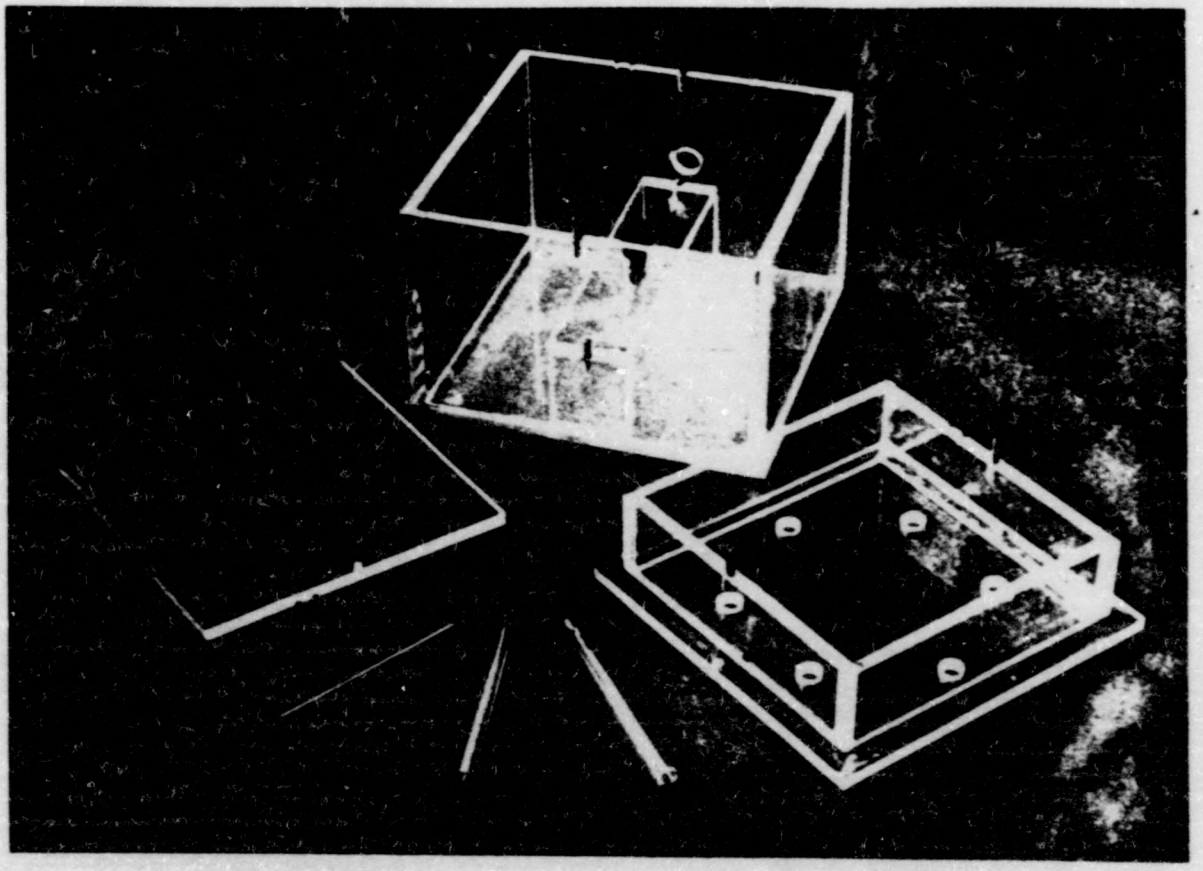
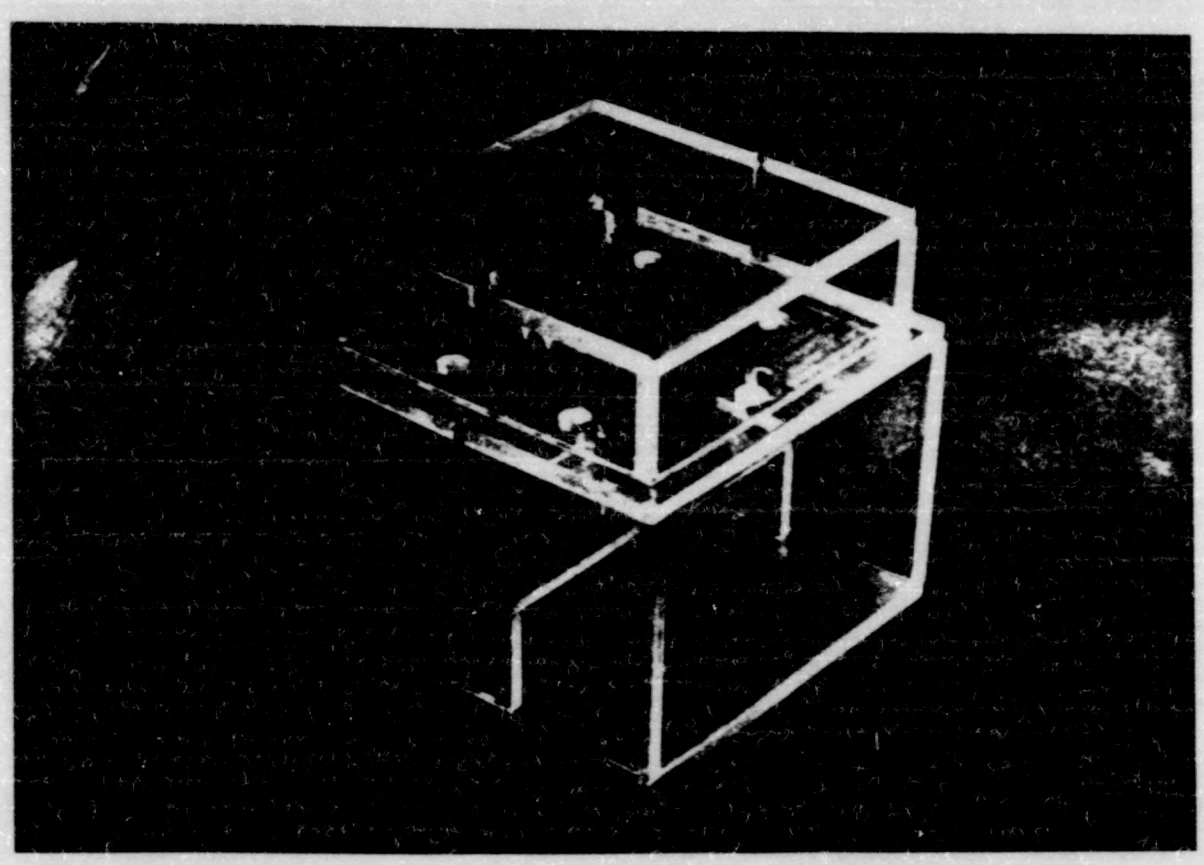




RESULTS

Analysis of the budbursting data showed definite species differences. L. formosana initiated budbursting nine days after being placed under growth chamber conditions and all specimens had shown initial budbursting 18 days after start of the program. L. styraciflua initiated budbursting later and took a longer period of time for all specimens to show budbursting. The range for L. styraciflua was 41 days compared to a range of nine days for L. formosana (Table 4).

The apical bud formation data showed a definite latitudinal cline. Under both long- and short-day conditions, the Missouri population developed dormant buds first. The Georgia population became dormant last with the Kentucky population being intermediate. Mean numbers of hours of darkness required for apical bud formation varied according to the light conditions (Tables 5 and 6) but the order of initial bud formation never changed. Statistically, there were no significant differences between the Missouri and Kentucky populations under any growth chamber conditions. Under the 12-hr light cycle, the Georgia population was highly significantly different ($p<.01$) from both the Kentucky and Missouri populations when 100% dormant bud formation was recorded. When measuring 50% dormant bud formation, however, the Georgia population was significantly

Table 4. Number of days from start of growth chamber program until initiation of budbursting

Tree Number	Date of Budbursting	Length of Time from 2/28/79
L. styraciflua		
1	4/11/79	41 days
2	4/22/79	52 days
3	3/29/79	29 days
4	3/22/79	22 days
5	4/29/79	59 days
6	4/17/79	47 days
7	4/01/79	31 days
8	3/18/79	18 days
L. formosana		
1	3/14/79	14 days
2	3/16/79	16 days
3	3/18/79	18 days
4	3/09/79	9 days
5	3/09/79	9 days
6	3/11/79	11 days
7	3/14/79	14 days
8	3/18/79	18 days

Table 5. Mean number of hours of darkness required for 50% dormant bud formation

	32-21 C 12-12 Hr.	32-21 C 16-8 Hr.
Missouri Collection Site[1]		
Mean	1325[a 2]	1445[b]
Standard Deviation	6.37	28.62
Kentucky Collection Site		
Mean	1330[a]	1454[bc]
Standard Deviation	10.04	30.68
Georgia Collection Site		
Mean	1361	1514[c]
Standard Deviation	26.29	64.10

[1]Each mean represents 10 observations.

[2]Means followed by the same letter are not statistically different.

Table 6. Mean number of hours of darkness required for 100% dormant bud formation

	32-21 C 12-12 Hr.	32-21 C 16-8 Hr.
Missouri Collection Site[1]		
Mean	1350[a 2]	1491[b]
Standard Deviation	32.12	62.74
Kentucky Collection Site		
Mean	1360[a]	1526[bc]
Standard Deviation	33.53	87.75
Georgia Collection Site		
Mean	1466[d]	1569[cd]
Standard Deviation	137.77	72.22

[1]Each mean represents 10 observations.

[2]Means followed by the same letter are not statistically different.

different ($p<.05$) from the other populations. Under the 16-8 hr light cycle, the Georgia population was significantly different from the Missouri population ($p<.05$) but there was no significant difference between the Georgia and Kentucky population. Using the paired "t" test to compare populations under both light conditions, there were highly significant differences ($p<.01$) found within each population when comparing the two light conditions. Populations under the 16-8 hr light cycle generally required more hours of darkness than those under the 12-12 hr light cycle. The only exception to this finding was the Georgia population. When measuring 100% dormant bud formation, no significant differences were found within the Georgia population.

Suggestions of species differences were seen in the first series of flavonoid patterns. (1) The chromatographs of the Texas and Mexico populations were virtually identical (Figure 5 and 6). (2) Differences between L. styraciflua (Figure 7) and L. formosana (Figure 8), while not striking, were present. Chromatographs from L. styraciflua contained two spots not found in L. formosana, a large spot of yellow pigment on the center right and a spot of light blue pigment near the lower central portion. (3) L. orientalis chromatographs (Figure 9) indicated the most pronounced differences. Two main deviations from other chromatographs were the presence of a large spot of orange pigment in the lower left corner and the absence of a spot of orange pigment in the upper margin.

Figure 5. Composite chromatograph of L. styraciflua.
This chromatograph summarizes three runs with two replications in each run.
Material: Mature leaves from an 11-year old tree representative of a Texas population.

Key: OR=orange
DR=dark red
P =purple
LB=light blue
G =green

Solid Line=spots present in all runs.
Dotted Line=spots present in most but not all runs.

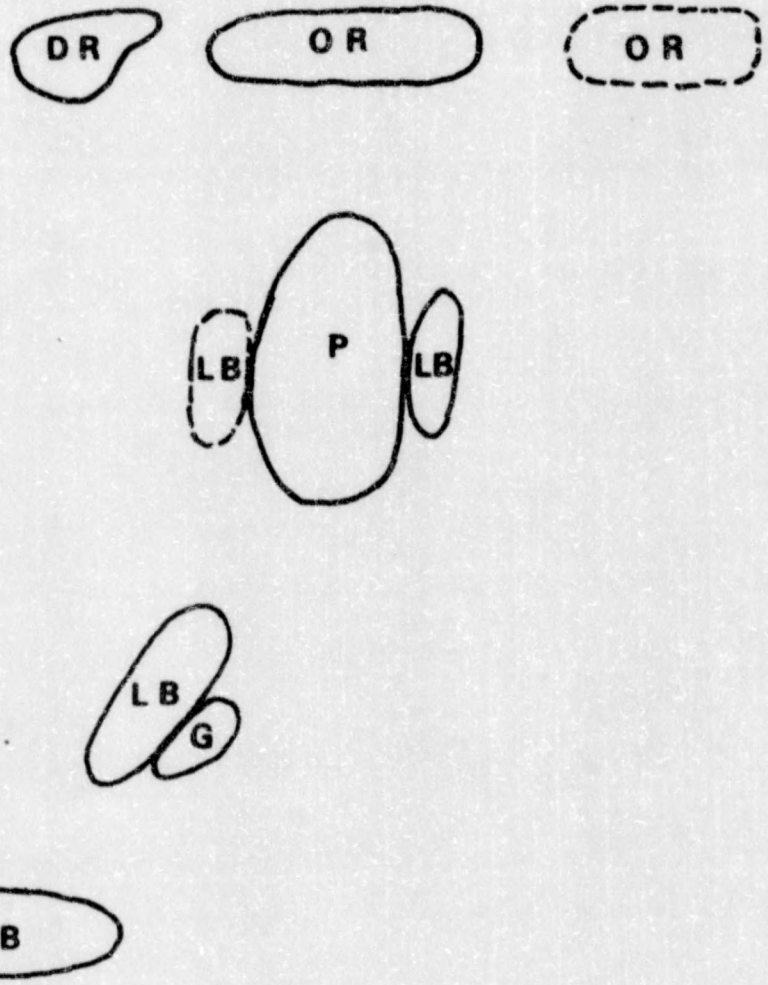

DR
OR
OR
LB
P
LB
LB
G
LB

Figure 6. Composite chromatograph of L. styraciflua
This chromatograph summarizes three runs with two replications in each run.
Material: Mature leaves from an 11-year old tree representative of a Mexico population.

Key: OR=orange
DR=dark red
P =purple
LB=light blue
G =green

Solid Line=spots present in all runs.
Dotted Line=spots present in most but not all runs.

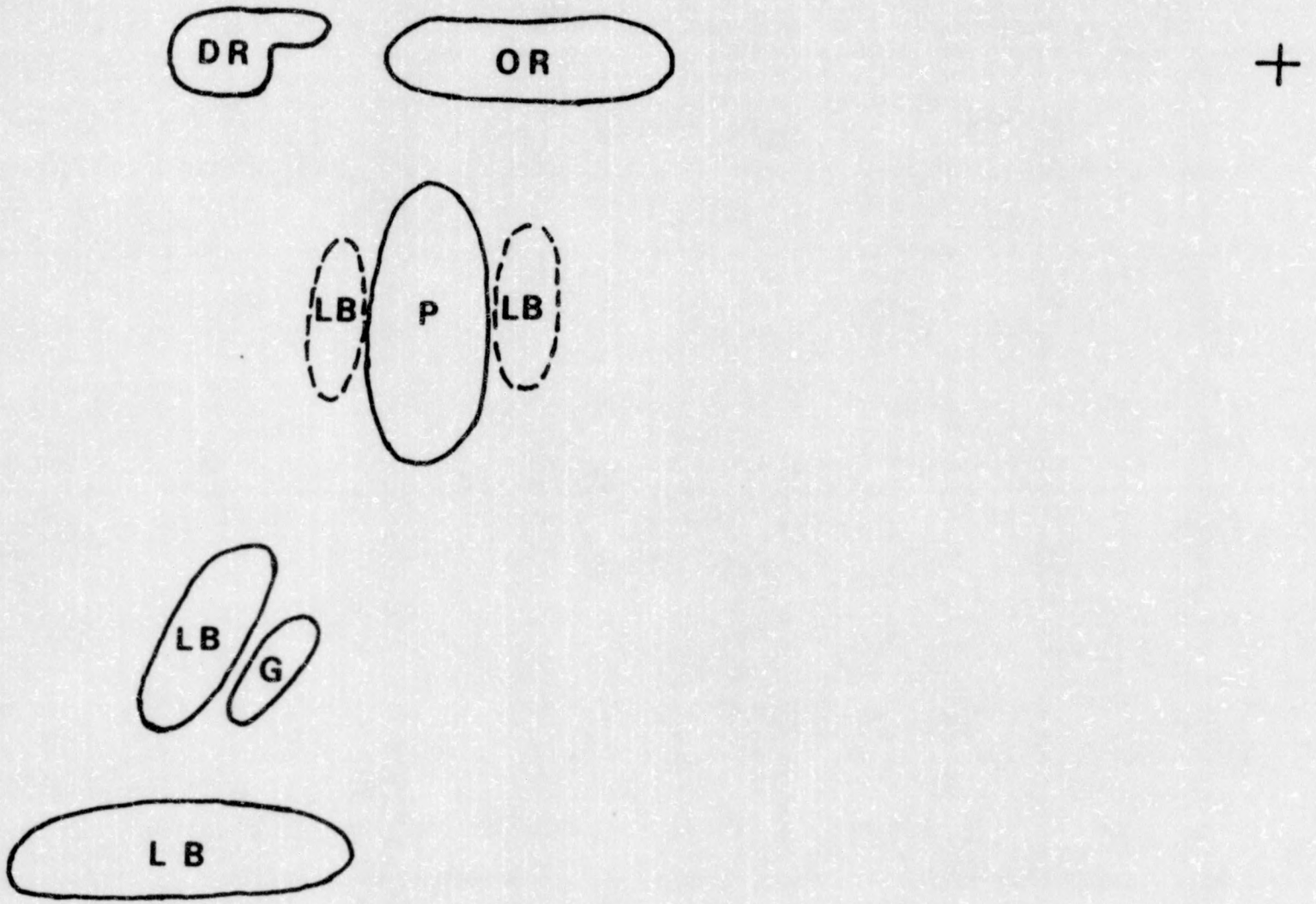

DR
OR
LB
P
LB
LB
G
L B

Figure 7. Composite chromatographs of L. styraciflua. This chromatograph summarizes three runs with three replications in each run.
Material: Mature leaves from two year old trees representing a Kentucky population.

Key: R=red
DR=dark red
OR=orange
Y =yellow
LB=light blue
YG=yellow green
DB=dark blue

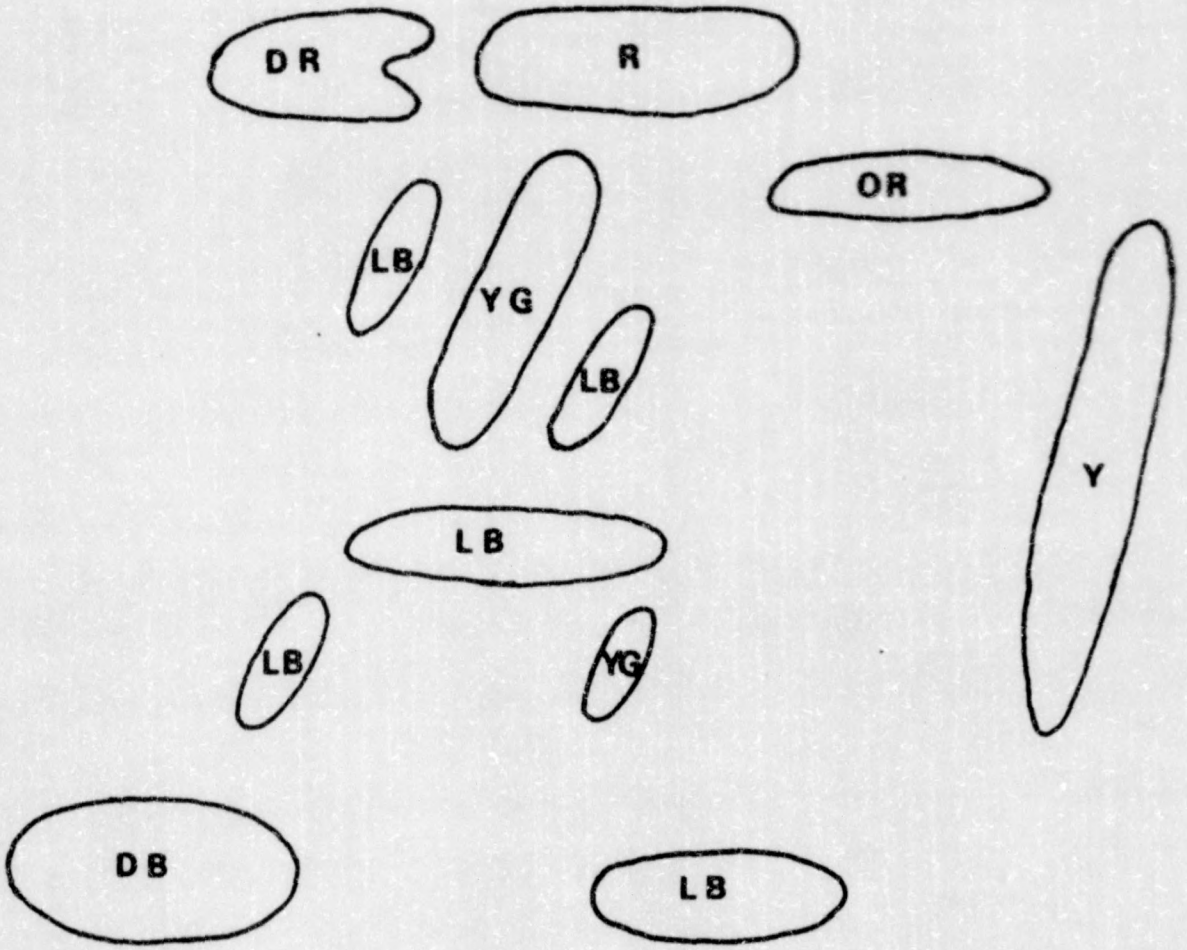

DR
R
OR
LB
YG
LB
Y
LB
LB
YG
DB
LB

Figure 8. Composite chromatograph of L. formosana.
This chromatograph summarizes three runs with three replications in each run.
Material: Mature leaves from two year old trees representing a Taiwan population.

Key:
R =red
DR=dark red
OR=orange
YG=yellow green
G =green
LB=light blue
DB=dark blue

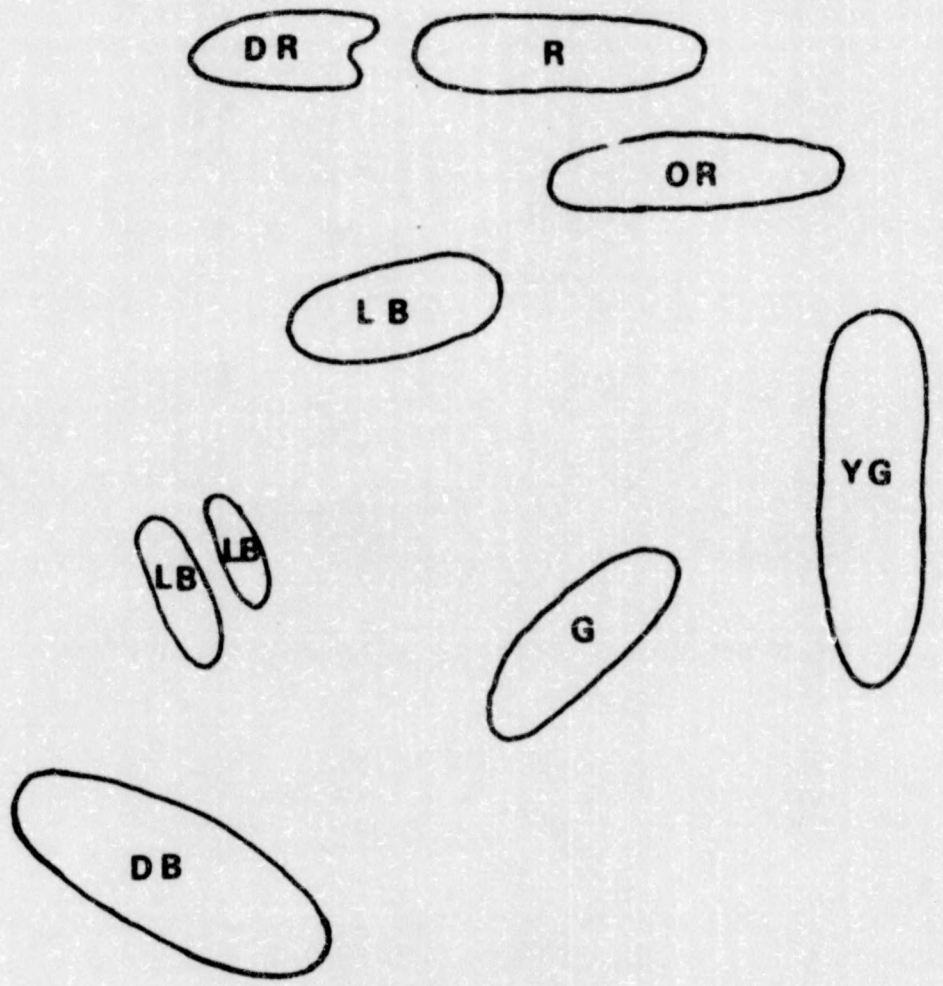
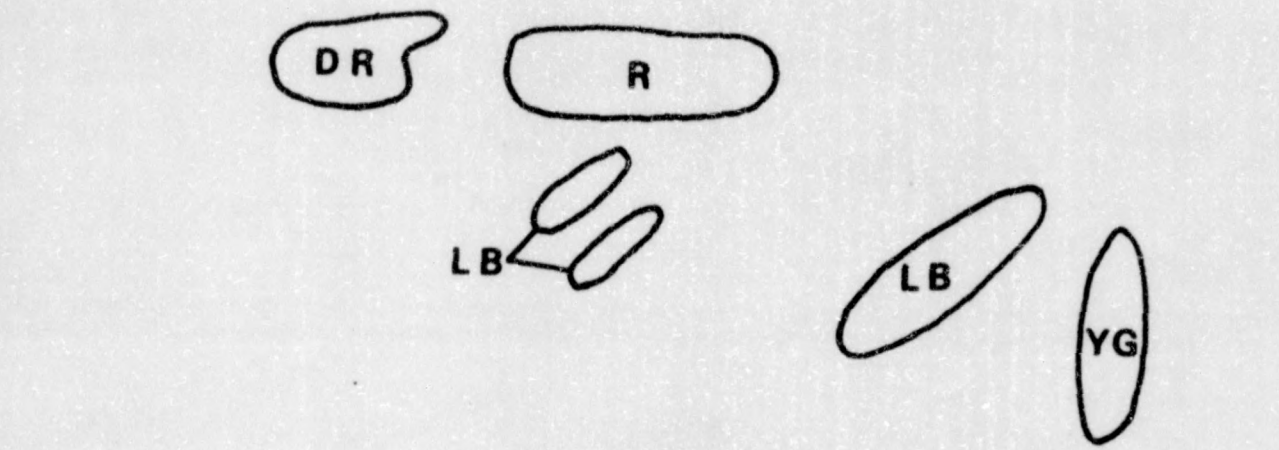
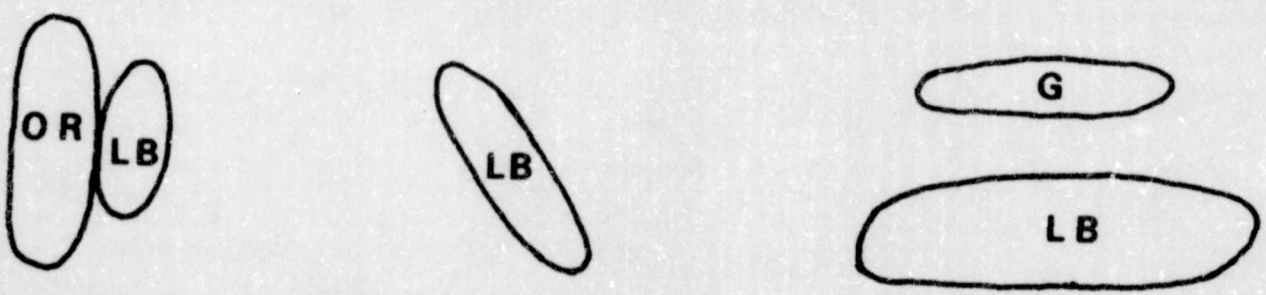

DR
R
OR
LB
LB
LB
YG
G
DB

Figure 9. Composite chromatograph of L. orientalis. This chromatograph summarizes three replications of a single sample.
Material: A single mature leaf sample from a tree near Istanbul, Turkey.

Key: R =red
DR=dark red
LB=light blue
YG=yellow green
G =green
OR=orange


DR
R
LB
LB
YG


OR
LB
LB
G
LB

This spot, along with two red spots also found in this chromatograph, is present in all other chromatographs.

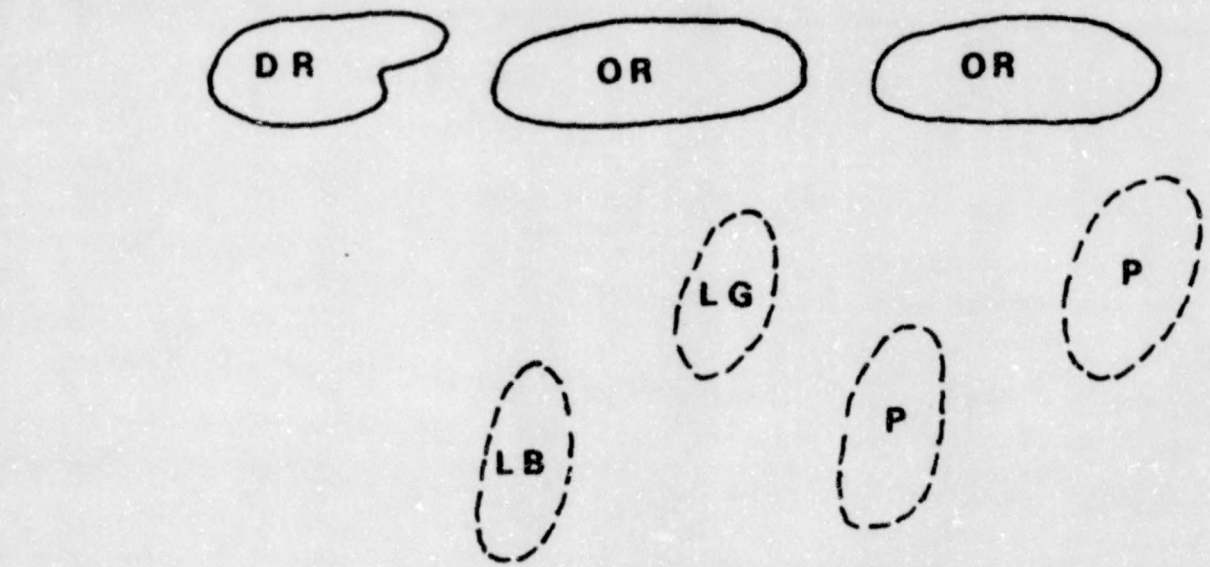
The results from the second series were not as conclusive. Comparisons of chromatographs before and after ether extraction (Figure 10 and 11) showed that many differences originally detected were due to the presence of phenols.

Results from 20 protein electrophoretic runs were discouraging. The initial experiments using Laemmli's (1970) reagents and buffers showed no indication of proteins being present. The KH_2PO_4 buffer was the only extraction buffer used which gave any indication of protein release from the leaf material and then only when used on very young seedlings. Seedlings, with no more than one set of true leaves, were ground with the KH_2PO_4 buffer. Traces of protein were seen on the first two gels run after the extract was made. Samples taken with the KH_2PO_4 buffer on older leaf material with the addition of 3% methanol and using dialysis also gave negative results.

Figure 10. Composite chromatograph of L. styraciflua. This chromatograph represents two runs with two replications in each run. The chromatograph summarizes samples treated with ether as well as an equal number of samples without the ether extraction.
Material: Mature leaves from 1-year old trees representing a Georgia population.

Key:
OR=orange
DR=dark red
LB=light blue
LG=light green
P =purple
B =blue

Solid Lines=spots present both with and without ether extraction.
Dotted Lines=spots present only without ether extraction.


DR
OR
OR
LG
P
LB
P

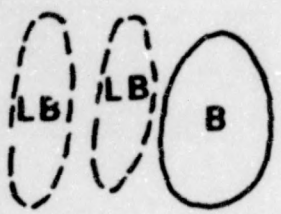
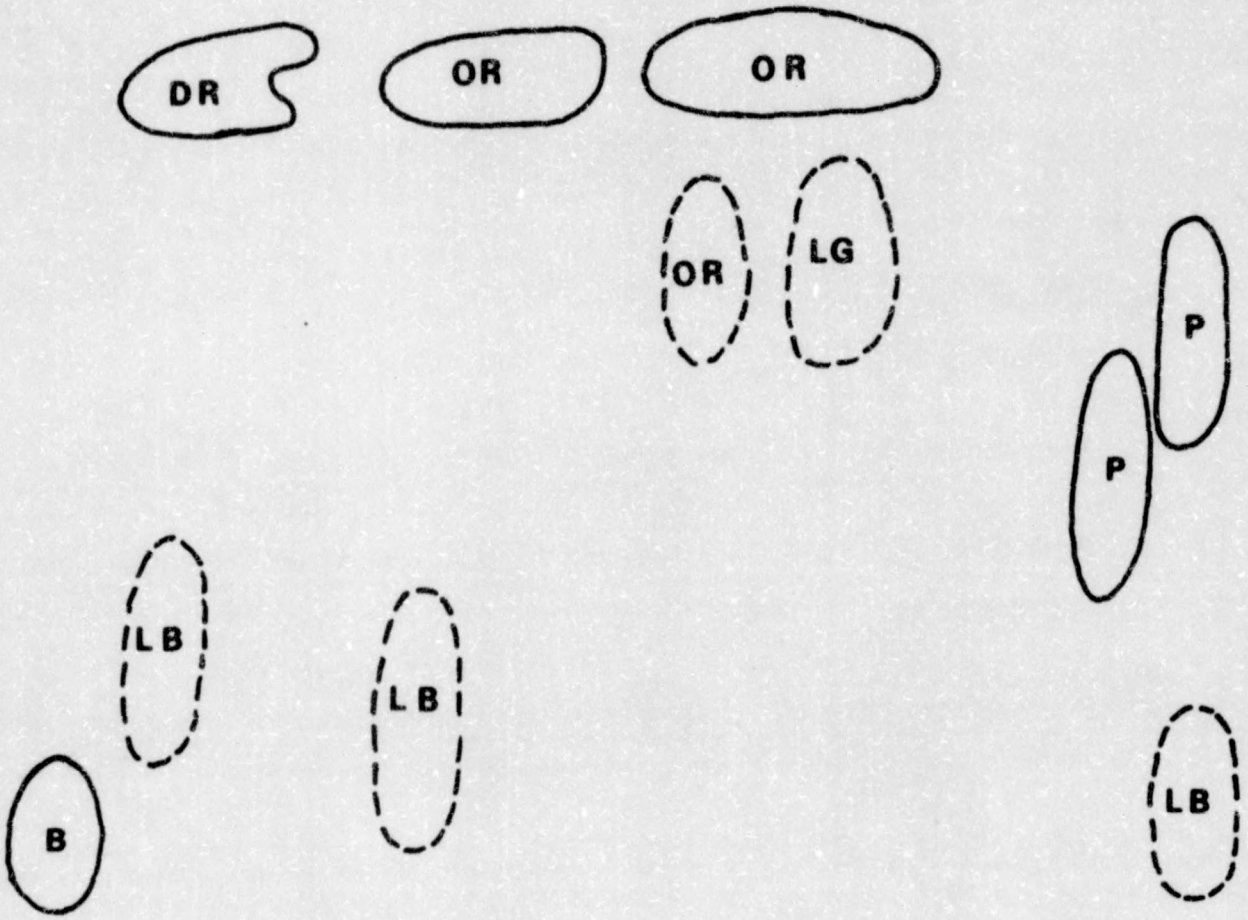

LB
LB
B

Figure 11. Composite chromatograph of L. formosana. This chromatograph represents two runs with two replications in each run. The chromatograph summarizes samples treated with ether as well as an equal number of samples without the ether extraction.
Material: Mature leaves from 1-year old trees representing a Taiwan population.

Key:
OR=orange
DR=dark red
LG=light green
P =purple
LB=light blue
B =blue

Solid Lines=spots present with and without ether extraction.

Dotted Lines=spots present only without ether extraction.


DR
OR
OR
OR
LG
P
P
LB
LB
B
LB

DISCUSSION

Data from the various studies were to be used to indicate species differences and possible indications of origin and migration of the genus. However, results from the budbursting, dormant bud formation and flavonoid patterns indicated response or adaptation to environment influences more than taxonomic differences.

Previous studies have shown ecotypic differences between northern and southern populations of Liquidambar styraciflua (Winstead, 1971, 1972; Winstead and McMillan, 1971; Randel and Winstead, 1976). Budbursting and dormant bud formation are factors included in these studies. Budbursting or leaf emergence, occurring in the spring, must be timed so that damage from frost is minimized. Frost in this area is not unusual into April, and freezing temperatues have been reported for as late as May 1. Therefore, budbursting must be delayed for a time after the first exposure to warm temperatures to prevent damage by late frost. Temperate populations of Liquidambar have been shown to have a cold requirement (Randel and Winstead, 1976) in initiating budbursting, while in subtropical or tropical populations budbursting is more in response to increasing temperatures.

Budbursting data collected from two-year-old trees of Liquidambar styraciflua and L. formosana tends to support

the premise that budbursting in temperate populations is delayed somewhat after introduction to warm temperatures while the subtropical species responds readily to warm temperatures. Specimens from L. formosana initiated budbursting nine days after being removed from the greenhouse and being placed in the growth chamber. All specimens from this species had initiated budbursting 18 days after the program was started; however, representatives of L. styraciflua delayed budbursting until the 18th day and continued through day 59. The mean day of budbursting of L. styraciflua was 37.4 compared to 13.6 days for L. formosana. Randel and Winstead (1976) showed in temperate populations of L. styraciflua length of cold treatment is inversely proportional to length of the time for budbursting to occur. In their study, limbs under cold treatment of zero days required 65 days for budbursting to occur, while those under cold treatment for 33 days required only 30 days and so on. The plants in the present study were maintained overwinter in the greenhouse thus effectively removing the cold treatment. The length of time for budbursting in L. styraciflua (the same species used by Randel) averaged 37.4 days which is much shorter than the 65 days required under similar conditions in their study. Possibly, the temperature in the greenhouse dropped enough to provide some exposure to low temperatures.

The absence of cold treatment for L. styraciflua could explain some of the differences in the length of time for

budbursting between L. styraciflua and L. formosana. However, due to the large differences in the two populations, it can be inferred that the subtropical species of L. formosana responds more readily to warm temperatures by initiating budbursting shortly after exposure. Budbursting in temperate species of Sweetgum is a response to exposure to a certain number of hours of cold temperature (McMillan and Winstead, 1976). Therefore, time of budbursting can vary from year to year. This need for cold treatment can delay budbursting and offer protection from frost. In tropical and subtropical species, on the other hand, budbursting seems to be a response to slight increases in temperature. This would explain the quick response of L. formosana to the growth chamber program.

Somewhat opposite in nature is dormant bud formation. Since dormancy is a way to protect delicate plant material, it must occur early enough to protect against early autumn frost. Dormant bud formation seems to be regulated by photoperiod. Exposure to a certain total number of hours of darkness is necessary for its occurrence. Randel and Winstead (1976) showed that more northern populations of Sweetgum required exposure to a lower total number of hours of darkness than southern populations. Results of the dormant bud data showed a definite population cline with representatives of the Missouri population going dormant first under both light conditions. While populations under the 12-12 hr light cycle required slightly fewer hours of darkness for dormancy in all

populations, the order of occurrence did not change. With one exception, each population required an increase in the total number of hours of darkness for dormant bud formation under the 12 hr light cycle. That one exception was the Georgia population when 100% dormant bud formation was recorded. In this one instance, there was a large amount of intrapopulation variation which was reflected in the large standard deviation. This large amount of variability within the one population may be masking significant differences in response to the different light conditions. It is interesting to note that the three representatives of L. formosana under the 16-8 hr light cycle at no time went dormant during the program. The climate in Taiwan is subtropical. Frosts are rare and not as prolonged as in temperate regions. Adaptations for frost tolerance are not as necessary for these plants. Their lack of mechanisms for frost tolerance was witnessed in both budbursting and dormant bud formation.

Results from the initial flavonoid studies indicated some species differences. In comparing the representatives from a Texas and Mexico population of Liquidambar styraciflua, few differences were noted. Those that were present were not found in all samples. If interpretations can be made from such a small sample size, the Mexico population seems to be closely related to the progeny examined from Texas. Harms (1930) wanted to separate the Central American populations from L. styraciflua and place them in a separate species.

These results indicate a close relationship between these two populations and on the basis of flavonoid patterns the separation of these two is not justified.

Leaves from two-year-old trees from L. styraciflua and L. formosana grown under controlled conditions showed only subtle differences. These patterns had fewer actual differences than are apparent when comparisons are made between the representative of L. styraciflua from Texas and L. styraciflua grown in the greenhouse. The trees in the first comparison (L. styraciflua of Texas and Mexico) represent older trees grown under harsher conditions than those in the second group. What may be more important than species or populational differences are environmental influences. It has been inferred that flavonoid patterns were not affected by environmental factors. However, this study indicates that this may not be the case. Beal (1967) working with two species of Spirodela showed in some plants environmental factors play an important role in flavonoid patterns. Certainly, more work needs to be done in this area.

The single sample from L. orientalis indicated the greatest number of differences. A single sample is shallow for making comparisons, but it does indicate that L. orientalis may not be as closely related to L. styraciflua as once indicated. This could contradict Harms (1930) placement of L. orientalis and L. styraciflua in the same subgroup. If the subgroups are to be retained, possibly L. orientalis should be placed in a separate subgroup and L. formosana and

L. styraciflua grouped together. An indepth study of the chemotaxonomy of L. orientalis is warranted.

Samples run with and without the ether extraction show that many of the differences in the original set of comparisons could be due to the presence of phenolic compounds. Samples were taken from representatives of a Georgia population of L. styraciflua and L. formosana which were grown under identical conditions. Again the patterns were more similar when compared to each other than when compared to members of the same species grown under different conditions.

It was planned to use protein banding patterns to determine any species differences as well as any populational differences of Liquidambar styraciflua. However, this procedure needs more work before protein banding patterns can be done.

The first runs were done using the method described by Laemmli (1970). This procedure has been successfully used by other investigators (Clarkson, 1973, 1977, Sharitz, 1978) using plant material. Runs were made varying gel concentrations, voltage and sample size. After protein determinations were performed on each sample which indicated proteins were present, several samples were concentrated before the electrophoretic tests. After 12 different runs proved unsuccessful, a different extraction buffer was obtained from Ester Iglich (Savannah River Ecology Lab). Changes were also made in the gel and reservoir buffers. Four runs were made using these changes. Samples were taken on very young seedlings as well

as fresh mature leaf material. Samples from the young seedlings indicated the presence of proteins but no real separation. No proteins were detected in the mature leaf material. Due to the increase in gel thickness, it was felt that switching to disc gel would improve separation. However, samples taken using the KH_2PO_4 buffer (Iglich) are not stable and poor results were obtained. Mature leaf material was tried but without success.

While working with maple leaves, Iglich encountered a similar problem. Acer and Liquidambar are both known to contain high phenol concentrations. Winstead reported problems with phenol interference while working with enzymes in Liquidambar (verbal communication). It was decided that possibly phenols could be interfering with the proteins. An extraction buffer which contained 3% methanol was used on fresh mature leaf material. It was hoped that addition of the methanol would prevent ionization of the phenols; therefore preventing reactions with the proteins. This procedure gave no positive results. Dialysing the sample against the reservoir buffer was also tried. This was done to increase the mobility of the sample hoping that whatever proteins were present would migrate more easily. Again, no positive results were obtained. It was decided to terminate the experiment at this point.

Recommendations for successive trials are the use of young seedlings containing no more than one set of true leaves and using samples as fresh as possible (samples must

stored frozen for no more than one week. Iglich reported that storage for two weeks was permissible but in the current study activity was lost after one week. If available, staining with napthol blue black instead of commassie blue should be tried. Napthol blue black is a more general stain and could possibly pick up some proteins that commassie blue misses.

SUMMARY

1. Populations of L. styraciflua show a delay in budbursting and a latitudinal cline in dormant bud formation. This temperate species shows adaptation to shorter growing season by delay of budburst as well as requiring shorter numbers of hours of darkness for dormant bud formation. The subtropical species, L. formosana, in comparison exhibits little evidence of such adaptations to frost tolerance. These differences were shown in both budbursting and dormant bud formation.

2. Flavonoid patterns indicated possible environmental influences. Patterns from different species grown under similar conditions were consistently more alike than patterns from the same species grown under different conditions.

3. Phenolic compounds can interfere with flavonoid patterns. Studies done on samples treated with ether show that some differences indicated in the flavonoid patterns were actually due to phenols.

4. Protein banding patterns studies were inconclusive. No usable results were obtained but several recommendations can be made:

a. Taking samples from only very young seedlings. Seedlings with no more than one set of true leaves should be used.

b. Using only fresh samples. Samples should be stored frozen for no longer than one week.

c. Alternate staining techniques should be attempted.

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VITA

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